Vaccines

The present invention relates to a novel adjuvant system comprising a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol. The present invention provides said novel adjuvants, vaccines comprising them, and methods for their manufacture and for 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. The adjuvants are particularly useful as mucosal adjuvants, but are also effective systemically. The adjuvants are especially useful in the context of influenza vaccines.

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 in 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 adjuvants which are suitable for use in humans, remain 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 sorbitan esters, polyoxyethylene ethers, bile salts, and other permeation enhancers, 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).

Other non-ionic surfactant formulations have been utilised. 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 is 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). In the examples shown in WO 94/17827, (in particular example 4) the concentration of TWEEN20™ that is required to enhance the immune response is very high (36%), whereas at 28% even in the presence of the caprylic/capric acid glyceride no enhancement of the immune response occurs.

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 entrap antigen within the inner aqueous phase or within the bilayer itself.

WO 96/36352 and US 5,653,987 describe 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, WO 95/17210).

Influenza virus vaccines have been prepared in the past by the use of Triton X-100 or a mixture of Tween and ether to split influenza virus. A clinical comparison of the systemic immunogenicity of the two splits shows that they are comparable (Gross et al. 1981. J. Clin Microbiol 14, 534-8). Other surfactants have also been investigated for their effect on the immunogenicity of the resulting split vaccine. In a comparative

study of parenteral administration Mukhlis et al. (1984 Vaccine 2, 199-203) showed that whole virus was more immunogenic than detergent disrupted virus, but that between different detergents Triton X-100 and cetyl trimethyl ammonium bromide (CTAB) gave marginally more immunogenic splits than the detergent empigen.

The applicant presents here the surprising finding that polyoxyethylene sorbitan esters in combination with an octoxynol together act as a potent adjuvant for vaccines. Advantageously, such compositions may be administered systemically, but are sufficient to induce systemic immune responses when administered mucosally. The immune responses induced by mucosal administration of vaccines of the present invention may be at least as high as or at least comparable to those observed after a systemic injection of conventional vaccine.

The present invention provides safe and potent adjuvants which are easy to manufacture, which may be administered either through mucosal or systemic routes.

In a first aspect the invention provides an adjuvant which comprises a polyoxyethylene sorbitan ester and an octoxynol.

In another aspect the invention provides a vaccine comprising an adjuvant according to the invention, together with an antigen.

Particularly preferred is a vaccine composition comprising an adjuvant according to the invention together with influenza virus antigen for administration to a mucosal surface, in particularly to the nasal mucosa. However, there are alternative routes of administration and other possible antigens for use in a vaccine according to the invention which will be described below.

Octoxynols and polyoxyethylene sorbitan esters are described in "Surfactant systems" Eds: Attwood and Florence (1983, Chapman and Hall). The octoxynol series, including t-octylphenoxypolyethoxyethanol (TRITON X-100™) is also described in Merck Index Entry 6858 (Page 1162, 12th Edition, Merck & Co. Inc., Whitehouse Station, N.J., USA; ISBN 0911910-12-3). 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 may be manufactured using methods described therein, or purchased from commercial sources such as Sigma Inc.

Preferred octoxynols for use in the adjuvants according to the invention include other non-ionic surfactants from the Triton series, such as Triton X-45, Triton X-102, Triton X-114, Triton X-165, Triton X-205, Triton X-305, Triton N-57, Triton N-101 and Triton N-128, but t-octylphenoxypolyethoxyethanol (Triton X-100) is particularly preferred.

The adjuvants of the present invention comprise a polyoxyethylene sorbitan ester and an octoxynol. Preferably the octoxynol is t-octylphenoxypolyethoxyethanol (TRITON –X-100™). Preferably the polyoxyethylene sorbitan ester is polyoxyethylene sorbitan monooleate (TWEEN80™).

The adjuvant according to the invention may advantageously further comprise a bile salt or a cholic acid derivative.

Accordingly the adjuvant may comprise a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80), an octoxynol such as t-octylphenoxy polyethoxyethanol (Triton X-100) and a bile salt or cholic acid derivative such as sodium deoxycholate or taurodeoxycholate. In a preferred embodiment, the invention provides an adjuvant formulation comprising polyoxyethylene sorbitan monooleate (Tween 80), t-octylphenoxy polyethoxyethanol (Triton X-100) and sodium deoxycholate.

Preferably, the total concentration of non-ionic surfactants present in the adjuvant formulation is less than 40%, more preferably up to about 20%. A preferred range is between about 0.001% to 20%, more preferably 0.01 to 10% and most preferably up to about 2% (w/v).

The individual non-ionic surfactants have preferred concentrations in the final vaccine composition as follows: octyl-or nonylphenoxy polyethoxyethanols 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); polyoxyethylene sorbitan esters such as Tween 80: 0.01 to 1%, most preferably about 0.0% (w/v).

