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Description

TECHNICAL FIELD

[0001] This invention concerns vaccines against *Neisseria meningitidis*. In particular, it
5 concerns vaccines based on conjugated capsular saccharides from multiple meningococcal serogroups.

BACKGROUND ART

[0002] Based on the organism's capsular polysaccharide, twelve serogroups of
10 *N.meningitidis* have been identified (A, B, C, H, I, K, L, 29E, W135, X, Y and Z). Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in USA and in most developed countries. Serogroups W135 and Y are responsible for the remaining cases in USA and developed countries.

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[0003] A tetravalent vaccine of capsular polysaccharides from serogroups A, C, Y and W135 has been known for many years [1,2]. Although effective in adolescents and adults, it induces a poor immune response and short duration of protection and cannot be used in infants [e.g. ref. 3] because polysaccharides are T cell-independent
20 antigens that induce a weak immune response which cannot be boosted. The polysaccharides in this vaccine are not conjugated [4].

[0004] Conjugate vaccines against serogroup C have been approved for human use, and include Menjugate™ [5], Meningitec™ and NeisVac-C™. Mixtures of conjugates
25 from serogroups A+C are known [6-8] and mixtures of conjugates from serogroups A+C+W135+Y have been reported [9-13].

[0005] While meningococcal conjugates are well known, they have not yet been fitted into existing pediatric immunisation schedules, which for developed countries typically
30 involve: hepatitis B vaccine at birth; and, starting at 2 months, all of diphtheria/tetanus/pertussis (D-T-P), *H.influenzae* type b (Hib) conjugate, inactivated poliovirus and pneumococcus conjugates at 2 months.

[0006] When adding conjugated vaccines to existing immunisation schedules,
35 however, the issue of carrier-induced epitopic suppression (or "carrier suppression", as it is generally known) must be addressed, particularly suppression arising from carrier

priming. "Carrier suppression" is the phenomenon whereby pre-immunisation of an animal with a carrier protein prevents it from later eliciting an immune response against a new antigenic epitope that is presented on that carrier [14].

5 [0007] As reported in reference 15, where several vaccine antigens contain the same protein component (being used as an immunogen and/or as a carrier protein in a conjugate) then there is the potential for interference between those antigens. In reference 15, the immune response against an antigen that was conjugated to a tetanus toxoid (Tt) carrier was suppressed by pre-existing immunity against Tt.

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[0008] Reference 16 reports how a combination of D-T-P vaccines with a Hib conjugate vaccine was adversely affected where the carrier for the Hib conjugate was the same as the tetanus antigen from the D-T-P vaccine. The authors concludes that this "carrier suppression" phenomenon, arising from interference by a common protein
15 carrier, should be taken into account when introducing vaccines that include multiple conjugates.

[0009] In contrast to references 15 and 16, reference 17 reported that priming with tetanus toxoid had no negative impact on the immune response against a
20 subsequently-administered Hib-Tt conjugate, but suppression was seen in patients with maternally acquired anti-Tt antibodies. In reference 18, however, an "epitopic suppression" effect was reported for a Tt-based peptide conjugate in patients having existing anti-Tt antibodies resulting from tetanus vaccination.

[0010] In reference 19, it was suggested that a conjugate having CRM197 (a detoxified mutant of diphtheria toxin) as the carrier may be ineffective in children that had not previously received diphtheria toxin as part of a vaccine (e.g. as part of a D-T-P or D-T vaccine). This work was further developed in reference 20, where a carrier
25 riming effect by D-T immunisation was seen to persist for subsequent immunisation with Hib conjugates.
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[0011] In reference 21, the authors found that pre-immunisation with a diphtheria or tetanus toxoid carrier protein reduced the increase in anti-Hib antibody levels after a subsequent immunisation with the Hib capsular saccharide conjugated to those
35 carriers, with IgG1 and IgG2 being equally affected. Responses to the carrier portions of the conjugates were also suppressed. Furthermore, a more general non-epitope-

specific suppression was seen, as pre-immunisation with one conjugate was seen to affect immune responses against both the carrier and saccharide portions of a second conjugate that was administered four weeks later.

5 [0012] The use of different carrier proteins in a single multivalent pneumococcal conjugate vaccine is reported in reference 22, with multiple carriers being used in order to avoid carrier suppression. The authors predict that there is a maximum load of a carrier protein that can be tolerated in a multivalent conjugate vaccine without giving rise to negative interference. In reference 23 it was reported that pneumococcal
10 conjugate vaccines including mixed carrier proteins elicited, in parallel to the anti-pneumococcus response, unintentional booster responses to the carriers.

[0013] In reference 24, an investigation of whether diphtheria and tetanus boosters could be administered with monovalent meningococcal serogroup C conjugates, it was
15 found that titres against the meningococcal conjugate were reduced where the carrier was tetanus toxoid carrier and the patient had received prior immunisation with a tetanus-containing vaccine.

[0014] Finally, reference 25 reports that "prior exposure to the carrier protein can
20 either enhance or suppress antibody response to polysaccharides administered in saccharide-protein conjugates". The conjugates used in reference 25 used tetanus toxoid or the CRM197 mutant as carrier protein.

[0015] The situation concerning carrier priming and/or suppression is thus confused,
25 and it remains unclear whether any particular conjugate will suffer from carrier suppression or will benefit from a carrier priming enhancement. Meningococcal conjugate vaccines will not be in a position to be integrated into or added to existing pediatric immunisation schedules until this issue is addressed. Furthermore, as meningococcal conjugates are to be administered as tetravalent mixtures (*i.e.* four
30 different conjugates) then the potential for carrier suppression becomes even more of a risk.

[0016] In addition to the problem of priming with a carrier having a negative impact on immune responses against saccharide conjugates, the reverse can also occur *i.e.*
35 immunisation with a conjugate can have a negative impact on immune responses against the carrier [26].

[0017] WO 03/094834 discloses immunogenic compositions comprising (a) a capsular saccharide antigen from serogroup C of *N.meningitidis*, and (b) a chitosan adjuvant. The compositions are said preferably to comprise (c) one or more further antigens
5 and/or (d) one or more further adjuvants. The compositions are said to be particularly suitable for mucosal delivery, including intranasal delivery. WO 03/094834 also discloses immunogenic compositions for mucosal delivery comprising capsular saccharides from at least two of serogroups A, C, W135 and Y of *N.meningitidis*. It is said to be preferred for the capsular saccharides in the compositions of the invention
10 to be conjugated to carrier protein(s) and/or to be oligosaccharides. Conjugated oligosaccharide antigens are said to be particularly preferred.

DISCLOSURE OF THE INVENTION

[0018] Reference 27 suggests that carrier suppression in meningococcal conjugate
15 vaccines should be dealt with by using more than one type of carrier protein. In particular, reference 27 suggests that *H.influenzae* protein D should be used as the carrier protein for meningococcal conjugates, with tetanus toxoid (Tt) also being a possibility. To avoid epitope suppression, protein D is also the carrier of choice in reference 28. Similarly, reference 29 suggests that *Bordetella pertussis* fimbriae
20 should be used as the carrier in order to avoid carrier suppression in multivalent conjugate vaccines. In contrast, it has been found that diphtheria toxoid (Dt) and its derivatives can safely be used as the carrier for meningococcal saccharide conjugates, even where multiple meningococcal conjugates are administered at the same time. None of the monovalent meningococcal serogroup C conjugates studied in
25 reference 24 used a Dt carrier.

