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(71) Demandeur/Applicant:
SMITHKLINE BEECHAM BIOLOGICALS S.A., BE

(72) Inventeurs/Inventors:
FRIEDE, MARTIN, BE;
HERMAND, PHILIPPE, BE;
HENDERICKX, VERONIQUE, BE

(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : ADJUVANT COMPRENANT UN ETHER OU ESTER D'ALKYLE POLYETHYLENE ET AU MOINS UN TENSIOACTIF NON IONIQUE

(54) Title: ADJUVANT COMPRISING A POLYXYETHYLENE ALKYL ETHER OR ESTER AND AT LEAST ONE NONIONIC SURFACTANT

(57) Abrégé/Abstract:
The present invention relates to a novel adjuvant system comprising a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant. Preferably said additional non-ionic surfactant is an Octoxynol (the TRITON$^{TM}$ series). The present invention provides said novel adjuvants, vaccines comprising them, and methods of their manufacture and their formulation into vaccines. The use of the adjuvants or vaccines of the present invention in the prophylaxis or therapy of disease is also provided.

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(71) Applicant *(for all designated States except US)*: **SMITHKLINE BEECHAM BIOLOGICALS S.A.** [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FRIEDE, Martin** [GB/BE]; SmithKline Beecham Biologicals s.a., Rue de l'Institut 89, B-1330 Rixensart (BE). **HERMAND, Philippe** [BE/BE]; Smithkline Beecham Biologicals s.a., Rue de l'institut 89, B-1330 Rixensart (BE). **HENDERICKX, Veronique** [BE/BE]; rue de l'Institut 89, B-1330 Rixensart (BE).

(74) Agent: **PRIVETT, Kathryn, Louise**; SmithKline Beecham, Corporate Intellectual Property, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).

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(54) Title: ADJUVANT COMPRISING A POLYXYETHYLENE ALKYL ETHER OR ESTER AND AT LEAST ONE NON-IONIC SURFACTANT

(57) Abstract: The present invention relates to a novel adjuvant system comprising a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant. Preferably said additional non-ionic surfactant is an Octoxynol (the TRITON™ series). The present invention provides said novel adjuvants, vaccines comprising them, and methods of their manufacture and their formulation into vaccines. The use of the adjuvants or vaccines of the present invention in the prophylaxis or therapy of disease is also provided.

# ADJUVANT COMPRISING A POLYXYETHYLENE ALKYL ETHER OR ESTER AND AT LEAST ONE NONIONIC SURFACTANT

The present invention relates to a novel adjuvant system comprising a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant. Preferably said additional non-ionic surfactant is an Octoxynol. The present invention provides said novel adjuvants, vaccines comprising them, and methods of their manufacture and their formulation into vaccines. The use of the adjuvants or vaccines of the present invention in the prophylaxis or therapy of disease are also provided. A method to enhance an immune response in a host using the adjuvant and vaccines of the present invention is also provided. The adjuvants are particularly useful as a mucosal adjuvant, but are also effective systemically.

Apart from bypassing the requirement for painful injections and the associated negative affect on patient compliance because of "needle fear", mucosal vaccination is attractive since it has been shown in animals that mucosal administration of antigens has a greater efficiency of inducing protective responses at mucosal surfaces, which is the route of entry of many pathogens. In addition, it has been suggested that mucosal vaccination, such as intranasal vaccination, may induce mucosal immunity not only in the nasal mucosa, but also in distant mucosal sites such as the genital mucosa (Mestecky, 1987, Journal of Clinical Immunology, 7, 265-276; McGhee and Kiyono, Infectious Agents and Disease, 1993, 2, 55-73). Despite much research in the field, safe and effective mucosal adjuvants which are suitable for use in humans, remains to be identified. The present invention provides a solution to this problem.

Medical uses of certain non-ionic surfactants have been described. For example, intranasal administration of polyoxyethylene ethers and esters for the enhancement of insulin uptake in the nasal cavity has been described (Hirai *et al*. 1981, International Journal of Pharmaceutics, 9, 165-172; Hirai *et al*. 1981, International Journal of Pharmaceutics, 9, 173-184).

Polyoxyethylene alkyl ethers have been described as components in oil emulsion, or acrylic acid polymer adjuvants (JP 05 201877; US 3,919,411).

Other non-ionic surfactants have been utilised in vaccine formulations. For example, vaccine preparations comprising an admixture of either polyoxyethylene castor oil or caprylic/capric acid glycerides, with polyoxyethylene sorbitan monoesters, and an antigen, are capable of inducing systemic immune responses after topical administration to a mucosal membrane (WO 94/17827). This patent application discloses the combination of the non-ionic surfactant TWEEN20™ (polyoxyethylene sorbitan monoester) and Imwitor742™ (caprylic/capric acid glycerides), or a combination of TWEEN20™ and polyoxyethylene castor oil are able to enhance the systemic immune response following intranasal immunisation. Details of the effect of this formulation on the enhancement of the immune response towards intranasally administered antigens have also been described in the literature (Gizurarson *et al.* 1996. Vaccine Research, 5, 69-75; Aggerbeck *et al.* 1997, Vaccine, 15, 307-316; Tebbey *et al.*,*Viral Immunol* 1999;12(1):41-5).

Non-ionic surfactants have also been formulated in such a way as to form non-ionic surfactant vesicles (commonly known as NISV, US 5,679,355). Such formulations of non-ionic surfactants, often in the presence of cholesterol, form lipid-bilayer vesicles which entrapp antigen within the inner aqueous phase or within the bilayer itself.

International Patent application WO 96/36352 (US 5,653,987) describes a liquid – pharmaceutical agent comprising at least two absorption enhancers and water, primarily for oral insulin delivery, wherein the amount of each absorption enhancing agent is present in a concentration of from 1 to 10 % w/w of the total formulation.

Surfactants are commonly used in the formulation of oil emulsion adjuvants for systemic administration, and function to stabilise the oil droplets. For example, polyoxyethylene sorbitan esters (TWEEN™) and sorbitan fatty acid esters (SPAN™) are used to stabilise oil in water emulsions (EP 0 399 843 B, WO 95/17210).

The applicant presents here the surprising finding that polyoxyethylene alkyl ethers or esters, in combination with at least one additional non-ionic surfactant, together act as a potent adjuvants for vaccines. Advantageously, such compositions may be administered systemically, but are also potent in the induction of systemic immune responses when the vaccine compositions are administered mucosally. The immune responses induced by mucosal administration of vaccines of the present invention may be at least as high as those observed after a systemic injection of conventional vaccine.

The present invention provides safe and potent adjuvants which are easily manufactured, and comprise at least one polyoxyethylene alkyl ether or ester and at least one additional non-ionic surfactant. The surfactants employed in the invention may be in aqueous solution or may form suspensions of particulate structures such as vesicles or micelles. Preferably the surfactants are in the form of an aqueous solution or a micelle.

The polyoxyethylene ethers or esters which may be formulated in the vaccines and adjuvant of the present invention comprise molecules of general formula (I):

$$HO(CH_2CH_2O)_n\text{-}A\text{-}R$$

wherein, n is 1-50, A is a bond or -C(O)-, R is $C_{1\text{-}50}$ alkyl or Phenyl $C_{1\text{-}50}$ alkyl.