Particularly prefered ranges for the concentrations of the non-ionic surfactants are:
Tween 80™: 0.01 to 1%, most preferably about 0.1% (v/v);
Triton X-100™: 0.001 to 0.1, most preferably 0.005 to 0.02 % (w/v).

One aspect of the present invention is a vaccine formulation comprising a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol, wherein the antigen present in the vaccine is not entrapped within a non-ionic surfactant vesicle.

Inflluenza virus antigens for use in the vaccine according to the invention may be any form of influenza antigens suitable for raising an immune response, including live or inactivated whole virus, split virus, or subunit antigens prepared from whole virus or by recombinant means. Influenza virus for production of the antigen may be grown in embryonated eggs in a conventional process or the virus may be grown in tissue culture. Suitable cell substrates for tissue culture of influenza include for example dog kidney cells such as MDCK cells, cells from a clone of MDCK, or 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 also 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.

Preferred is an influenza virus antigen preparation which comprises split virus which has undergone a series of purification steps. Thus the antigen preparation may be produced by a number of different commercially applicable processes, for example the split flu process described in patent no. DD 300 833 and DD 211 444, incorporated herein by reference. Commercially available split influenza vaccine includes Fluarix™ which is sold by SmithKline Beecham.

Accordingly a preferred vaccine formulation according to the invention comprises egg or tissue culture derived influenza antigen, preferably split influenza antigen, together with a polyoxyethylene sorbitan ester and an octoxynol, optionally further comprising a bile salt or derivative of cholic acid. Most preferably such a formulation comprises split influenza virus antigen, polyoxyethylene sorbitan monooleate (Tween 80), t-octylphenoxypolyethoxyethanol (Triton X-100) and sodium deoxycholate.

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 vaccines 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.

The vaccine preparations of the present invention are preferably 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 the preferred, intranasal route of 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 consistent 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, EP 311 863 B and EP 516 636 B, incorporated herein by reference. 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. Generally the two sub-doses are in a single chamber and the construction of the device allows the efficient delivery of a single sub-dose at a time.

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. In a preferred embodiment of this aspect of the invention, the intranasal administration device is filled with an influenza vaccine.

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 a derivative thereof, particular in the form of a salt. These include derivatives of cholic acid and salts thereof, in particular sodium salts of cholic acid or cholic acid derivatives. Examples of bile acids and derivatives thereof include cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid, ursodeoxycholic acid, hyodeoxycholic acid and derivatives such as glyco-, tauro-,

amidopropyl-1-propanesulfonic-, amidopropyl-2-hydroxy-1-propanesulfonic derivatives of the aforementioned bile acids, or N,N-bis (3Dgluconoamidopropyl) deoxycholamide. A particularly preferred example is sodium deoxycholate (NaDOC) which may be present in the final vaccine dose.

Preferably, the adjuvant formulations 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 easy to administer to the nasal mucosa in the form of a spray without degradation of the complex physical structure of the adjuvant.

In a further aspect of the present invention, there is provided a method of preparing a vaccine which method comprises admixing an adjuvant according to the invention with an antigen.

In a still further aspect, there is provided a method of inducing or enhancing an immune response in a subject, comprising admixing the antigen and the adjuvant according to the invention, and administering said admixture to the subject.

Preferably, the route of administration to the subject is via a mucosal surface and more preferably via the nasal mucosa. When the vaccine is administered via the nasal mucosa, the vaccine is preferably administered as a spray. In a preferred method of inducing or enhancing an immune response, a systemic response is induced by a nasal administration of the vaccine. Thus, a mucosal vaccine according to the invention is preferably capable of inducing a systemic immune response when administered via a mucosal route.

The present invention further provides the use of a polyoxyethylene sorbitan ester, and an octoxynol in the manufacture of an adjuvant formulation, in particular an adjuvant formulation for application to the mucosa of a patient. The present invention also relates to the use of a polyoxyethylene sorbitan ester, an octoxynol and an antigen, in the manufacture of a vaccine formulation, especially a vaccine formulation for application to the mucosa. Preferably the antigen is influenza virus antigen.

Particularly preferred are adjuvants and vaccines for administration to the nasal mucosa.

Preferably the administering of a vaccine according to the invention comprises the administration of a priming or a boosting dose of the vaccine, such as a priming or a boosting dose of influenza vaccine comprising an influenza antigen preparation.

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 *Moraxella 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 particular 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 proteins 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 protein D(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 may 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. Its 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 alternatively contain an anti-tumour antigen and be useful for the immunotherapeutic treatment of 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 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.

The vaccines of the present invention may also be administered via the oral route. In such cases the pharmaceutically acceptable excipient may also include antacid

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.