[0019] Moreover, reference 27 also suggests that meningococcal conjugate vaccines should be administered at the same time as D-T-P-Hib vaccines (e.g. see example 3), such that there is no previous exposure to the carrier protein from the meningococcal
30 conjugates. In contrast, it has now been found that meningococcal conjugates can be administered to patients even where they have already received the carrier protein, either in the form of a previous immunogen (e.g. in a D-T-P or a D-T immunisation) or as a previous carrier protein (e.g. in a Hib conjugate or pneumococcal conjugate vaccine). The previous study of carrier-induced epitopic suppression in monovalent
35 serogroup C conjugate vaccines [24] did not look at the effect of any prior administration of conjugates.

[0020] As well as contrasting with reference 27, the ability of a patient to raise an immune response against a meningococcal conjugate, even where they have already received a different conjugate, contrasts with reference 21.

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[0021] Thus the invention provides a composition that comprises: (a) a conjugate of (i) the capsular saccharide of serogroup A *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; (b) a conjugate of (i) the capsular saccharide of serogroup C *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; (c) a conjugate of (i) the capsular saccharide of serogroup W135 *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; and (d) a conjugate of (i) the capsular saccharide of serogroup Y *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid, for use in a method for immunising a patient against a disease caused by *Neisseria meningitidis*, comprising the step of administering to the patient the composition, wherein the patient has been pre-immunised with (a) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid and/or (b) a conjugate of (i) a capsular saccharide of an organism other than *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid.

[0022] The invention also provides the use of: (a) a conjugate of (i) the capsular saccharide of serogroup A *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; (b) a conjugate of (i) the capsular saccharide of serogroup C *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; (c) a conjugate of (i) the capsular saccharide of serogroup W135 *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; and (d) a conjugate of (i) the capsular saccharide of serogroup Y *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid, in the manufacture of a medicament for immunising a patient against a disease caused by *Neisseria meningitidis* wherein the patient has been pre-immunised with (a) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with

diphtheria toxoid and/or (b) a conjugate of (i) a capsular saccharide of an organism other than *N.meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid.

- 5 [0023] The meningococcal disease is preferably meningitis, more preferably bacterial meningitis, and most preferably meningococcal meningitis. Thus the invention can be used to protect against meningococcal infections that cause meningitis.

[0024] Where the pre-immunisation antigen is a derivative of a diphtheria toxoid that
10 remains immunologically cross-reactive with Dt, then that derivative is preferably CRM197.

The pre-immunised patient

[0025] The patient to be immunised has been pre-immunised with: (a) a diphtheria
15 toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid; and/or (b) a conjugate of (i) a capsular saccharide of an organism other than *Neisseria meningitidis* and (ii) a diphtheria toxoid or derivative thereof that remains immunologically cross-reactive with diphtheria toxoid. Typical pre-immunisation will have included: a diphtheria toxoid antigen; a Hib capsular saccharide conjugate using
20 a diphtheria toxoid or CRM197 carrier; and/or a pneumococcal capsular saccharide conjugate using a diphtheria toxoid or CRM197 carrier.

[0026] The patient will have received at least one (e.g. 1, 2, 3 or more) dose of the
25 pre-immunisation antigen(s), and that dose (or the earliest of multiple doses) will have been administered to the patient at least six (e.g. 6, 9, 12, 15, 18, 21, 24, 36, 48, 60, 120, 180, 240, 300 or more) months before the immunisation with the meningococcal conjugates according to the invention. In a preferred group of patients, the pre-immunisation took place within 3 years of birth e.g. within 2 years of birth, within 1 year of birth, within 6 months of birth, or even within 3 months, 2 months or 1 month of birth.

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[0027] The patient to be immunised according to the invention will typically be a
human. The human will generally be at least 1 month old e.g. at least 2 months old, at
least 3 months old, at least 4 months old, at least 6 months old, at least 2 years old, at
least 5 years old, at least 11 years old, at least 17 years old, at least 40 years old, at
35 least 55 years old, etc. A preferred set of patients is at least 6 months old. Another preferred set of patients is in the age group 2-55 years old, and another preferred set

of patients is in the age group 11-55 years old. A further preferred set of patients is less than 11 years old e.g. 2-11 years old. In all cases, however, regardless of age, the patient will have been pre-immunised as defined herein.

5 **[0028]** Where the pre-immunisation antigen is a diphtheria toxoid then the patient will typically have received the toxoid as the 'D' antigen in a D-T-P or a D-T pre-immunisation. Such immunisations are typically given to newborn children at ages 2, 3, and 4 months. Where the immunisation includes a pertussis vaccine, that vaccine may be a whole cell or cellular pertussis vaccine ('Pw'), but is preferably an acellular
10 pertussis vaccine ('Pa'). Pre-immunisation Pa vaccines will generally include one, two or three of the following well-known and well-characterised *B.pertussis* antigens: (1) pertussis toxoid ('PT'), detoxified either by chemical means or by site-directed mutagenesis e.g. the '9K/129G' mutant [30]; (2) filamentous haemagglutinin ('FHA'); (3) pertactin (also known as '69 kiloDalton outer membrane protein'). Acellular
15 pertussis vaccines may also include agglutinin 2 and/or agglutinin 3. The 'T' antigen in a D-T-P pre-immunisation is typically a tetanus toxoid.

[0029] Where the pre-immunisation antigen is a diphtheria toxoid then the patient may also or alternatively have received the toxoid as the carrier protein of a protein-saccharide conjugate. Such conjugates include the 'PRP-D' Hib conjugate [see Table
20 14-7 of ref. 32] e.g. the ProHIBIT™ product.

[0030] Where the pre-immunisation antigen is CRM197 then the patient will typically have been pre-immunised with a Hib conjugate and/or a multivalent pneumococcal
25 conjugate. Such immunisations are typically given to newborn children at ages 2, 3, and 4 months. Hib conjugates that use a CRM197 carrier include the 'HbOC' conjugates [Table 14-7 of ref. 32] e.g. the HibTITER™ product. Pneumococcal conjugates that use a CRM197 carrier include the 7-valent PCV7 mixtures e.g. the PreVNar™ vaccine [31]. The patient may also have been pre-immunised with a
30 serogroup C meningococcal ('MenC') conjugate. MenC conjugates that use CRM197 carrier include Meninvact™/Menjugate™ [5] and Meningitec™. Preferably, however, the patient has been pre-immunised with Hib and/or pneumococcal conjugate, but not with a MenC conjugate. If the patient has been pre-immunised with a MenC conjugate then the vaccine administered according to the invention may or may not include a
35 serogroup C conjugate.

[0031] Where pre-immunisation was with a conjugated antigen then the patient will almost inevitably have also received a small amount of free diphtheria toxoid (or derivative) as a result of low-level contamination of the conjugate (e.g. caused by hydrolysis of the conjugate during storage), but this small amount will not typically have been adequate to provide a significant immune response.

[0032] Diphtheria toxoid is a well known and well characterised protein [e.g. see chapter 13 of ref. 32] that can be obtained by treating the ADP-ribosylating exotoxin of *Corynebacterium diphtheriae* with an inactivating chemical, such as formalin or formaldehyde. CRM197 is also well known and well characterised [33-36], and has been widely used as a carrier in conjugated saccharide vaccines. CRM197 and Dt share many carrier epitopes.

[0033] The result of the pre-immunisation is that the patient's immune system has been exposed to the pre-immunisation antigens. For pre-immunisation with diphtheria toxoid (Dt), this generally means that the patient will have raised an anti-Dt antibody response (typically to give an anti-Dt titer >0.01 IU/ml) and will possess memory B and/or T lymphocytes specific for Dt *i.e.* pre-immunisation with Dt is typically adequate to elicit an anamnestic anti-Dt immune response in the patient. For pre-immunisation where Dt (or derivative) is a carrier for a saccharide within a conjugate then the pre-immunisation will have raised an anti-saccharide response and the patient will possess memory B and/or T lymphocytes specific for the saccharide *i.e.* the pre-immunisation is typically adequate to elicit an anamnestic anti-saccharide immune response in the patient. The pre-immunisation was preferably adequate to elicit protective immunity in the patient e.g. against diphtheria disease.