Thus, one embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene alkyl ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, more preferably 6-12, and most preferably 9; the *R* component is $C_{1\text{-}50}$, preferably $C_4$-$C_{20}$ alkyl and most preferably $C_{12}$ alkyl. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Suitable polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

Most preferably, said polyoxyethylene alkyl ether is polyoxyethylene-9-lauryl ether (laureth 9). Alternative terms or names for polyoxyethylene lauryl ether are disclosed in the CAS registry. The CAS registry number of polyoxyethylene-9 lauryl ether is: ***9002-92-0***. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12th ed: entry 7717, Merck & Co. Inc., Whitehouse Station, N.J., USA; ISBN 0911910-12-3), where therapeutic uses are stated to include: topical anesthetic; anti-pruritic; and sclerosing agent activities. As a class, such polyoxyethylene ethers, or esters, are non-ionic surfactants. Laureth 9 is formed by reacting ethylene oxide with dodecyl alcohol, and has an average of nine ethylene oxide units. In the case that one such mixture of surfactants of formula (I) are used, it is intended in the context of the present invention that n = the average number of ethylene oxide units present in all of the surfactants in the mixture.

The ratio of the length of the polyoxyethylene section to the length of the alkyl chain in the surfactant (*i.e.* the ratio of n: alkyl chain length), affects the solubility of this class of detergent in an aqueous medium. Thus, the adjuvants of the present invention may be in solution or may form particulate structures such as micelles or vesicles. As a solution, the adjuvants of the present invention safe, easily sterilisable by for example passing through a 0.22μm membrane, simple to administer, and may be manufactured in a simple fashion without the GMP and QC issues associated with the formation of uniform particulate structures. Some polyoxyethylene ethers, such as laureth 9, are capable of forming non-vesicular solutions. However, polyoxyethylene-8 palmitoyl ether ($C_{18}E_8$) is capable of forming vesicles. Accordingly, the use of vesicles of polyoxyethylene-8 palmitoyl ether in combination with at least one additional non-ionic surfactant, to form adjuvants of the present invention is specifically contemplated.

Preferably, the polyoxyethylene alkyl ether element present in the adjuvant combinations of the present invention has haemolytic activity. The haemolytic activity of a polyoxyethylene alkyl ether may be measured *in vitro*, with reference to

the following assay, and is as expressed as the highest concentration of the detergent which fails to cause lysis of the red blood cells:

1. Fresh blood from guinea pigs is washed with phosphate buffered saline (PBS) 3 times in a desk-top centrifuge. After resuspension to the original volume the blood is further diluted 10 fold in PBS.
2. 50 $\mu$l of this blood suspension is added to 800 $\mu$l of PBS containing two-fold dilutions of detergent.
3. After 8 hours the haemolysis is assessed visually or by measuring the optical density of the supernatant. The presence of a red supernatant, which absorbs light at 570 nm indicates the presence of haemolysis.
4. The results are expressed as the concentration of the first detergent dilution at which hemolysis no longer occurs.

Within the inherent experimental variability of such a biological assay, the polyoxyethylene alkyl ethers, or surfactants of general formula (I), of the present invention preferably have a haemolytic activity, of approximately between 0.5-0.0001%, more preferably between 0.05-0.0001%, even more preferably between 0.005-0.0001%, and most preferably between 0.003-0.0004%. Ideally, said polyoxyethylene ethers or esters should have a haemolytic activity similar (*i.e.* within a ten-fold difference) to that of either polyoxyethylene-9 lauryl ether or polyoxyethylene-8 stearyl ether.

To the polyoxyethylene alkyl ether or ester is added at least one additional non-ionic surfactant, which may be any detergent with suitable surface active properties. Suitable detergents are described in "Surfactant systems" Ed: Attwood and Florence (1983, Chapman and Hall).

Preferred non-ionic surfactants are not ones that fall within the general formula (I), e.g. Octoxynols and Polyoxyethylene sorbitan esters. Particularly preferred Octoxynols include Triton X-45, t-octylphenoxy polyethoxyethanol (Triton X-100), Triton X-102, Triton X-114, Triton X-165, Triton X-205, Triton X-305, Triton N-

57, Triton N-101, Triton N-128. Triton X-100 is particularly preferred. The Octoxynol series, including t-octylphenoxypolyethoxyethanol (TRITON X100™) is described in Merck Index Entry 6858 (Page 1162, 12th Edition, Merck & Co. Inc., Whitehouse Station, N.J., USA; ISBN 0911910-12-3).

Other preferred non-ionic surfactants are polyoxyethylene sorbitan esters. The polyoxyethylene sorbitan esters, including polyoxyethylene sorbitan monooleate (TWEEN80™) are described in Merck Index Entry 7742 (Page 1308, 12th Edition, Merck & Co. Inc., Whitehouse Station, N.J., USA; ISBN 0911910-12-3). Both Octoxynols and polyoxyethylene sorbitan esters may be purchased from Sigma Inc. The preferred polyoxyethylene sorbitan ester is polyoxyethylene sorbitan monooleate (Tween 80™).

The most prefered adjuvants of the present invention comprise a polyoxyethylene alkyl ether and an Octoxynol, such as t-octylphenoxypolyethoxyethanol (TRITON X100™). Optionally said combination may further comprise a polyoxyethylene sorbitan ester, such as polyoxyethylene sorbitan monooleate (TWEEN80™). Most preferably, said polyoxyethylene alkyl ether is polyoxyethylene-9-lauryl ether, and said Octoxynol is t-octylphenoxypolyethoxyethanol (TRITON X100™). To these formulations, an ionic detergent may be added such as a bile salt or derivative of cholic acid.

Accordingly the adjuvant formulation may comprise a polyoxyethylene alkyl ether or ester (formula I), an octoxynol, optionally comprising a polyoxyethylene sorbitan ester, and optionally comprising a bile salt or cholic acid derivative. The preferred embodiment of this formulation comprises a combination of polyoxyethylene-9 lauryl ether, t-octylphenoxypolyethoxyethanol (TRITON X100™), polyoxyethylene sorbitan monooleate and sodium deoxycholate.

The concentration of polyoxyethylene alkyl ether or ester, such as polyoxyethylene-9 lauryl ether, in the adjuvants of the present invention will typically be in the range

of 0.001 to 20 %, preferably 0.001 to 10 % and more preferably 0.001 to 1 %, and most preferably between 0.001 and 0.8% or about 0.5% (w/v). To this is added the additional non-ionic surfactant which is not a polyoxyethylene ether or ester. The or each of the additional non-ionic surfactants will typically be present in the final vaccine formulation at a concentration of between 0.001 to 20%, more preferably 0.01 to 10%, and most preferably up to about 2% (w/v). Where two of said additional non-ionic surfactants are present, these are preferably present in the final formulation at a concentration of up to about 2% each, typically at a concentration of up to about 0.6% each. If three or more additional non-ionic surfactants are present, they are generally present at up to a concentration of about 1% each and typically in traces up to about 0.2% or 0.1 % each. Any mixture of surfactants may be present in the vaccine formulations according to the invention. Non-ionic surfactants such as those discussed above have preferred concentrations in the final vaccine composition as follows: octyl- or nonylphenoxy polyoxyethanols such as Triton X-100™ or other detergents in the Triton series: from 0.001% to 20%, preferably 0.001% to 10%, more preferably from 0.001 to 1%, and most preferably 0.005 to 0.1 % (w/v); and if present polyoxyethylene sorbitan esters such as Tween 80™: 0.01 to 1%, most preferably about 0.1% (w/v).

The total concentration of detergent in the vaccine or adjuvant formulations of the present invention, including the polyoxyethylene ether or ester and one or more additional non-ionic surfactants, is typically in the range of 0.001 to 40%, preferably between 0.001 and 20%, more preferably between 0.001 and 10%, more preferably still between 0.001 and 1%, and most preferably between 0.001 to 0.7%. (w/v).