In an alternative, related embodiment of the present invention the adjuvant of the present invention may further be combined with other adjuvants including Cholera toxin and its B subunit, Monophosphoryl Lipid A and its non-toxic derivative 3-de-O-acylated monophosphoryl lipid A (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).

The present invention is illustrated by, but not limited to, the following examples. In the examples below we used whole egg-grown flu virus inactivated with formaldehyde, or TWEEN-ether split virus or NaDOC split egg-grown virus supplemented with Triton X-100. The concentrations of the Tween-80 and Triton X-100 are shown in the examples.

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

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

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 µl 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 µl/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 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 is stopped by adding 50 µl/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 mice

Sera (25 µl) are first treated for 20 minutes at room temperature (RT) with 100 µl borate 0.5M buffer (pH 9) and 125 µl Dade Behring-purchased kaolin. After centrifugation (30 minutes, 3000 RPM or 860 g), 100 µl supernatant (corresponding to a 1/10 dilution of the serum) are taken and incubated for 1 hour at 4°C with 0.5% chicken red blood cells. Supernatant is collected after centrifugation for 10 minutes at 3200 RPM (970 g). Both operations are done for eliminating the natural hemagglutinating factors contained in the sera. Then, 25 µl treated-sera are diluted in 25 µl PBS (serial 2-fold dilutions starting at 1/20) 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 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.

Example2. *Effect of TWEEN80 and Triton on the intranasal immunogenicity of inactivated whole influenza virus in mice*

In the past, the pre-clinical evaluations of alternative influenza vaccines (e.g. adjuvanted parenteral vaccines, DNA-based vaccines or mucosally delivered vaccines) have mainly been performed in naïve animals. In general, the promising results obtained from these studies were not confirmed in humans. This was probably due to the fact that the majority of adults have been immunologically "primed" through natural infections before vaccination, unlike the naïve animals. Therefore, the best way to evaluate intranasal influenza vaccines in animal models would be to test their ability to boost pre-established immune responses in nasally primed animals. We assess in the present example the effect of TWEEN-80 and Triton X-100 on such responses.

The priming was done in female Balb/c mice (8 weeks old) at day 0 by administering with a pipette (under anesthesia) in each nostril 2.5 µg HA of BPL-inactivated A/Beijing/262/95 influenza virus contained in 10 µl PBS. After 28 days, mice (6 animals/group) were boosted intranasally (under anesthesia) with 20 µl of solution (10 µl per nostril, delivered as droplets by pipette) containing 5 µg HA of BPL-inactivated A/Beijing/262/95 influenza virus in either A: PBS; B: TWEEN80 (0.11%) plus Triton X-100 (0.074%) ; or by C: intramuscular injection of 1.5 µg HA of influenza vaccine. Antigens were supplied by SSD GmBH manufacturer (Dresden, Germany). HAI Ab responses were measured in sera as described in Example 1. As shown in the Figure 1, when formulated with TWEEN80 and Triton, inactivated influenza virus delivered intranasally is capable of boosting pre-established systemic HAI Ab responses as efficiently as the classical parenteral influenza vaccine. However, the same antigen given intranasally in the absence of TWEEN80 and Triton is significantly less immunogenic.

Example 3: *A comparison of the immunogenicity of an intranasal split influenza vaccine with TWEEN 80 & TRITON X-100 compared to a licensed conventional parenteral vaccine (*Fluarix™*) in healthy adult subjects.*

Formulations used in the study

Two formulations (A,B) of egg-derived split influenza antigens have been evaluated. A is an intranasal formulation and B is the Fluarix™/α-Rix® given intramuscularly. The formulations contain three inactivated split virion antigens prepared from the WHO recommended strains of the 1998/1999 season.

The device used for intranasal delivery is the Accuspray™ intranasal syringes from Becton Dickinson. 100µl of the A formulation is sprayed in each nostril.

Composition of the formulations.

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

1. 30µg HA A/Beijing/262/95 (H1N1)
2. 30µg HA A/Sydney/5/97 (H3N2)
3. 30µg HA of B/Harbin/7/94

and phosphate buffered saline pH 7.4± 0.1, Tween 80 0.1%, Triton X-100 0.015%, Na deoxycholate 0.0045% and thiomersal below 35µg/ml.

The volume of one dose is 200µl (100µl sub-doses for each nostril).

The comparator Fluarix™/α-Rix® is the SmithKline Beecham Biologicals' commercial inactivated trivalent split influenza vaccine. The dose of 500µl is administered intramuscularly.

This dose contains;

15µg HA of the three strains mentioned above, Tween 80 between 500 and 1000 µg per ml (0.05%-0.1%), Triton X-100 between 50 and 170µg/ml (0.005%-0.017%), sodium deoxycholate maximum 100µg/ml, thiomersal 100µg/ml and phosphate buffered saline pH between 6.8 and 7.5.