[0034] Thus the patients to be immunised according to the invention are distinct from patients in general, as they are members of a subset of the general population whose immune systems have already mounted an immune response to the pre-immunisation antigens, such that immunisation according to the invention with a meningococcal conjugate that includes a diphtheria toxoid (or derivative) carrier elicits a different immune response in the subset than in patients who have not previously mounted an immune response to the pre-immunisation antigens. Patients who have been pre-immunised with Dt (or derivative) as the carrier of a conjugate (particularly of a Hib conjugate) are preferred. Particularly preferred patients have been pre-immunised with

Dt (or derivative) as the carrier of a conjugate and also with Dt as an unconjugated immunogen.

[0035] As well as having been pre-immunised with a diphtheria toxoid (or derivative),
5 in conjugated or non-conjugated form, the patient may have been pre-immunised with
other antigens. Such antigens include, but are not limited to: pertussis antigen(s) - see
above; tetanus toxoid - see above; *Haemophilus influenzae* type B - see above;
hepatitis B surface antigen (HBsAg); poliovirus, such as an inactivated poliovirus
vaccine (IPV); *Streptococcus pneumoniae* - see above; influenza virus; BCG; hepatitis
10 A virus antigens; measles virus; mumps virus; rubella virus; varicella virus; *etc.*

[0036] The patient may or may not have been pre-immunised with one or more
meningococcal conjugate(s). In some preferred embodiments, at the time when a
patient first receives a meningococcal conjugate, they have already been pre-
15 immunised with Dt (or derivative). In other embodiments, a meningococcal conjugate
is administered to a patient who has already been pre-immunised with both (i) Dt or a
derivative and (ii) a meningococcal conjugate.

The conjugates

20 [0037] The invention immunises patients with conjugated saccharides. Conjugation is
used to enhance the immunogenicity of saccharides, as it converts them from T-
independent antigens to T-dependent antigens, thus allowing priming for
immunological memory. Conjugation is particularly useful for pediatric vaccines [*e.g.*
ref. 37] and is a well known technique [*e.g.* reviewed in refs. 38 to 46].

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[0038] The composition used according to the invention comprises at least two
meningococcal conjugates, wherein each conjugate comprises a diphtheria toxoid (or
derivative) carrier protein, and the capsular saccharide. The capsular saccharides are
chosen from meningococcal serogroups A, C, W135 and Y, such that the
30 compositions include saccharides from 2, 3, or all 4 of these four serogroups. Specific
compositions comprise saccharides from: serogroups A & C; serogroups A & W135;
serogroups A & Y; serogroups C & W135; serogroups C & Y; serogroups W135 & Y;
serogroups A & C & W135; serogroups A & C & Y; serogroups A & W135 & Y;
serogroups C & W135 & Y; serogroups A & C & W135 & Y. Compositions including
35 saccharides from all four serogroups are most preferred.

[0039] The capsular saccharides of each of these four serogroups are well characterised. The capsular saccharide of serogroup A meningococcus is a homopolymer of (α 1 \rightarrow 6)-linked N-acetyl-D-mannosamine-1-phosphate, with partial O-acetylation in the C3 and C4 positions. The acetyl groups can be replaced with blocking groups to prevent hydrolysis [47], and such modified saccharides are still serogroup A saccharides within the meaning of the present invention. The serogroup C capsular saccharide is a homopolymer of (α 2 \rightarrow 9)-linked sialic acid (N-acetyl neuraminic acid, or 'NeuNAc'). Most serogroup C strains have O-acetyl groups at C-7 and/or C-8 of the sialic acid residues, but about 15% of clinical isolates lack these O-acetyl groups [48,49]. The saccharide structure is written as \rightarrow 9)-Neu p NAc 7/8 OAc (α 2 \rightarrow . The serogroup W135 saccharide is a polymer of sialic acid-galactose disaccharide units. Like the serogroup C saccharide, it has variable O-acetylation, but at sialic acid 7 and 9 positions [50]. The structure is written as: \rightarrow 4)-D-Neup5Ac(7/90Ac)- α -(2 \rightarrow 6)-D-Gal- α -(1 \rightarrow . The serogroup Y saccharide is similar to the serogroup W135 saccharide, except that the disaccharide repeating unit includes glucose instead of galactose. Like serogroup W135, it has variable O-acetylation at sialic acid 7 and 9 positions [50]. The serogroup Y structure is written as: \rightarrow 4)-D-Neup5Ac(7/90Ac)- α -(2 \rightarrow 6)-D-Glc- α -(1 \rightarrow .

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[0040] The saccharides used according to the invention may be O-acetylated as described above (e.g. with the same O-acetylation pattern as seen in native capsular saccharides), or they may be partially or totally de-O-acetylated at one or more positions of the saccharide rings, or they may be hyper-O-acetylated relative to the native capsular saccharides.

25

[0041] The saccharides used according to the invention are preferably shorter than the native capsular saccharides seen in bacteria. Thus the saccharides are preferably depolymerised, with depolymerisation occurring after purification but before conjugation. Depolymerisation reduces the chain length of the saccharides. A preferred depolymerisation method involves the use of hydrogen peroxide [9]. Hydrogen peroxide is added to a saccharide (e.g. to give a final H₂O₂ concentration of 1%), and the mixture is then incubated (e.g. at about 55°C) until a desired chain length reduction has been achieved. Another depolymerisation method involves acid hydrolysis [10]. Other depolymerisation methods are known to the skilled person. The saccharides used to prepare conjugates for use according to the invention may be

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obtainable by any of these depolymerisation methods. Depolymerisation can be used in order to provide an optimum chain length for immunogenicity and/or to reduce chain length for physical manageability of the saccharides.

5 [0042] Typical carrier proteins for use in conjugates are bacterial toxins or toxoids, such as diphtheria toxin (or its CRM₁₉₇ mutant) and tetanus toxin. Other known carrier proteins include the *N.meningitidis* outer membrane protein, synthetic peptides, heat shock proteins, pertussis proteins, cytokines, lymphokines, hormones, growth factors, artificial proteins comprising multiple human CD4⁺ T cell epitopes from various
10 pathogen-derived antigens, protein D from *H.influenzae*, pneumococcal surface protein PspA, iron-uptake proteins, toxin A or B from *C.difficile*, etc. According to the invention, however, the meningococcal conjugates include a diphtheria toxoid (or derivative, such as CRM197) carrier protein. Covalent conjugation is preferred.

15 [0043] It is possible to use more than one carrier protein in the compositions. Thus different carrier proteins can be used for different serogroups e.g. serogroup A saccharides might be conjugated to CRM197 while serogroup C saccharides might be conjugated to diphtheria toxoid. It is also possible to use more than one carrier protein for a particular saccharide antigen e.g. serogroup A saccharides might be in two
20 groups, with some conjugated to CRM197 and others conjugated to diphtheria toxoid. In general, however, it is preferred to use the same carrier protein for all meningococcal saccharides in the composition, and more preferably for all saccharides (i.e. including any non-meningococcal conjugates that may be present). It is preferred that compositions of the invention do not include any tetanus toxoid carrier
25 protein. Where the composition includes a diphtheria toxoid carrier protein then it is preferred that it does not include any CRM 197 carrier protein.