The vaccine preparations of the present invention may be used to protect or treat a mammal susceptible to, or suffering from disease, by means of administering said vaccine via a mucosal route, such as the oral/bucal/intestinal/vaginal/rectal or nasal route. Such administration may be in a droplet, spray, or dry powdered form. Nebulised or aerosolised vaccine formulations also form part of this invention. Enteric formulations such as gastro resistant capsules and granules for oral

administration, suppositories for rectal or vaginal administration also form part of this invention. The present invention may also be used to enhance the immunogenicity of antigens applied to the skin (transdermal or transcutaneous delivery). In addition, the adjuvants of the present invention may be parentally delivered, for example intramuscular, or subcutaneous administration. When used for intranasal vaccination, the vaccines of the present invention are preferably haemolytic in nature.

Depending on the route of administration, a variety of administration devices may be used. For example, for intranasal administration a spray device such as the commercially available Accuspray™ (Becton Dickinson) may be used.

Preferred spray devices for intranasal use are devices for which the performance of the device is not dependent upon the pressure applied by the user. These devices are known as pressure threshold devices. Liquid is released from the nozzle only when a threshold pressure is attained. These devices make it easier to achieve a spray with a regular droplet size. Pressure threshold devices suitable for use with the present invention are known in the art and are described for example in WO 91/13281 and EP 311 863 B. Such devices are commercially available from Pfeiffer GmbH.

Preferred intranasal devices produce droplets (measured using water as the liquid) in the range 1 to 200μm, preferably 10 to 120μm. Below 10μm there is a risk of inhalation, therefore it is desirable to have no more than about 5% of droplets below 10μm. Droplets above 120μm do not spread as well as smaller droplets, so it is desirable to have no more than about 5% of droplets exceeding 120μm.

Bi-dose delivery is a further preferred feature of an intranasal delivery device for use with the vaccines according to the invention. Bi-dose devices contain two subdoses of a single vaccine dose, one sub-dose for administration to each nostril.

The invention provides in a further aspect a kit comprising an intranasal administration device as described herein containing a vaccine formulation according to the invention.

For certain vaccine formulations, other vaccine components may be included in the formulation. As such the adjuvant formulations of the present invention may also comprise a bile acid or derivative of cholic acid. Preferably the derivative of cholic acid is a salt thereof, and more preferably a sodium salt thereof. Examples of bile acids and derivatives thereof include cholic acid itself, deoxycholic acid, taurodeoxycholate, chenodeoxy colic acid, lithocholic acid ursodeoxycholic acid, hyodeoxycholic acid and derivatives like glyco-, tauro-, amidopropyl-1-propanesulfonic-, amidopropyl-2-hydroxy-1-propanesulfonic derivatives of the aforementioned bile acids, or N,N-bis (3DGluconoamidopropyl) deoxycholamide. A particular preferred example is sodium deoxycholate (NaDOC) which may be present in the final vaccine dose.

Preferably, the adjuvant formulation of the present invention are advantageous when in the form of an aqueous solution or a suspension of non-vesicular forms. Such formulations are easy to manufacture reproducibly, and also to sterilise (terminal filtration through a 450 or 220 nm pore membrane) and are also easy to administer to the nasal mucosa in the form of a spray without degradation of complex physical structure of the adjuvant. Polyoxyethylene-9 lauryl ether in combination with TRITON-X 100™ forms an aqueous solution (small micelles may also be present).

In one aspect of the present invention, there is provided a method of inducing or enhancing an immune response in a host, comprising admixing the antigen and the adjuvants of the present invention, and administering said admixture to the host. Preferably, the route of administration to said host is via a mucosal surface, and more preferably via the nasal mucosa. When the admixture is administered via the nasal mucosa, the admixture is preferably administered as a spray. In a prefered methods of inducing an immune response, systemic immune response is induced by a nasal administration of the vaccines of the present invention. It is preferred that

the methods to enhance an immune response may be either a priming or boosting dose of the vaccine, and that the vaccine comprises an influenza antigen or antigenic preparation. The prefered adjuvant formulation for administering to the nasal mucosa in these methods, are combinations of polyoxyethylene alkyl ether and an octoxynol, such as a preferred combination of polyoxyethylene-9 lauryl ether and t-octylphenoxypolyethoxyethanol (TRITON X100™), optionally said adjuvant combination additionally comprising a polyoxyethylene sorbitan ester (such as the monooleate, TWEEN 80™) and/or a bile salt or cholic acid derivative such as sodium deoxycholate.

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from a wide variety of sources. For example, antigens may include human, bacterial, or viral nucleic acid, pathogen derived antigen or antigenic preparations, tumour derived antigen or antigenic preparations, host-derived antigens, including GnRH and IgE peptides, recombinantly produced protein or peptides, and chimeric fusion proteins.

Preferably the vaccine formulations of the present invention contain an antigen or antigenic composition capable of eliciting an immune response against a human pathogen, which antigen or antigenic composition is derived from HIV-1, (such as tat, nef, gp120 or gp160), human herpes viruses, such as gD or derivatives thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2, cytomegalovirus ((esp Human)(such as gB or derivatives thereof), Rotavirus (including live-attenuated viruses), Epstein Barr virus (such as gp350 or derivatives thereof), Varicella Zoster Virus (such as gpI, II and IE63), or from a hepatitis virus such as hepatitis B virus (for example Hepatitis B Surface antigen or a derivative thereof), hepatitis A virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as paramyxoviruses: Respiratory Syncytial virus (such as F and G proteins or derivatives thereof), parainfluenza virus, measles virus, mumps virus, human papilloma viruses (for example HPV6, 11, 16, 18, ..), flaviviruses (e.g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or Influenza virus (whole live or inactivated virus, split

influenza virus, grown in eggs or MDCK cells, or Vero cells or whole flu virosomes (as described by R. Gluck, Vaccine, 1992, 10, 915-920) or purified or recombinant proteins thereof, such as HA, NP, NA, or M proteins, or combinations thereof), or derived from bacterial pathogens such as *Neisseria spp,* including *N. gonorrhea* and *N. meningitidis* (for example capsular polysaccharides and conjugates thereof, transferrin-binding proteins, lactoferrin binding proteins, PilC, adhesins); *S. pyogenes* (for example M proteins or fragments thereof, C5A protease, lipoteichoic acids), *S. agalactiae, S. mutans; H. ducreyi; Moraxella spp, including M catarrhalis, also known as Branhamella* catarrhalis (for example high and low molecular weight adhesins and invasins*); Bordetella spp, including B. pertussis* (for example pertactin, pertussis toxin or derivatives thereof, filamenteous hemagglutinin, adenylate cyclase, fimbriae), *B. parapertussis and B. bronchiseptica; Mycobacterium spp., including M. tuberculosis* (for example ESAT6, Antigen 85A, -B or -C), *M. bovis, M. leprae, M. avium, M. paratuberculosis, M. smegmatis; Legionella spp,* including *L. pneumophila; Escherichia spp,* including *enterotoxic E. coli* (for example colonization factors, heat-labile toxin or derivatives thereof, heat-stable toxin or derivatives thereof), enterohemorragic *E. coli,* enteropathogenic *E. coli* (for example shiga toxin-like toxin or derivatives thereof); *Vibrio spp,* including *V. cholera* (for example cholera toxin or derivatives thereof*); Shigella spp,* including *S. sonnei, S. dysenteriae, S. flexnerii; Yersinia spp,* including *Y.* enterocolitica (for example a Yop protein) , *Y. pestis, Y. pseudotuberculosis; Campylobacter spp,* including *C. jejuni* (for example toxins, adhesins and invasins) and *C. coli; Salmonella spp,* including *S. typhi, S. paratyphi, S. choleraesuis, S. enteritidis; Listeria spp.,* including *L. monocytogenes; Helicobacter spp,* including *H.* pylori (for example urease, catalase, vacuolating toxin); *Pseudomonas spp,* including *P. aeruginosa; Staphylococcus spp.,* including *S. aureus, S. epidermidis; Enterococcus spp.,* including *E. faecalis, E. faecium; Clostridium spp.,* including *C.* tetani (for example tetanus toxin and derivative thereof), *C. botulinum* (for example botulinum toxin and derivative thereof*), C. difficile* (for example clostridium toxins A or B and derivatives thereof); *Bacillus spp.,* including *B. anthracis* (for example botulinum toxin and derivatives thereof*);* *Corynebacterium spp.,* including *C. diphtheriae* (for example diphtheria toxin and