Immunogenicity Study

An open, controlled and randomised study evaluated the immunogenicity of an intranasal split influenza vaccine formulated with Tween 80 & 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 of the intranasal influenza vaccine. The intranasal formulation (200µl) contained the following inactivated virions: 30µg of haemagglutinin A/Beijing/262/95 (H1N1), 30µg of haemagglutinin A/Sydney/5/97 (H3N2), 30µg of haemagglutinin B/Harbin/7/94 with phosphate buffered saline (pH 7.4 ± 0.1), Tween 80 (0.1%), Triton X-100 (0.015%), sodium deoxycholate (0.0045%) and thiomersal (<35µg/ml).

There was an eight-day follow-up period for solicited local and general symptoms and both vaccines were well-tolerated regarding 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 the 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). Generally, an influenza vaccine needs to have > or equal to 40% seroconversion rate, > or equal to 70% seroprotection rate and a conversion factor of > or equal to 2.5, for each strain, in order to meet the international regulatory requirements. This applies for adults between 18-60 years; different criteria apply for the elderly.

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 1.

Table 1:

HI seropositivity, seronversion and seroprotection rates at 21 days post dose 1

Strain	Group	Timing	N	Seropositivity		Seroprotection		Seroconversion	
				n	%	n	%	n	%
A/Beijing	Intranasal vaccine plus Tween 80 & Titron X100	Day 0	20	4	20.0	0	0.0		
		Day 21	20	17	85.0	15	75.0	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 Tween 80 & Titron X100	Day 0	20	13	65.0	3	15.0		
		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 Tween 80 & Titron X100	Day 0	20	10	50.0	7	35.0		
		Day 21	20	20	100.0	18	90.0	14	70.0
	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

In all cases, the conversion factor (fold increase in serum HI GMTs after vaccination) was greater than 2.5, the level required for a successful influenza vaccine.

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 2.

Table 2:

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	Tween & Triton	20	55.0	30.0
	Fluarix™	19	52.6	26.3
A/Sydney	Tween & Triton	20	65.0	45.0
	Fluarix™	19	47.4	5.3
B/Harbin	Tween & Triton	20	40.0	30.0
	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 those produced by the conventional parenteral vaccine (Fluarix™) twenty-one days after one dose. The intranasal formulation produced a better mucosal IgA response after one dose than the conventional parenteral vaccine (Fluarix™).

CLAIMS

1. The use of a combination of a polyoxyethylene sorbitan ester and an octoxynol in the preparation of an adjuvant for application to a mucosal surface of a patient

2. The use according to claim 1, wherein said polyoxyethylene sorbitan ester is polyoxyethylene sorbitan monooleate (TWEEN80™).

3. The use according to claim 1 or claim 2, wherein said octoxynol is t-octylphenoxypolyethoxyethanol (TRITON X-100™).

4. The use according to any one of claims 1 to 3, further comprising a bile salt or a cholic acid derivative.

5. The use of an adjuvant according to any one of claims 1 to 4, together with an antigen, in the manufacture of a vaccine for mucosal administration.

6. The use according to claim 5 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.

7. The use according to claim 6, wherein said antigen is an antigen or antigenic preparation from influenza virus.

8. The use according to claim 7, wherein the antigenic preparation is a split influenza virus preparation.

9. The use according to claim 7 or claim 8, for the manufacture of a vaccine for prophylaxis against influenza.

10. The use according to any one of claims 5 to 9, for the manufacture of a vaccine for use in medicine.

11. A method of producing a vaccine which method comprises admixing (a) a polyoxyethylene sorbitan ester, (b) an octoxynol and (c) an antigen, and providing the vaccine in the form of a vaccine dose for mucosal administration.

12. The method according to claim 11, wherein the vaccine is provided in an intranasal aerosol or spray device.

13. A spray or aerosol device, more particularly a bi-dose device, filled with a

vaccine comprising a polyoxyethylene sorbitan ester, an octoxynol and an antigen.

14. The spray or aerosol device according to claim 13, wherein the antigen is an influenza antigen or antigenic preparation.

15. The spray or aerosol device according to claim 14, wherein the antigenic preparation is a split influenza virus preparation.

16. A method of treating a mammal suffering from or susceptible to a pathogenic infection, or cancer, or allergy, which method comprises administering to the mucosa of the mammal a safe and effective amount of a vaccine composition comprising a polyoxyethylene sorbitan ester, an octoxynol and an antigen.

17. The method according to claim 16 wherein the vaccine is administered intranasally.

18. The method according to claim 16 or claim 17 wherein the vaccine is an influenza virus vaccine comprising an influenza antigen or antigenic preparation, such as a split influenza virus preparation.