[0044] A single carrier protein might carry more than one saccharide antigen [51]. For example, a single carrier protein might have conjugated to it saccharides from
30 serogroups A and C. To achieve this goal, saccharides can be mixed prior to the conjugation reaction. In general, however, it is preferred to have separate conjugates for each serogroup. Conjugates are preferably mixed to give substantially a 1:1:1:1 ratio (measured as mass of saccharide) e.g. the mass of each serogroup's saccharide is within $\pm 10\%$ of each other. A typical quantity of meningococcal antigen per
35 serogroup in a composition is between 1 μ g and 20 μ g e.g. between 2 and 10 μ g per

serogroup, or about 4 μ g. As an alternative to a 1:1:1:1 ratio, a double serogroup A dose may be used (2:1:1:1).

5 [0045] Conjugates with a saccharide:protein ratio (w/w) of between 1:15 (*i.e.* excess protein) and 15:1 (*i.e.* excess saccharide), preferably between 1:10 and 10:1, more preferably between 1:5 and 5:1, are preferred. Excess carrier protein is preferred. Conjugates with saccharide:protein ratio of about 1:12 or about 1:6 or about 1:3 are preferred, particularly where the carrier is Dt. A 1:3 ratio is most preferred.

10 [0046] Conjugates may be used in conjunction with free carrier protein [52]. When a given carrier protein is present in both free and conjugated form in a composition of the invention, however, the unconjugated form is preferably no more than 5% of the total amount of the carrier protein in the composition as a whole, and more preferably present at less than 2% by weight. Similarly, unconjugated saccharide is preferably no
15 more than 15% by weight of the total amount of saccharide.

[0047] Any suitable conjugation reaction can be used, with any suitable linker where necessary.

20 [0048] The saccharide will typically be activated or functionalised prior to conjugation. Activation may involve, for example, cyanylating reagents such as CDAP (*e.g.* 1-cyano-4-dimethylamino pyridinium tetrafluoroborate [53, 54, *etc.*]). Other suitable techniques use carbodiimides, hydrazides, active esters, norborane, *p*-nitrobenzoic acid, N-hydroxysuccinimide, S-NHS, EDC, TSTU; see also the introduction to
25 reference 44).

[0049] Linkages via a linker group may be made using any known procedure, for example, the procedures described in references 55 and 56. One type of linkage involves reductive amination of the polysaccharide, coupling the resulting amino group
30 with one end of an adipic acid linker group, and then coupling a protein to the other end of the adipic acid linker group [42, 57, 58]. Other linkers include B-propionamido [59], nitrophenyl-ethylamine [60], haloacyl halides [61], glycosidic linkages [62], 6-aminocaproic acid [63], ADH [64], C₄ to C₁₂ moieties [65] *etc.* As an alternative to using a linker, direct linkage can be used. Direct linkages to the protein may comprise
35 oxidation of the polysaccharide followed by reductive amination with the protein, as described in, for example, references 66 and 67.

[0050] A process involving the introduction of amino groups into the saccharide (e.g. by replacing terminal =O groups with -NH₂) followed by derivatisation with an adipic diester (e.g. adipic acid N-hydroxysuccinimido diester) and reaction with carrier protein is preferred.

[0051] In one preferred conjugation method, a saccharide is reacted with adipic acid dihydrazide. For serogroup A, carbodiimide may also be added at this stage. After a reaction period, sodium cyanoborohydride is added. Derivatized saccharide can then be prepared e.g. by ultrafiltration. The derivatized saccharide is then mixed with carrier protein (e.g. with a diphtheria toxoid), and carbodiimide is added. After a reaction period, the conjugate can be recovered. Further details of this conjugation method can be found in reference 10. Conjugates obtainable by this method are preferred conjugates for use according to the invention e.g. conjugates comprising a diphtheria toxoid carrier and an adipic acid linker.

[0052] Conjugates are preferably prepared separately and then mixed. After mixing, the concentration of the mixed conjugates can be adjusted e.g. with sterile pyrogen-free, phosphate-buffered saline. Each conjugate, before mixing, preferably contains no more than 15µg of carrier.

[0053] The result of administering meningococcal conjugates according to the invention is preferably that, for each administered serogroup, the patient raises a serum bactericidal antibody (SBA) response, with the increase in SBA titre (compared to the pre-immunised patient before receiving the mixed meningococcal conjugates) being at least 4-fold, and preferably at least 8-fold. The SBA test is a standard correlate for meningococcal protection. Further details of serologic correlates for meningococcal vaccines are given in reference 68.

30 ***Further antigenic components of compositions used according to the invention***

[0054] In addition to meningococcal conjugates, compositions used according to the invention may optionally include 1, 2 or 3 of the following further antigens:

1. A conjugated capsular saccharide from *S.pneumoniae* [e.g. chapter 23 of ref. 32; refs. 69-71].

5 It is preferred to include saccharides from more than one serotype of *S.pneumoniae*. For example, mixtures of polysaccharides from 23 different serotype are widely used, as are conjugate vaccines with polysaccharides from between 5 and 11 different serotypes [72]. For example, PreVNar™ [31] contains antigens from seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) with each saccharide individually conjugated
10 to CRM197 by reductive amination, with 2µg of each saccharide per 0.5ml dose (4µg of serotype 6B), and with conjugates adsorbed on an aluminium phosphate adjuvant. Where pneumococcal conjugates are included in a compositions for use with the invention, the composition preferably includes at least serotypes 6B, 14, 19F and 23F.

15

2. A conjugated capsular saccharide from *H.influenzae* B [e.g. chapter 14 of ref. 32].

20 The carrier protein for the conjugate may be CRM197, Dt, a tetanus toxoid or an outer membrane complex of *N.meningitidis*. The saccharide moiety of the conjugate may be a polysaccharide (e.g. full-length polyribosylribitol phosphate (PRP)), but it is preferred to depolymerise the capsular polysaccharides to form oligosaccharides (e.g. MW from ~1 to ~5 kDa). A preferred Hib conjugate comprises an oligosaccharide covalently linked to CRM197 via an adipic acid linker [73,74].
25 Administration of the Hib antigen preferably results in an anti-PRP antibody concentration of >0.15µg/ml, and more preferably >1µg/ml. Where a composition includes a Hib saccharide antigen, it preferably does not also include an aluminium hydroxide adjuvant. If the
30 composition includes an aluminium phosphate adjuvant then the Hib antigen may be adsorbed to the adjuvant [75] or it may be non-adsorbed [27]. Prevention of adsorption can be achieved by selecting the correct pH during antigen/adjuvant mixing, an adjuvant with an appropriate point of zero charge, and an appropriate order of mixing for the various
35 different antigens in a composition [76].

3. A protein antigen from *Neisseria meningitidis* serogroup B [e.g. ref. 77].

[0055] The composition may comprise one or more of these further antigens.

- 5 [0056] Such antigens may or may not be adsorbed to an aluminium salt.

[0057] If meningococcal conjugates are being administered in a series of doses then none, some or all of the doses may include these extra antigens.

- 10 [0058] Compositions containing the meningococcal conjugates preferably do not include tetanus toxoid. They preferably do not include pertussis antigens. They preferably do not include hepatitis B virus surface antigen. They preferably do not include poliovirus. A composition preferably contains no more than 50µg of diphtheria toxoid per meningococcal conjugate, and more preferably no more than 50µg of
15 diphtheria toxoid for all meningococcal conjugates combined.

The vaccine composition

[0059] The composition used according to the invention will typically include a pharmaceutically acceptable carrier. Such carriers include any carrier that does not
20 itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary
25 skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable carriers and excipients is available in reference 78.

30

[0060] Compositions used according to the invention may include an antimicrobial, particularly if packaged in a multiple dose format.