derivatives thereof); *Borrelia spp.*, including *B. burgdorferi* (for example OspA, OspC, DbpA, DbpB), *B. garinii* (for example OspA, OspC, DbpA, DbpB), *B. afzelii* (for example OspA, OspC, DbpA, DbpB), *B. andersonii* (for example OspA, OspC, DbpA, DbpB), *B. hermsii; Ehrlichia spp.*, including *E. equi* and the agent of the Human Granulocytic Ehrlichiosis; *Rickettsia spp*, including *R. rickettsii; Chlamydia spp., including C. trachomatis* (for example MOMP, heparin-binding proteins), *C. pneumoniae* (for example MOMP, heparin-binding proteins), *C. psittaci; Leptospira spp.*, including *L. interrogans; Treponema spp.*, including *T. pallidum* (for example the rare outer membrane proteins), *T. denticola, T. hyodysenteriae;* or derived from parasites such as *Plasmodium spp.*, including *P. falciparum; Toxoplasma spp.*, including *T. gondii (for example SAG2, SAG3, Tg34); Entamoeba spp.*, including *E. histolytica; Babesia spp.*, including *B. microti; Trypanosoma spp.*, including *T. cruzi; Giardia spp.*, including *G. lamblia; Leshmania spp.*, including *L. major; Pneumocystis spp.*, including *P. carinii; Trichomonas spp.*, including *T. vaginalis; Schisostoma spp.*, including *S. mansoni,* or derived from yeast such as *Candida spp.*, including *C. albicans; Cryptococcus spp.*, including *C. neoformans*.

Preferred bacterial vaccines comprise antigens derived from *Streptococcus spp,* including *S. pneumoniae* (for example capsular polysaccharides and conjugates thereof, PsaA, PspA, streptolysin, choline-binding proteins)and the protein antigen Pneumolysin (Biochem Biophys Acta, 1989, 67, 1007; Rubins et al., Microbial Pathogenesis, 25, 337-342), and mutant detoxified derivatives thereof (WO 90/06951; WO 99/03884). Other preferred bacterial vaccines comprise antigens derived from *Haemophilus spp., including H. influenzae type B* (for example PRP and conjugates thereof), *non typeable H. influenzae,* for example OMP26, high molecular weight adhesins, P5, P6, protein D and lipoprotein D, and fimbrin and fimbrin derived peptides (US 5,843,464) or multiple copy varients or fusion proteins thereof. Other preferred bacterial vaccines comprise antigens derived from *Morexella Catarrhalis* (including outer membrane vesicles thereof, and OMP106 (WO97/41731)) and from *Neisseria mengitidis B* (including outer membrane vesicles thereof, and NspA (WO 96/29412).

Derivatives of Hepatitis B Surface antigen are well known in the art and include, inter alia, those PreS1, PreS2 S antigens set forth described in European Patent applications EP-A-414 374; EP-A-0304 578, and EP 198-474. In one preferred aspect the vaccine formulation of the invention comprises the HIV-1 antigen, gp120, especially when expressed in CHO cells. In a further embodiment, the vaccine formulation of the invention comprises gD2t as hereinabove defined.

In a preferred embodiment of the present invention vaccines containing the claimed adjuvant comprise antigen derived from the Human Papilloma Virus (HPV) considered to be responsible for genital warts, *(*HPV 6 or HPV 11 and others*)*, and the HPV viruses responsible for cervical cancer (HPV16, HPV18 and others*)*.

Particularly preferred forms of genital wart prophylactic, or therapeutic, vaccine comprise L1 particles or capsomers, and fusion protein*s* comprising one or more antigens selected from the HPV 6 and HPV 11 proteins E6, E7, L1, and L2.

The most preferred forms of fusion protein are: L2E7 as disclosed in WO 96/26277, and proteinD(1/3)-E7 disclosed in GB 9717953.5 (PCT/EP98/05285).

A preferred HPV cervical infection or cancer, prophylaxis or therapeutic vaccine, composition may comprise HPV 16 or 18 antigens. For example, L1 or L2 antigen monomers, or L1 or L2 antigens presented together as a virus like particle (VLP) or the L1 alone protein presented alone in a VLP or capsomer structure. Such antigens, virus like particles and capsomer are per se known. See for example WO94/00152, WO94/20137, WO94/05792, and WO93/02184.

Additional early proteins may be included alone or as fusion proteins such as preferably E7, E2 or E5 for example; particularly preferred embodiments of this includes a VLP comprising L1E7 fusion proteins (WO 96/11272).

Particularly preferred HPV 16 antigens comprise the early proteins E6 or E7 in fusion with a protein D carrier to form Protein D - E6 or E7 fusions from HPV 16, or combinations thereof; or combinations of E6 or E7 with L2 (WO 96/26277).

Alternatively the HPV 16 or 18 early proteins E6 and E7, may be presented in a single molecule, preferably a Protein D- E6/E7 fusion. Such vaccine may optionally contain either or both E6 and E7 proteins from HPV 18, preferably in the form of a Protein D - E6 or Protein D - E7 fusion protein or Protein D E6/E7 fusion protein.

The vaccine of the present invention may additionally comprise antigens from other HPV strains, preferably from strains HPV 6, 11, 31, 33, or 45.

Vaccines of the present invention further comprise antigens derived from parasites that cause Malaria. For example, preferred antigens from *Plasmodia falciparum* include RTS,S and TRAP. RTS is a hybrid protein comprising substantially all the C-terminal portion of the circumsporozoite (CS) protein of *P.falciparum* linked via four amino acids of the preS2 portion of Hepatitis B surface antigen to the surface (S) antigen of hepatitis B virus. It's full structure is disclosed in the International Patent Application No. PCT/EP92/02591, published under Number WO 93/10152 claiming priority from UK patent application No.9124390.7. When expressed in yeast RTS is produced as a lipoprotein particle, and when it is co-expressed with the S antigen from HBV it produces a mixed particle known as RTS,S. TRAP antigens are described in the International Patent Application No. PCT/GB89/00895, published under WO 90/01496. A preferred embodiment of the present invention is a Malaria vaccine wherein the antigenic preparation comprises a combination of the RTS,S and TRAP antigens. Other plasmodia antigens that are likely candidates to be components of a multistage Malaria vaccine are *P. faciparum* MSP1, AMA1, MSP3, EBA, GLURP, RAP1, RAP2, Sequestrin, PfEMP1, Pf332, LSA1, LSA3, STARP, SALSA, PfEXP1, Pfs25, Pfs28, PFS27/25, Pfs16, Pfs48/45, Pfs230 and their analogues in Plasmodium spp.