[0061] Compositions used according to the invention may comprise detergent e.g. a
35 Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels e.g. <0.01%.

[0062] Compositions used according to the invention may include sodium salts (e.g. sodium chloride and/or sodium phosphate). These can be used for tonicity. A concentration of 10 ± 2 mg/ml NaCl is-typical e.g. about 8.8mg/ml. A concentration of 1.2mg/ml sodium phosphate is typical.

5

[0063] Compositions used according to the invention will generally include a buffer e.g. a phosphate buffer.

[0064] Compositions used according to the invention may comprise a sugar alcohol (e.g. mannitol) or a disaccharide (e.g. sucrose or trehalose) e.g. at about 15-30 mg/ml (e.g. 25 mg/ml), particularly if they are to be lyophilised or if they include material which has been reconstituted from lyophilised material. Preferred compositions, however, are not lyophilised i.e. all meningococcal conjugates are present in aqueous form, from the packaging stage to the administration stage.

15

[0065] Compositions 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 (e.g. 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.

20

[0066] Meningococcal conjugates from multiple serogroups are administered in admixture within a single composition. The composition may be administered as a single dose, or may be administered more than once in 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 of the meningococcal conjugates. Suitable timing between priming doses (e.g. between 4-16 weeks), and between priming and boosting, can be routinely determined. The conjugates may conveniently be administered at the same time as other vaccines e.g. at the same time as a D-T-P vaccine, or at the same time as a pneumococcal conjugate vaccine, or at the same time as an influenza vaccine, or at the same time as a MMR or MMRV vaccine. These vaccines will generally be administered separately but during the same visit to the doctor.

30

35

[0067] Bacterial infections can affect various areas of the body and so compositions may be prepared in various forms. For example, the compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared (e.g. a lyophilised composition). The composition may be prepared for topical administration e.g. as an ointment, cream or powder. The composition be prepared for oral administration e.g. as a tablet or capsule, or as a syrup (optionally flavoured). The composition may be prepared for pulmonary administration e.g. as an inhaler, using a fine powder or a spray. The composition may be prepared as a suppository or pessary. The composition may be prepared for nasal, aural or ocular administration e.g. as spray, drops, gel or powder [e.g. refs 79 & 80]. In general, however, the meningococcal conjugates are formulated for intramuscular injection.

[0068] Compositions used according to the invention may or may not include a vaccine adjuvant. Adjuvants which may be used in compositions of the invention include, but are not limited to:

A. Mineral-containing compositions

[0069] 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. 81], or mixtures of different mineral compounds, with the compounds taking any suitable form (e.g. gel, crystalline, amorphous, etc.), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [82].

[0070] Aluminium phosphates are particularly preferred, and a typical adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at about 0.6mg Al^{3+}/ml . Adsorption with a low dose of aluminium phosphate may be used e.g. between 50 and 100 μg Al^{3+} per conjugate per dose. Where a composition includes conjugates from multiple bacterial species then not all conjugates need to be adsorbed.

B. Oil Emulsions

[0071] Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 81; see also ref. 83] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles
5 using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 81]

[0072] Saponin formulations may also be used as adjuvants in the invention. Saponins
10 are a heterologous group of sterol glycosides and triterpenoid glycosides that are found in the bark, leaves, stems, roots and even flowers of a wide range of plant species. Saponin from the bark of the *Quillaia saponaria* Molina tree have been widely studied as adjuvants. Saponin can also be commercially obtained from *Smilax ornata* (sarsapilla), *Gypsophilla paniculata* (brides veil), and *Saponaria officianalis* (soap
15 root). Saponin adjuvant formulations include purified formulations, such as QS21, as well as lipid formulations, such as ISCOMs. QS21 is marketed as Stimulon™.

[0073] Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17,
20 QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 84. Saponin formulations may also comprise a sterol, such as cholesterol [85].

[0074] Combinations of saponins and cholesterol can be used to form unique
25 particles called immunostimulating complexes (ISCOMs) [chapter 23 of ref. 81]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA & QHC. ISCOMs are further described in refs. 85-87. Optionally, the ISCOMS may be devoid of additional detergent [88].

30

[0075] A review of the development of saponin based adjuvants can be found in refs. 89 & 90.

D. Virosomes and virus-like particles

[0076] Virosomes and virus-like particles (VLPs) can also be used as adjuvants in the invention. These structures generally contain one or more proteins from a virus optionally combined or formulated with a phospholipid. They are generally non-
5 pathogenic, non-replicating and generally do not contain any of the native viral genome. The viral proteins may be recombinantly produced or isolated from whole viruses. These viral proteins suitable for use in virosomes or VLPs include proteins derived from influenza virus (such as HA or NA), Hepatitis B virus (such as core or capsid proteins), Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-
10 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. 91-96. Virosomes are discussed further in, for example, ref. 97.

E. Bacterial or microbial derivatives

[0077] 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.

20

[0078] Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 98. Such "small particles" of
25 3dMPL are small enough to be sterile filtered through a 0.22 μ m membrane [98]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives e.g. RC-529 [99,100].

[0079] Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as
30 OM-174. OM-174 is described for example in refs. 101 & 102.

[0080] 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
35 guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

[0081] The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 103, 104 and 105 disclose possible analog substitutions e.g. replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG
5 oligonucleotides is further discussed in refs. 106-111.

[0082] The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [112]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell
10 response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 113-115. Preferably, the CpG is a CpG-A ODN.

[0083] Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences
15 may be attached at their 3' ends to form "immunomers". See, for example, refs. 112 & 116-118.

[0084] 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*
20 heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 119 and as parenteral adjuvants in ref. 120. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant
25 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. 121-128. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 129.

30

F. Human immunomodulators

[0085] 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 [130], etc.) [131], interferons (e.g. interferon- γ), macrophage colony stimulating factor, and
35 tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

[0086] Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [132] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrrolidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [133].

H Microparticles

[0087] 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(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 81)

[0088] Examples of liposome formulations suitable for use as adjuvants are described in refs. 134-136.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

[0089] Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [137]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [138] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [139]. 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.

L. Muramyl peptides

[0090] 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).

K Polyphosphazene (PCPP)

[0091] PCPP formulations are described, for example, in refs. 140 and 141.

M. Imidazoquinolone Compounds.

- 5 [0092] Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (e.g. "Resiquimod 3M"), described further in refs. 142 and 143.

N. Thiosemicarbazone Compounds.

- 10 [0093] Examples of thiosemicarbazone compounds, as well as methods of formulating, manufacturing, and screening for compounds all suitable for use as adjuvants in the invention include those described in ref. 144. The thiosemicarbazones are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF- α .

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O. Tryptanthrin Compounds.

- [0094] Examples of tryptanthrin compounds, as well as methods of formulating, manufacturing, and screening for compounds all suitable for use as adjuvants in the invention include those described in ref. 145. The tryptanthrin compounds are particularly effective in the stimulation of human peripheral blood mononuclear cells for the production of cytokines, such as TNF- α .
- 20

- [0095] 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 [146]; (2) a saponin (e.g. QS21) + a non-toxic LPS derivative (e.g. 3dMPL) [147]; (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) [148]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [149]; (6) SAF, containing 10% squalane, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) Ribit™ 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™); (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL); and
- 25
- 30
- 35

(9) one or more mineral salts (such as an aluminum salt) + an immunostimulatory oligonucleotide (such as a nucleotide sequence including a CpG motif).

5 [0096] Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 81.

10 [0097] The use of an aluminium hydroxide or aluminium phosphate adjuvant is particularly preferred, and conjugates are generally adsorbed to these salts [e.g. examples 7 & 8 of ref. 9; example J of ref. 10]. Mixing with aluminium salts with no adsorption is also possible [27, 76]. Calcium phosphate is another preferred adjuvant. Conjugates may be mixed with (and optionally adsorbed to) the adjuvants separately and then the conjugates may be mixed together, or the conjugates may be mixed together and then mixed with adjuvant.

15 [0098] The pH of compositions used according to the invention is preferably between 6 and 8, preferably about 7. Stable pH may be maintained by the use of a buffer. Where a composition comprises an aluminium hydroxide salt, it is preferred to use a histidine buffer [150]. The composition may be sterile and/or pyrogen-free. Compositions may be isotonic with respect to humans.

20

[0099] Compositions may include a preservative (e.g. thiomersal, 2-phenoxyethanol), or may be preservative-free. Preferred compositions of the invention do not include any mercurial material e.g. they are thiomersal-free.

25 [0100] To prevent interference between antigens, particularly conjugate antigens, it is possible to include a polyanionic polymer, such as poly-L-glutamic acid [151].

30 [0101] Compositions may be presented in vials, or they may be presented in ready-filled syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses. Injectable compositions will usually be liquid solutions or suspensions. Alternatively, they may be presented in solid form (e.g. freeze-dried) for solution or suspension in liquid vehicles prior to injection.

35 [0102] Compositions may be packaged in unit dose form or in multiple dose form. For multiple dose forms, vials are preferred to pre-filled syringes. Effective dosage

volumes can be routinely established, but a typical human dose of the composition for injection has a volume of 0.5ml.

[0103] Where a composition is to be prepared extemporaneously prior to use (*e.g.* where a component is presented in lyophilised form) and is presented as a kit, the kit may comprise two vials, or it may comprise one ready-filled syringe and one vial, with the contents of the syringe being used to reactivate the contents of the vial prior to injection. For compositions that include a serogroup A capsular saccharide then the serogroup A saccharide may be lyophilised, whereas saccharide(s) from other serogroup(s) may be present in liquid form.

[0104] Compositions will comprise an immunologically effective amount of the meningococcal conjugates, 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, elicits a protective anti-meningococcal immune response in patients. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate, *etc.*), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials, and a typical quantity of each meningococcal antigen per dose is between 1 µg and 20µg per serogroup (measured in terms of saccharide) *e.g.* between 2 and 10 µg per serogroup. A dose of about 4µg per serogroup is preferred (*i.e.* a total of 16µg in a tetravalent mixture).

[0105] The total amount of carrier protein in a composition preferably does not exceed 100µg/dose *e.g.* it is <90µg/dose, <80µg/dose, <70µg/dose, <60µg/dose, ≤50µg/dose, *etc.* The total amount of carrier protein in a composition will generally be at least 10µg/dose.

General

[0106] 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.

[0107] The term "about" in relation to a numerical value x means, for example, $x \pm 10\%$.

[0108] 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,
5 the word "substantially" may be omitted from the definition of the invention.

MODES FOR CARRYING OUT THE INVENTION

Lack of carrier suppression using tetravalent A/C/W135/Y conjugate mixture

10 [0109] Mixtures of meningococcal conjugates for serogroups A+C, C+W+Y or A+C+W+Y can be prepared as described in references 9 and 10. If desired, these can be mixed with aluminium hydroxide or aluminium phosphate adjuvants, as also described in references 9 and 10. These vaccines have either CRM197 or diphtheria toxoid (Dt) as the carrier protein, covalently linked to the saccharides.

15

[0110] Patients who received pediatric D-T-P vaccination (either D-T-Pa or D-T-Pw), including those who received vaccines containing D-T-P and other antigens (e.g. D-T-P-Hib tetravalent, D-T-P-HBsAg tetravalent, D-T-P-Hib-HBsAg pentavalent, D-T-P-Hib-HBsAg-IPV hexavalent, etc.) are selected to receive the mixture of conjugates that
20 has a Dt carrier. This tetravalent mixture of conjugates is immunogenic in humans [9, 11, 13].

[0111] Patients who received pediatric Hib vaccination (either as a monovalent Hib, with or without D-T-Pa or D-T-Pw; or as part of a combination vaccine such as D-T-P-Hib tetravalent, D-T-P-Hib-HBsAg pentavalent, D-T-P-Hib-HBsAg-IPV hexavalent,
25 etc.) are selected to receive the mixture of conjugates that has a CRM197 carrier.

[0112] A control group of patients is selected to receive one of the two conjugate mixtures. The control patients have not previously received either diphtheria toxoid or
30 CRM197, either as separate antigens or as carrier proteins in conjugates.

[0113] The ability of the tetravalent conjugates to elicit an immune response in the patients is assessed. Carrier suppression is indicated if the test groups show significantly lower anti-meningococcal immune responses than the control patients,
35 and in particular if the conjugates fail to elicit a useful SBA response in the patients.

[0114] In clinical trial V59P2, conducted in Finland and Germany with 620 subjects aged 12-16 months, five formulations were tested. The vaccines used CRM197 carrier and an aluminium phosphate adjuvant [10]. Doses of each serogroup saccharide, expressed as µg saccharide mass per 0.5ml dose, were as follows:

Group	MenA	MenC	MenW135	MenY
1	10	10	10	10
2	0	10	10	10
3	10	5	5	5
4	5	5	5	5
5	2.5	2.5	2.5	2.5

[0115] Subjects received an injection at time zero, and 25% of the subjects then received a second dose of the vaccine 4 weeks later.

[0116] Sera of patients were collected 1 month after vaccine administration and were tested in a SBA assay against *N.meningitidis* from each serogroup, using human complement. SBA titre increase relative to time zero sera was assessed, with criteria being $\geq 1:4$ and $\geq 1:8$, Anti-capsule titres (GMT) were also measured for each serogroup. Results are shown in Table 1 below.

[0117] Thus the trivalent and tetravalent vaccines were both immunogenic in toddlers. The conjugates are immunogenic at saccharide doses as low as 2.5µg per conjugate. The immune response are boostable, with large SBA titre increases after the second dose. No evidence of carrier suppression was seen in this trial.

Lack of suppression of anti-Dt responses

[0118] A Belgian study from 1999 [26] demonstrated impaired tetanus immunity in infants, persisting into early childhood, following receipt of a primary course of Hib vaccine conjugated to tetanus toxoid. The conjugate's carrier protein was therefore having a negative immunological impact. In contrast, the opposite effect was seen in a study of pneumococcal conjugate vaccines [152]. The possibility that a meningococcal conjugate might interfere with diphtheria immunity [153] was studied using a conjugate of meningococcal serogroup C saccharide to a CRM197 carrier (Menjugate™).

[0119] Children were split into five groups, who were immunised in the first year of life

as follows: (1) four doses of Menjugate™; (2) three doses of Menjugate™, plus one dose of a bivalent unconjugated A/C mixture; (3) three doses of HBsAg vaccine then one dose of Menjugate™; (4) three doses of HBsAg vaccine then one dose of a bivalent unconjugated A/C mixture; (5) controls to receive no meningococcal vaccines.

- 5 All children also received three doses of diphtheria vaccine during the first four months of life, but had not received a pre-school diphtheria booster.