The formulations may also contain an anti-tumour antigen and be useful for the immunotherapeutic treatment cancers. For example, the adjuvant formulation finds utility with tumour rejection antigens such as those for prostrate, breast, colorectal, lung, pancreatic, renal or melanoma cancers. Exemplary antigens include MAGE 1 and MAGE 3 or other MAGE antigens for the treatment of melanoma, PRAME, BAGE or GAGE (Robbins and Kawakami, 1996, Current Opinions in Immunology 8, pps 628-636; Van den Eynde et al., International Journal of Clinical & Laboratory Research (submitted 1997); Correale et al. (1997), Journal of the National Cancer Institute 89, p293. Indeed these antigens are expressed in a wide range of tumour types such as melanoma, lung carcinoma, sarcoma and bladder carcinoma. Other Tumor-Specific antigens are suitable for use with adjuvant of the present invention and include, but are not restricted to Prostate specific antigen (PSA) or Her-2/neu, KSA (GA733), MUC-1 and carcinoembryonic antigen (CEA). Accordingly in one aspect of the present invention there is provided a vaccine comprising an adjuvant composition according to the invention and a tumour rejection antigen.

Additionally said antigen may be a self peptide hormone such as whole length Gonadotrophin hormone releasing hormone (GnRH, WO 95/20600), a short 10 amino acid long peptide, in the treatment of many cancers, or in immunocastration.

It is foreseen that compositions of the present invention will be used to formulate vaccines containing antigens derived from *Borrelia sp.*. For example, antigens may include nucleic acid, pathogen derived antigen or antigenic preparations, recombinantly produced protein or peptides, and chimeric fusion proteins. In particular the antigen is OspA. The OspA may be a full mature protein in a lipidated form virtue of the host cell (E.Coli) termed (Lipo-OspA) or a non-lipidated derivative. Such non-lipidated derivatives include the non-lipidated NS1-OspA fusion protein which has the first 81 N-terminal amino acids of the non-structural protein (NS1) of the influenza virus, and the complete OspA protein, and another, MDP-OspA is a non-lipidated form of OspA carrying 3 additional N-terminal amino acids.

Vaccines of the present invention may be used for the prophylaxis or therapy of allergy. Such vaccines would comprise allergen specific (for example Der p1) and allergen non-specific antigens (for example peptides derived from human IgE, including but not restricted to the stanworth decapeptide (EP 0 477 231 B1)).

The most preferred vaccines, and the methods of inducing an immune response, of the present invention comprise Influenza virus antigen. Non-live influenza virus preparations may be derived from the conventional embryonated egg method, or they may be derived from any of the new generation methods using tissue culture to grow the virus. Suitable cell substrates for growing the virus include for example dog kidney cells such as MDCK or cells from a clone of MDCK, MDCK-like cells, monkey kidney cells such as AGMK cells including Vero cells, or any other cell type suitable for the production of influenza virus for vaccine purposes. Suitable cell substrates include human cells e.g. MRC-5 cells. Suitable cell substrates are not limited to cell lines; for example primary cells such as chicken embryo fibroblasts are also included. The influenza virus antigen preparation may be produced by any of a number of commercially applicable processes, for example the split flu process described in patent no. DD 300 833. Commercially available split influenza includes Fluarix™ which is sold by SmithKline Beecham, as such Fluarix in combination with the adjuvant of the present invention constitute a preferred vaccine of the present invention.

The influenza vaccine according to the invention is preferably a multivalent influenza vaccine comprising two or more strains of influenza. Most preferably it is a trivalent vaccine comprising three strains. Conventional influenza vaccine generally comprise three strains of influenza, two A strains and one B strain. However, monovalent vaccines, which may be useful for example in a pandemic situation, are not excluded from the invention. A monovalent, pandemic flu vaccine will most likely contain influenza antigen from a single A strain.

Accordingly a preferred vaccine formulation comprises egg or tissue culture influenza antigen, preferably split influenza antigen, a polyoxyethylene alkyl ether and at least one additional non-ionic surfactant, optionally comprising a bile salt or derivative of cholic acid. Preferred embodiments of this vaccine comprise split influenza virus antigen, polyoxyethylene-9 lauryl ether and TRITON-X 100™. Optionally, this most preferred vaccine may further comprise a polyoxyethylene sorbitan ester, such as TWEEN80™, and/or sodium deoxycholate.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented. Generally, it is expected that each dose will comprise 1-1000 μg of protein, preferably 1-500 μg, preferably 1-100μg, most preferably 1 to 50μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of appropriate immune responses in subjects. Following an initial vaccination, subjects may receive one or several booster immunisation adequately spaced.

In a preferred aspect of the present invention is when the adjuvant affect of the polyoxyethylene alkyl ether is enhanced synergistically by the additional non-ionic surfactant. In this regard, the synergy may be observed in the magnitude of the immune response from the combined adjuvant formulation being greater than the sum of the immune responses generated by each individual component when used alone. Alternatively, synergism may also be observed when low doses of polyoxyethylene ether and additional non-ionic surfactant generate significant immune responses, even when one or each component may not generate significant or detectable immune responses when used alone.

One aspect of the present invention is adjuvant and vaccine formulations comprising a polyoxyethylene alkyl ether or ester and at least one additional non-ionic surfactant, wherein the antigen present in the vaccine is not entrapped within a non-ionic surfactant vesicle.

The vaccines of the present invention may also be administered via the oral route. In such cases the pharmaceutically acceptible excipient may also include alkaline buffers, or enteric capsules or microgranules. The vaccines of the present invention may also be administered by the vaginal route. In such cases, the pharmaceutically acceptable excipients may also include emulsifiers, polymers such as CARBOPOL®, and other known stablilisers of vaginal creams and suppositories. The vaccines of the present invention may also be administered by the rectal route. In such cases the excipients may also include waxes and polymers known in the art for forming rectal suppositories.

The formulations of the present invention may be used for both prophylactic and therapeutic purposes. Accordingly, the present invention provides for a method of treating a mammal susceptible to or suffering from an infectious disease or cancer, or allergy, or auto-immune disease. In a further aspect of the present invention there is provided an adjuvant combination and a vaccine as herein described for use in medicine. Vaccine preparation is generally described in New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978.

One embodiment of the present invention relates to the use of non-ionic surfactants such as a polyoxyethylene alkyl ether or ester of general formula (I), and an octoxynol, in the manufacture of an adjuvant formulation. The present invention also relates to the use of a polyoxyethylene alkyl ether or ester of general formula (I), an octoxynol, and an antigen, in the manufacture of vaccine formulations. Optionally said adjuvant and vaccines manufactured as described, may further comprise an polyoxyethylene sorbitan ester. In all of these aspects of the present invention, the preferred polyoxyethylene alkyl ether is polyoxyethylene-9 lauryl ether, and the preferred Octoxynol is t-octylphenoxy polyethoxyethanol (Triton X-100™).

In an alternative related embodiment of the present invention the adjuvants of the present invention may further be combined with other adjuvants including Cholera toxin and its B subunit, E.Coli heat labile enterotoxin LT, its B subunit LTB and detoxified versions thereof such as mLT; Monophosphoryl Lipid A and its non-toxic derivative 3-O-deacylated monophosphoryl lipid A (3D-MPL, as described in UK patent no. GB 2,220,211), immunologically active saponin fractions *e.g.* Quil A derived from the bark of the South American tree Quillaja Saponaria Molina and derivatives thereof (for example QS21, US Patent No.5,057,540), and the oligonucleotide adjuvant system CpG (as described in WO 96/02555), especially $^{5'}$TCG TCG TTT TGT CGT TTT GTC GTT$^{3'}$ (SEQ ID NO. 1).