[0120] Patients received a single dose of Menjugate™ at 4 years of age, and blood was sampled pre- and post- (~30 days) this Menjugate™ injection. Diphtheria antibodies were measured by ELISA and geometric mean concentrations were assessed. The percentage of subjects with an antibody level ≥ 0.1 IU/ml was also assessed. Results were as follows:

Group	Pre-vaccination		Post-vaccination	
	GMC	% ≥ 0.1	GMC	% ≥ 0.1
1	0.35	94%	9.00	100%
2	0.18	87%	8.55	100%
3	0.20	81%	2.82	100%
4	0.10	51%	4.55	99%

[0121] The baseline anti-Dt titres were higher in patients who had previously received Menjugate™ than in those who had not (e.g. compare groups 1 and 4). Simple regression analysis revealed significant linear relationships between the number of previous Menjugate™ doses (4, 3, 1 and 0 for groups 1, 2, 3 and 4, respectively) and (a) pre-vaccination anti-Dt titres and (b) post-vaccination anti-Dt titres.

20 [0122] Thus there is enhanced persistence of immunity to diphtheria at 4 years of age in children who received four Menjugate™ doses in infancy. Furthermore, there is a trend towards higher anti-Dt antibody responses following a booster dose of Menjugate™ in patients who had received at least three Menjugate™ doses as infants. No evidence of immunological interference between meningococcal conjugates and diphtheria immunity was found.

Bivalent A/C conjugate mixture shows no interference with DTP

[0123] A mixture of capsular saccharides from serogroups A and C [8] has been administered to infants (5 to 11 weeks old) in Niger [154] who had not previously received DTP vaccine. Children received either unconjugated saccharides (50µg of

each serogroup) or unadjuvanted Dt-conjugated saccharides (4µg of each), with six different schedules:

- (1) Four conjugate doses: 6 weeks, 10 weeks, 14 weeks, 9 months.
- 5 (2) Three conjugate doses: 6 weeks, 10 weeks, 14 weeks.
- (3) Two conjugate doses: 14 weeks, 9 months.
- (4) One conjugate doses: 14 weeks.
- (5) One conjugate dose: 9 months.
- (6) One unconjugated dose: 9 months.

10

[0124] The children received DTP and oral polio vaccines at 6, 10 and 14 weeks, with a booster at 9 months, and the meningococcal vaccines were administered at the same time as these existing vaccines. To assess anamnestic responses, the children were also given an unconjugated vaccine at 24 months.

15

[0125] Serum bactericidal antibody responses were measured at 18 weeks (*i.e.* 4 weeks after the third DTP/polio vaccination), at 10 months (*i.e.* 1 month after the 9 month DTP/polio booster) and 1 week after the administration of unconjugated material at 24 months. The percentages of patients showing a ≥ 128 -fold increase in

20 SBA titres were as follows:

%	18 weeks		10 months		24¼ months	
	MenA	MenC	MenA	MenC	MenA	MenC
(1)	56	84	89	73	100	95
(2)	56	86	6	9	96	82
(3)	68	64	85	85	100	95
(4)	61	57	4	8	100	93
(5)	3	7	62	26	100	99
(6)	2	2	5	11	97	52

[0126] There was no difference in antibody responses against diphtheria toxoid between the six groups [8].

25 **[0127]** Thus there is no evidence of interference resulting from the use of diphtheria toxoid as both a protective antigen and as a carrier for the conjugates. For example, patients in group 5 had received diphtheria toxoid in DTP vaccines at 6, 10 and 14

weeks before receiving the first dose of meningococcal conjugate, but showed an anti-meningococcal SBA response of >99% at 24 months.

No negative impact on anti-diphtheria responses

- 5 [0128] As mentioned above, patients receiving meningococcal conjugate vaccines at the same time as DTP vaccines showed no reduction in immune responses against diphtheria toxoid. In another study, meningococcal conjugates and DTP have been administered at different times. A three dose DTP schedule at 2, 3 and 4 months of age had been followed by a single dose of a bivalent A/C vaccine either with Dt-
10 conjugated saccharides or with unconjugated saccharides. Immune responses against diphtheria toxoid were measured by ELISA, and GM titres were as follows:

Antigens	Pre-immunisation	Post-immunisation
Conjugated	0.05	21.2
Unconjugated	0.06	0.06

- [0129] The unconjugated saccharides did not cause any anti-Dt response (unsurprisingly), but the conjugated saccharides resulted in a strong anti-DT response.
15 Administration of these conjugates may thus provide anti-diphtheria immunity in naive patients, or may take the place of a Dt booster.

- [0130] It will be understood that the invention is described above by way of example only and modifications may be made while remaining within the scope of the invention,
20 which is defined in the appended claims.

TABLE 1- Results of trial V59P2

Group	A	C	W135	Y
	GMT (1 month after 1 dose)			
1	3.9	6.4	7.1	8.9
2	2	6.1	8.3	8.5
3	5.7	5.2	6.9	12
4	3.8	4.5	7.0	9.6
5	3.9	5.3	7.0	12
	GMT (1 month after 2 doses)			
1	27	89	22	37
2	2	80	20	57
3	29	76	28	58
4	14	47	20	35
5	17	71	23	52
	% patients with SBA >1:4 (1 month after 1 dose)			
1	33	56	57	58
2	0	57	60	61
3	55	49	53	70
4	37	42	54	64
5	40	51	57	67
	% patients with SBA ≥1:4 (1 month after 2 doses)			
1	100	100	96	96
2	0	100	73	92
3	91	96	95	95
4	84	96	88	96
5	80	100	80	92
	% patients with SBA ≥1:8 (1 month after 1 dose)			
1	25	44	46	48
2	0	40	50	49
3	39	34	45	64
4	23	30	44	51
5	26	35	40	60
	% patients with SBA ≥1:8 (1 month after 2 doses)			
1	92	100	85	93
2	0	100	64	92
3	87	96	95	82
4	60	92	77	92
5	72	92	72	88

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PATENTKRAV

1. Sammensætning, der indeholder: (a) et konjugat af (i) det kapsulære saccharid af serogruppe A *N.meningitidis* og (ii) et differitoxid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxid; (b) et konjugat af (i) det kapsulære saccharid af serogruppe C *N.meningitidis* og (ii) et differitoxid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxid; (c) et konjugat af (i) det kapsulære saccharid af serogruppe W135 *N.meningitidis* og (ii) et differitoxid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxid; og (d) et konjugat af (i) det kapsulære saccharid af serogruppe Y *N.meningitidis* og (ii) et differitoxid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxid, til anvendelse ved en fremgangsmåde til immunisering af en human patient mod en sygdom forårsaget af *Neisseria meningitidis*, omfattende trinnet indgivelse af sammensætningen til den humane patient, hvor patienten er blevet præimmuniseret med (a) et differitoxid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxidet og/eller (b) et konjugat af (i) et kapsulært saccharid af en anden organisme end *N.meningitidis* og (ii) et differitoxid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxid.
2. Sammensætning ifølge krav 1 til anvendelse ifølge krav 1, hvor sammensætningen indeholder: (a) et konjugat af (i) det kapsulære saccharid af serogruppe A *N.meningitidis* og (ii) et differitoxid; (b) et konjugat af (i) det kapsulære saccharid af serogruppe C *N.meningitidis* og (ii) et differitoxid; (c) et konjugat af (i) det kapsulære saccharid af serogruppe W135 *N.meningitidis* og (ii) et differitoxid; og (d) et konjugat af (i) det kapsulære saccharid af serogruppe Y *N.meningitidis* og (ii) et differitoxid, og hvor patienten er blevet præimmuniseret med (a) et differitoxid og/eller (b) et konjugat af (i) et kapsulært saccharid af en anden organisme end *N.meningitidis* og (ii) et differitoxid eller CRM197.
3. Sammensætning ifølge krav 1 til anvendelse ifølge krav 1, hvor sammensætningen indeholder: (a) et konjugat af (i) det kapsulære saccharid af serogruppe A *N.meningitidis* og (ii) CRM197; (b) et konjugat af (i) det kapsulære saccharid af serogruppe C *N.meningitidis* og (ii) CRM197; (c) et konjugat af (i) det kapsulære saccharid af serogruppe W135 *N.meningitidis* og (ii) CRM197; og (d) et konjugat af (i) det kapsulære saccharid af serogruppe Y *N.meningitidis* og (ii) CRM197, og hvor

patienten er blevet præimmuniseret med et konjugat af (i) et kapsulært saccharid af en anden organisme end *N.meningitidis* og (ii) CRM197 eller et differitoxoid.

4. Anvendelse af (a) et konjugat af (i) det kapsulære saccharid af serogruppe A
5 *N.meningitidis* og (ii) et differitoxoid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxoid; (b) et konjugat af (i) det kapsulære saccharid af serogruppe C *N.meningitidis* og (ii) et differitoxoid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxoid; (c) et konjugat af (i) det kapsulære saccharid af serogruppe W135 *N.meningitidis* og (ii) et differitoxoid eller derivat deraf,
10 der forbliver immunologisk krydsreaktivt med differitoxoid; og (d) et konjugat af (i) det kapsulære saccharid af serogruppe Y *N.meningitidis* og (ii) et differitoxoid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxoid, ved fremstilling af et lægemiddel til immunisering af en human patient mod en sygdom forårsaget af *Neisseria meningitidis*, hvor patienten er blevet præimmuniseret med (a) et
15 differitoxoid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxoidet og/eller (b) et konjugat af (i) et kapsulært saccharid af en anden organisme end *N.meningitidis* og (ii) et differitoxoid eller derivat deraf, der forbliver immunologisk krydsreaktivt med differitoxoid.

20 5. Anvendelse ifølge krav 1, hvor der anvendes: (a) et konjugat af (i) det kapsulære saccharid af serogruppe A *N.meningitidis* og (ii) et differitoxoid; (b) et konjugat af (i) det kapsulære saccharid af serogruppe C *N.meningitidis* og (ii) et differitoxoid; (c) et konjugat af (i) det kapsulære saccharid af serogruppe W135 *N.meningitidis* og (ii) et differitoxoid; og (d) et konjugat af (i) det kapsulære saccharid af serogruppe Y
25 *N.meningitidis* og (ii) et differitoxoid, og hvor patienten er blevet præimmuniseret med (a) et differitoxoid og/eller (b) et konjugat af (i) et kapsulært saccharid af en anden organisme end *N.meningitidis* og (ii) et differitoxoid eller CRM197.

6. Anvendelse ifølge krav 4, hvor der anvendes: (a) et konjugat af (i) det kapsulære
30 saccharid af serogruppe A *N.meningitidis* og (ii) CRM197; (b) et konjugat af (i) det kapsulære saccharid af serogruppe C *N.meningitidis* og (ii) CRM197; (c) et konjugat af (i) det kapsulære saccharid af serogruppe W135 *N.meningitidis* og (ii) CRM197; og (d) et konjugat af (i) det kapsulære saccharid af serogruppe Y *N. meningitidis* og (ii) CRM197, og hvor patienten er blevet præimmuniseret med et konjugat af (i) et
35 kapsulært saccharid af en anden organisme end *N.meningitidis* og (ii) CRM197 eller et differitoxoid.

7. Sammensætning ifølge ethvert af kravene 1-3 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-6, hvor konjugaterne er blandet til
5 opnåelse af et forhold på 1:1:1:1 or 2:1:1:1 (målt som masse of saccharid).
8. Sammensætning ifølge ethvert af kravene 1-3 og 7 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-7, hvor hvert meningokok-antigen pr. dosis ligger mellem 2 og 10 µg pr. serogruppe (målt som
10 saccharid).
9. Sammensætning ifølge ethvert af kravene 1-3 og 7-8 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-8, hvor patienten er blevet præimmuniseret med en vaccine indeholdende et differitoxid.
15
10. Sammensætning ifølge ethvert af kravene 1-3 og 7-9 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-9, hvor patienten er blevet præimmuniseret med en vaccine indeholdende et Hib-konjugat.
- 20 11. Sammensætning ifølge ethvert af kravene 1-3 og 7-10 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-10, hvor patienten er blevet præimmuniseret med en vaccine indeholdende mindst ét pneumokok- konjugat.
- 25 12. Sammensætning ifølge ethvert af kravene 1-3 og 7-11 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-11, hvor patienten er præimmuniseret mindst 6 måneder inden fremgangsmåden eller anvendelsen.
- 30 13. Sammensætning ifølge krav 12 til anvendelse ifølge krav 12, eller anvendelse ifølge krav 12, hvor patienten er præimmuniseret mindst 8 år før fremgangsmåden eller anvendelsen.
- 35 14. Sammensætning ifølge ethvert af kravene 1-3 og 7-13 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-13, hvor præimmuniseringen fandt sted inden for 1 år efter patientens fødsel.

15. Sammensætning ifølge ethvert af kravene 1-3 og 7-14 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-14, hvor sacchariderne i meningokok-konjugaterne (a) til (d) er blevet depolymeriseret således, at de er kortere end de native kapsulære saccharider, der iagttages i meningokokker.
- 5
16. Sammensætning ifølge ethvert af kravene 1-3 og 7-15 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-15, hvor meningokok-konjugaterne indeholder en difteritoxoidbærer og en adipinsyre-linker.
- 10
17. Sammensætning ifølge krav 16 til anvendelse ifølge krav 16, eller anvendelse ifølge krav 16, indeholdende ikke mere end 60 µg difteritoxoidbærer.
- 15
18. Sammensætning ifølge ethvert af kravene 1-3 og 7-17 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-17, hvor sammensætningen eller lægemidlet yderligere indeholder et konjugeret kapsulært saccharid fra *Streptococcus pneumoniae*.
- 20
19. Sammensætning ifølge ethvert af kravene 1-3 og 7-18 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-18, hvor sammensætningen eller lægemidlet yderligere indeholder et konjugeret kapsulært saccharid fra *Haemophilus influenzae* type B.
- 25
20. Sammensætning ifølge ethvert af kravene 1-3 og 7-19 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-19, hvor sammensætningen eller lægemidlet yderligere indeholder et proteinantigen fra serogruppe B af *Neisseria meningitidis*.
- 30
21. Sammensætning ifølge ethvert af kravene 1-3 og 7-20 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-20, hvor sammensætningen eller lægemidlet indeholder et aluminiumhydroxid-adjuvans og/eller et aluminiumphosphat-adjuvans.
- 35
22. Sammensætning ifølge ethvert af kravene 1-3 og 7-21 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-21, hvor sygdommen forårsaget af *Neisseria meningitidis* er meningokok-meningitis.

23. Sammensætning ifølge ethvert af kravene 1-3 til anvendelse ifølge det pågældende krav, eller anvendelse ifølge ethvert af kravene 4-6, hvor:
- patienten er blevet præimmuniseret mindst 5 år inden fremgangsmåden eller anvendelsen med en vaccine indeholdende et differitoxoid;
- 5
- meningokok-konjugaterne indeholder et differitoxoid-bærestof og eventuelt en adipinsyre-linker;
 - meningokok-konjugaterne er til stede i en mængde på 8 µg/ml (målt som meningokok-saccharid) pr. serogruppe;
 - vægtforholdet mellem saccharid og bærestof for hvert konjugat er 1:3;
- 10
- sammensætningen eller lægemidlet indeholder 96 µg/ml differitoxoid;
 - sammensætningen eller lægemidlet indeholder 1,2 mg/ml natriumphosphat; og
 - sammensætningen eller lægemidlet indeholder 8,8 mg/ml natriumchlorid.