In this embodiment, an adjuvant combination of a polyoxyethylene alkyl ether (such as polyoxyethylene-9 lauryl ether), additional non-ionic surfactant (such as t-octylphenoxy polyethoxyethanol (Triton X-100™)), and 3-O-deacylated monophosphoryl lipid A (3D-MPL) is particularly preferred. This preferred embodiment may optionally further comprise a polyoxyethylene sorbitan ester such as TWEEN80™, and/or a bile salt or cholic acid derivative such as sodium deoxycholate. Vaccines comprising this adjuvant formulation and influenza antigens, especially split influenza antigens are particularly preferred.

The present invention is illustrated by, but not limited to, the following examples.

## EXAMPLES

**Example 1**, *Methods used to measure antibody (Ab) responses in sera*

ELISA for the measurement of influenza-specific serum Ig Abs in monkeys:

Maxisorp Nunc immunoplates are coated overnight at 4°C with 50 µl/well of 1 µg/ml HA of β-propiolactone (BPL) inactivated influenza virus (supplied by SSD GmBH manufacturer, Dresden, Germany) diluted in PBS. Free sites on the plates are blocked (1 hour, 37°C) using saturation buffer : PBS containing 1%BSA, 0.1%

polyoxyethylene sorbitan monolaurate (TWEEN 20). Then, serial 2-fold dilutions (in saturation buffer, 50 $\mu$l per well) of a reference serum added as a standard curve (serum having a mid-point titer expressed as ELISA Unit/ml, and put in row A ) and serum samples (starting at a 1/100 dilution and put in rows B to H) are incubated for 1hr 30mins at 37°C. The plates are then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, biotinylated goat anti-human Ig (Amersham) diluted 1/3000 in saturation buffer are incubated (50 $\mu$l/well) for 1hr 30mins, at 37°C. After 3 washings, and subsequent addition of streptavidin-horseradish peroxidase conjugate (Amersham), plates are washed 5 times and incubated for 20 min at room temperature with 50 $\mu$l/well of revelation buffer (OPDA 0.4 mg/ml (Sigma) and $H_2O_2$ 0.03% in 50mM pH 4.5 citrate buffer). Revelation is stopped by adding 50 $\mu$l/well $H_2SO_4$ 2N. Optical densities are read at 492 and 630 nm by using Biorad 3550 immunoreader. Antibody titre are calculated by the 4 parameter mathematical method using SoftMaxPro software.

Hemagglutination Inhibition (HAI) activity of Flu-specific serum Abs in monkeys

In order to eliminate the non-specific inhibitors of hemagglutination present in the primate sera, these (25 µl) are incubated overnight at 37°C with 100 µl calcium chlorure/borate/sodium borate mix solution containing 400 receptor destroying enzyme units per ml of *V. cholerae* neuraminidase (Boerhinger Mannheim). After addition of 75 µl sodium citrate 2.5%, sera are heated for 30 minutes at 56°C. A solution of 50 µl PBS is added to give a final serum dilution of $1/10^{th}$. Then, 25 µl treated-sera are diluted in 25 µl PBS (serial 2-fold dilutions starting at 1/10) in 96 well Greiner plates. BPL inactivated whole virus is added (25 µl / well) at a concentration of 4 Hemagglutination Units (i.e. at a dilution which is 4-fold lower than the last one provoking an agglutination of red blood cells) for 30 minutes at room temperature (RT) under agitation. Chicken red blood cells are then added (25 µl / well) for 1 hour at RT. Plates are finally kept overnight at 4°C before to be read. The HAI titer corresponds to the inverse of the last serum dilution inhibiting the virus-induced hemagglutination.

ELISA for the measurement of tetanus toxoid (TT) specific serum IgG in mice:

Maxisorp Nunc immunoplates were coated overnight at 4°C with 50 µl/well of 1 µg/ml antigen (TT provided by Behring) diluted in PBS (in rows B to H of plate), or with 50 µl of 5 µg/ml purified goat anti-mouse Ig (Boerhinger), in PBS (row A). Free sites on the plates were blocked (1 hour, 37°C) using saturation buffer : PBS containing 1%BSA, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20), and 4% Normal Bovine Serum (NBS). Then, serial 2-fold dilutions (in saturation buffer, 50 µl per well) of IgG isotype mixture added as a standard curve (mixture of mouse monoclonal antibodies IgG1, IgG2a and IgG2b from Sigma, starting at 200 ng/ml and put in row A) and serum samples (starting at a 1/100 dilution and put in rows B to H) were incubated for 1hr 30mins at 37°C. The plates were then washed (×3) with washing buffer (PBS, 0.1% polyoxyethylene sorbitan monolaurate (TWEEN 20)). Then, biotinylated goat anti-mouse IgG (Amersham) diluted 1/5000 in saturation buffer were incubated (50 µl/well) for 1hr 30mins, at 37°C. After 3 washings, and subsequent addition of streptavidin-horseradish peroxidase conjugate (Amersham), plates were washed 5 times and incubated for 20 min at room temperature with 50 µl/well of revelation buffer (OPDA 0.4 mg/ml (Sigma) and $H_2O_2$ 0.03% in 50mM pH 4.5 citrate buffer). Revelation was stopped by adding 50 µl/well $H_2SO_4$ 2N. Optical densities were read at 492 and 630 nm by using Biorad 3550 immunoreader. Antibody titre were calculated by the 4 parameter mathematical method using SoftMaxPro software.

**Example 2,** *Effect of Laureth 9 together with a combination of TWEEN80 and TritonX100 on the immunogenicity of an intranasal influenza vaccine in primed Rhesus monkeys*

The priming was done in Rhesus monkeys by administering with a spray device (under anesthesia) in each nostril 25 µg HA per strain of β-propiolactone-inactivated A/Beijing/262/95 and B/Harbin/7/94 influenza virus contained in 100 µl PBS. After 28 days, monkeys (4 or 5 animals/group) were boosted intranasally (under anesthesia) with 200 µl of solution (100 µl per nostril, delivered with a spray device) containing

30 µg HA/strain of BPL-inactivated A/Beijing/262/95 and B/Harbin/7/94 influenza virus in either A: polyoxyethylene-9-lauryl ether 0.5% (L9); B: polyoxyethylene-9-lauryl ether 0.5% + TWEEN80 (0.11%) + triton-X-100 (0.074%); or by C: intramuscular injection of 15 µg HA/strain of an influenza vaccine containing the same strains as in A and B. Viral antigens were grown in eggs from seed stocks by the supplier (SSD GmBH, Dresden, Germany). HAI and Ig Ab responses were measured in sera as described in example 1. Results are expressed as percentages of animals having experienced a 4-fold Ab rise upon boosting.

Previous experience with 0.5% polyoxyethylene-9-lauryl ether has demonstrated that this formulation is potent in the induction of anti-influenza systemic immune responses. However, as shown in the table 1, this level of adjuvanticity is significantly improved by the addition of additional non-ionic surfactants. Thus when polyoxyethylene-9-lauryl ether is supplemented with TWEEN80 and triton-X-100, this formulation is capable of boosting pre-established systemic Ig Ab responses as efficiently as the classical parenteral influenza vaccine.

The haemagluttination inhibition (HAI) response is was also measured (table 2), once again, the best intranasal formulation is Polyoxyethylene-9-lauryl ether supplemented with TWEEN80 and triton-X-100. This formulation was equally as immunogenic as the conventional parenteral vaccine.

Table 1, serum Ig responses in monkeys

| **Ig ELISA antibodies** | **4-fold Ab rise seroconversion (%) to:** | |
|---|---|---|
| **Group** | **A/Beijing/262/95** | **B/Harbin/7/94** |
| **A** | **0** | **0** |
| **B** | **100** | **100** |
| **C** | **75** | **75** |

Table 2, serum HAI titers in monkeys

| **HAI antibodies** | **4-fold Ab rise seroconversion (%) to:** | |
|---|---|---|
| **Group** | **A/Beijing/262/95** | **B/Harbin/7/94** |
| **A** | **0** | **0** |
| **B** | **20** | **0** |
| **C** | **25** | **0** |

**Example 3:** *A comparison of the immunogenicity of an intranasal split influenza vaccine formulated with laureth 9 with TWEEN80 and triton-X-100, with the immunogenicity of a licensed conventional parenteral vaccine (Fluarix™) in healthy adult subjects.*

An intranasal formulation of egg-derived split influenza antigens, formulated with laureth 9 + TWEEN80 and triton-X-100 (A) was evaluated and compared with Fluarix™/α-Rix® (B). The formulations contained three inactivated split virion antigens prepared from the WHO recommended strains of the 1998/1999 season. The device used for administration of the vaccines was the Accuspray™ intranasal syringe from Becton Dickinson. The device works on a similar basis to a conventional syringe, but has a special tip containing spiral channels which result in the production of a spray when even pressure is exerted on the plunger. 100µl of the formulation was sprayed in each nostril.

Composition of the formulation

The intranasal formulation (A) contained the following inactivated split virions:

1. 30$\mu$g HA A/beijing/262/95 (H1N1)
2. 30$\mu$g HA A/Sydney/5/97 (H3N2)
3. 30$\mu$g HA of B/Harbin/7/94

and phosphate buffered saline pH 7.4± 0.1, TWEEN80 0.1%, Triton-X-100 0.015% , sodium deoxycholate 0.0045% and thiomersal below 35µg/ml.

The volume of one dose was 200$\mu$l (100$\mu$l sub-doses for each nostril).

Formulation A was adjuvanted with laureth 9 to obtain a final concentration of 0.5% (w/v).

The comparator Fluarix™/α-Rix® (B) is SmithKlineBeecham Biologicals' commercial inactivated trivalent split influenza vaccine, which is administered intramuscularly in a dose of 500μl.

Immunogenicity Study

An open, controlled and randomised study evaluated the immunogenicity of an intranasal split influenza vaccine formulated with laureth 9 supplemented with TWEEN80 and triton-X-100 compared to the conventional parenteral vaccine (i.e. Fluarix™). Twenty healthy adult subjects (aged 18-40 years) received one dose of Fluarix™ and ten subjects received one dose (two sub-doses, one per nostril) of the intranasal influenza vaccine.

There was an eight-day follow-up period for solicited local and general symptoms and both vaccines were well-tolerated in relation to safety and reactogenicity. No serious adverse events related to vaccination were reported.

The immunogenicity of the vaccines was examined by assessing the serum haemagglutination inhibition (HI) titres to determine seroconversion rate (defined as the percentage of vaccinees who have at least a 4-fold increase in serum HI titres on day 21 compared to day 0, for each vaccine strain), conversion factor (defined as the fold increase in serum HI Geometric Mean Titres (GMTs) on day 21 compared to day 0, for each vaccine strain) and seroprotection rate (defined as the percentage of vaccinees with a serum HI titre ≥40 after vaccination (for each vaccine strain) that is accepted as indicating protection ). In addition, the mucosal IgA antibody response was assessed by Enzyme Linked Immunosorbent Assay (ELISA).

HI seropositivity, serconversion and seroprotection rates twenty-one days after one dose of Fluarix™ or the intranasal formulation can be seen in Table 3.

**Table 3:** HI seropositivity, serconversion and seroprotection rates at 21 days post dose 1:

| Strain | Group | Timing | N | Seropositivity | | Seroprotection | | Seroconversion | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | n | % | n | % | n | % |
| A/Beijing | Intranasal vaccine plus Laureth 9 | Day 0 | 20 | 5 | 25.0 | 1 | 5.0 | | |
| | | Day 21 | 20 | 19 | 95.0 | 10 | 50.0 | 15 | 75.0 |
| | Fluarix™ | Day 0 | 19 | 4 | 21.1 | 3 | 15.8 | | |
| | | Day 21 | 19 | 19 | 100.0 | 18 | 94.7 | 19 | 100.0 |
| A/Sydney | Intranasal vaccine plus Laureth-9 | Day 0 | 20 | 16 | 80.0 | 4 | 20.0 | | |
| | | Day 21 | 20 | 20 | 100.0 | 19 | 95.0 | 15 | 75.0 |
| | Fluarix™ | Day 0 | 19 | 14 | 73.7 | 1 | 5.3 | | |
| | | Day 21 | 19 | 19 | 100.0 | 18 | 94.7 | 16 | 84.2 |
| B/Harbin | Intranasal vaccine plus Laureth-9 | Day 0 | 20 | 18 | 90.0 | 11 | 55.0 | | |
| | | Day 21 | 20 | 20 | 100.0 | 19 | 95.0 | 12 | 60.0 |
| | Fluarix™ | Day 0 | 19 | 17 | 89.5 | 11 | 57.9 | | |
| | | Day 21 | 19 | 19 | 100.0 | 19 | 100.0 | 15 | 78.9 |

Seropositivity (n,%) : number and percentage of subjects with titer ≥ 10

Seroprotection (n,%) : number and percentage of subjects with titer ≥ 40

Seroconversion (n,%) : number and percentage of subjects with at least a 4-fold increase in titres from day 0 to day 21

The percentage of subjects with a two-fold or a four-fold increase in the specific/total mucosal IgA antibody ratio between day 21 and day 0 (1 dose) can be seen in Table 4.

**Table 4**: Percentages of subjects with a two-fold or a four-fold increase in the specific/total IgA ratio between day 21 and day 0 (1 dose).

| Strain | Group | N | 2 fold increase (%) | 4 fold increase (%) |
|---|---|---|---|---|
| **A/Beijing** | Laureth-9 | 20 | 50.0 | 20.0 |
| | Fluarix™ | 19 | 52.6 | 26.3 |
| **A/Sydney** | Laureth-9 | 20 | 55.0 | 25.0 |
| | Fluarix™ | 19 | 47.4 | 5.3 |
| **B/Harbin** | Laureth-9 | 20 | 15.0 | 10.0 |
| | Fluarix™ | 19 | 26.3 | 5.3 |

Summary

The immunogenicity results tabulated above show that the intranasal formulation produced similar levels of seropositivity, seroconversion and seroprotection to the conventional parenteral vaccine (Fluarix™) twenty-one days after one dose. The intranasal formulation generally produced a better mucosal IgA response after one dose than the conventional parenteral vaccine (Fluarix™).

**Example 4,** *Effect of Laureth-9 together with Triton X100 on the immunogenicity of an intranasal tetanus toxoid vaccine in primed mice*

In the present example, we evaluated the effect of adding Triton X100 to a low and sub-optimal dose of Laureth-9 on the intranasal boosting of tetanus toxoid (TT)-specific serum antibodies. Female balb/c mice were primed intra-muscularly with 20% (2x50 µl) of the human dose of the commercial DTPa vaccine (Diptheria, Tetanus, acellular Pertussis vaccine: INFANRIX™ SmithKline Beecham, Belgium). One month later the mice were boosted intranasally (5 µl in each nostril, under anesthesia) with 5 µg TT in A: PBS; B: 0.5% polyoxyethylene-9 lauryl ether; C: 0.1% polyoxyethylene-9 lauryl ether; D: 0.1% polyoxyethylene-9 lauryl ether + 0.02% Triton X100 or; E: by intramuscular injection of the DTPa vaccine (2×50 µl). Two weeks after the boosting the sera were analyzed for their TT-specific IgG.

As shown in the figure 1, Laureth-9 low dose (0.1%) was ineffective in enhancing the boosting response to TT, contrary to the 0.5% dose. However, the adjuvanticity of that formulation was strongly improved by supplementing it with Triton X100 ($p$<0.0001). The antibody response elicited was similar to the one induced by the commercial DTPa vaccine.

## Claims

1. An adjuvant composition comprising (a) polyoxyethylene alkyl ether or ester of general formula (I):

$HO(CH_2CH_2O)_n$-A-R

wherein, n is 1-50, A is a bond or -C(O)-, R is $C_{1-50}$ alkyl or Phenyl $C_{1-50}$ alkyl; and (b) at least one additional non-ionic surfactant.

2. An adjuvant composition as claimed in claim 1, wherein said additional non-ionic surfactant is an Octoxynol.

3. An adjuvant composition as claimed in claim 2, wherein said Octoxynol is t-octylphenoxypolyethoxyethanol (TRITON X100™).

4. An adjuvant composition as claimed in any one of claims 1 to 3, additionally comprising one or both of a polyoxyethylene sorbitan ester or cholic acid or derivative thereof.

5. An adjuvant composition as claimed in any one of claims 1 to 4, characterised in that the polyoxyethylene alkyl ether or ester of formula (I) is haemolytic.

6. An adjuvant composition as claimed in claim 5, characterised in that the degree of haemolytic activity of the polyoxyethylene alkyl ether or ester is in the range of 0.05-0.0001% as measured in the Guinea Pig blood haemolysis assay.

7. An adjuvant as claimed in claim 5 or claim 6, wherein the polyoxyethylene alkyl ether or ester of formula (I) has a haemolytic activity within a ten fold difference to that of polyoxyethylene-9 lauryl ether or polyoxyethylene-8 stearyl ether, as measured in the Guinea Pig blood haemolysis assay.

8. An adjuvant composition as claimed in any one of claims 1 to 7, comprising a polyoxyethylene alkyl ether or ester of formula (I), wherein *n* is 4 to 24.

9. An adjuvant composition as claimed in claim 8, wherein, *n* is 9.

10. An adjuvant composition as claimed in any one of claims 1 to 7, comprising a polyoxyethylene alkyl ether or ester of formula (I), wherein R is $C_{8-20}$ alkyl or Phenyl $C_{8-20}$ alkyl.

11. An adjuvant composition as claimed in claim 10, wherein R is $C_{12}$ alkyl.

12. An adjuvant composition as claimed in any one of claims 1 to 11, comprising a polyoxyethylene alkyl ether or ester of formula (I), wherein A is a bond, thereby forming an ether.

13. An adjuvant composition as claimed in any one of claims 1 to 12, comprising a polyoxyethylene alkyl ether or ester of formula (I), wherein A is -C(O)-, thereby forming an ester.

14. An adjuvant composition as claimed in any one of claims 1 to 13, wherein the polyoxyethylene ether or ester of formula (I) is selected from the group comprising: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-lauryl ester, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, polyoxyethylene-23-lauryl ether.

15. An adjuvant combination comprising polyoxyethylene-9 lauryl ether and t-octylphenoxypolyethoxyethanol (TRITON X100™).

16. An adjuvant composition as claimed in any one of claims 1 to 15, wherein the total concentration of the detergent present is in the range 0.001-10%.

17. An adjuvant composition as claimed in claim 16, wherein the total concentration of the detergent is in the range 0.001-1%.

18. An adjuvant composition as claimed in claim 17, wherein the total concentration of detergent is in the range of 0.001 to 0.7%.

19. An adjuvant combination, comprising an adjuvant as claimed in any one of claims 1 to 17, in combination with at least one additional immunostimulant.

20. An adjuvant combination as claimed in claim 19, wherein the at least one additional immunostimulant is selected from the group comprising: LT, CT, 3D-MPL, CpG, and QS21.

21. An adjuvant composition as claimed in claim 20, wherein the CpG adjuvant is: TCC ATG ACG TTC CTG ACG TT (SEQ ID NO. 1).

22. An adjuvant combination comprising polyoxyethylene-9 lauryl ether, t-octylphenoxypolyethoxyethanol (TRITON X100™), and 3D-MPL.

23. A vaccine comprising an adjuvant as claimed in any one of claims 1 to 22, further comprising an antigen.

24. A vaccine as claimed in claim 23, wherein said antigen is selected from the group comprising: Human Immunodeficiency Virus, Varicella Zoster virus, Herpes Simplex Virus type 1, Herpes Simplex virus type 2, Human cytomegalovirus, Dengue virus, Hepatitis A, B, C or E, Respiratory Syncytial virus, human papilloma virus, Influenza virus, Hib, Meningitis virus, Salmonella, Neisseria, Borrelia, Chlamydia, Bordetella, Streptococcus, Mycoplasma, Mycobacteria, Haemophilus, Plasmodium or Toxoplasma, stanworth decapeptide; or Tumour associated antigens (TMA), MAGE, BAGE, GAGE, MUC-1, Her-2 neu, LnRH, CEA, PSA, KSA, or PRAME.

25. A vaccine as claimed in claim 24, wherein said antigen in an antigen or antigenic preparation from Influenza virus.

26. A vaccine composition comprising polyoxyethylene-9 lauryl ether, t-octylphenoxypolyethoxyethanol (TRITON X100™) and an influenza virus antigen.

27. A vaccine as claimed in any one of claims 23 to 26, wherein the vaccine is in the form of an aerosol or spray.

28. A vaccine as claimed in any one of claims 23 to 27, for use in medicine.

29. Use of an adjuvant composition as claimed in any one of claims 1 to 22, in the manufacture of a medicament for application onto a mucosal surface or the skin of a patient.

30. Use of a combination of polyoxyethylene-9 lauryl ether and t-octylphenoxypolyethoxyethanol (TRITON X100™) in the manufacture of a vaccine for application onto a mucosal surface of a patient.

31. A spray device, more particularly a bi-dose spray device, filled with a vaccine, as claimed in any one of claims 23 to 27.

32. Use of vaccine composition as defined in any of claims 23 to 27, for the manufacture of a vaccine for the treatment of viral, bacterial, parasitic infections, allergy, or cancer.

33. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the administration of a safe and effective amount of a vaccine composition according to any of claims 23 to 27 to the mammal.

34. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the mucosal administration of a safe and effective amount of a vaccine composition according to any of claims 23 to 27.

35. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, comprising the intranasal administration of a safe and effective amount of a vaccine composition according to any of claims 23 to 27.

36. A process for making a vaccine composition according to any one of claims 23 to 27, comprising admixing (a) an adjuvant composition as claimed in any one of claims 1 to 22, (b) a pharmaceutically acceptable excipient, and (c) an antigen or antigenic composition.

FIG. 1, *Serum IgG to TT in balb/c mice*

