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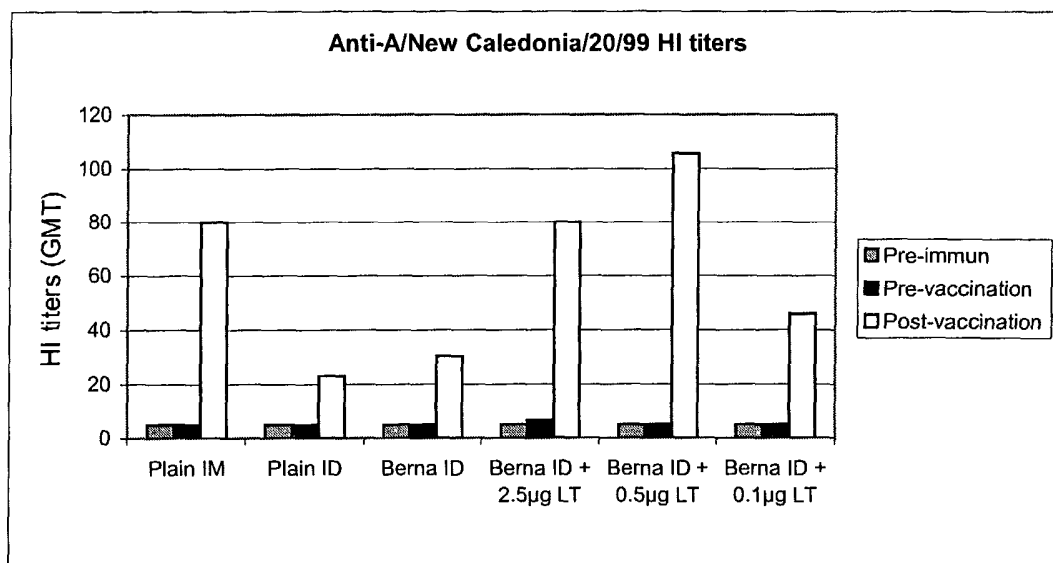
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(54) Title: INTRADERMAL INFLUENZA VACCINE COMPOSITIONS CONTAINING AN ADP-RIBOSYLATING FACTOR



(57) Abstract: The invention relates to an intradermal influenza vaccine comprising an influenza antigen and an ADP-ribosylating toxin or a functional derivative thereof, along with kits comprising an intradermal influenza vaccine, an ADP-ribosylating toxin or a functional derivative thereof and a delivery means for said toxin or derivative.

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INTRADERMAL INFLUENZA VACCINE COMPOSITIONS CONTAINING AN ADP-RIBOSYLATING FACTOR

This invention relates to influenza vaccine formulations for intradermal delivery, methods for preparing them and their use in prophylaxis or therapy.

5

Influenza virus is one of the most ubiquitous viruses present in the world, affecting both humans and livestock. The economic impact of influenza is significant.

The influenza virus is an RNA enveloped virus with a particle size of about 125 nm in diameter. It consists basically of an internal nucleocapsid or core of ribonucleic acid (RNA) associated with nucleoprotein, surrounded by a viral envelope with a lipid bilayer structure and external glycoproteins. The inner layer of the viral envelope is composed predominantly of matrix proteins and the outer layer mostly of the host-derived lipid material. The surface glycoproteins neuraminidase (NA) and haemagglutinin (HA) appear as spikes, 10 to 12 nm long, at the surface of the particles. It is these surface proteins, particularly the haemagglutinin, that determine the antigenic specificity of the influenza subtypes.

Typical influenza epidemics cause increases in incidence of pneumonia and lower respiratory disease as witnessed by increased rates of hospitalisation or mortality. The elderly or those with underlying chronic diseases are most likely to experience such complications, but young infants also may suffer severe disease. These groups in particular therefore need to be protected.

Currently available influenza vaccines are either inactivated or live attenuated influenza vaccines. Inactivated flu vaccines comprise one of three types of antigen preparation: inactivated whole virus, sub-virions where purified virus particles are disrupted with detergents or other reagents to solubilise the lipid envelope (so-called "split" vaccine) or purified HA and NA (subunit vaccine). These inactivated vaccines are generally given intramuscularly (i.m.).

Influenza vaccines, of all kinds, are usually trivalent vaccines. They generally contain antigens derived from two influenza A virus strains and one influenza B strain. A standard 0.5 ml injectable dose in most cases contains 15 µg of haemagglutinin

antigen component from each strain, as measured by single radial immunodiffusion (SRD) (J.M. Wood et al.: An improved single radial immunodiffusion technique for the assay of influenza haemagglutinin antigen: adaptation for potency determination of inactivated whole virus and subunit vaccines. J. Biol. Stand. 5 (1977) 237-247; J. M. Wood et al., International collaborative study of single radial diffusion and immunoelectrophoresis techniques for the assay of haemagglutinin antigen of influenza virus. J. Biol. Stand. 9 (1981) 317-330).

In certain circumstances, such as the occurrence of a pandemic influenza strain, it may be desirable to have a vaccine which contains only the single strain. This will help the speed of response to a pandemic situation.

The influenza virus strains to be incorporated into influenza vaccine each season are determined by the World Health Organisation in collaboration with national health authorities and vaccine manufacturers.

Current efforts to control the morbidity and mortality associated with yearly epidemics of influenza are based on the use of intramuscularly administered inactivated influenza vaccines. The efficacy of such vaccines in preventing respiratory disease and influenza complications ranges from 75% in healthy adults to less than 50% in the elderly.

It would be desirable to provide an alternative way of administering influenza vaccines, in particular a way that is pain-free or less painful than i.m. injection, and does not involve the associated negative affect on patient compliance because of "needle fear". It would also be desirable to target the cell mediated immune system for example by targeting the antigen to the dendritic cells that reside in the skin, particularly in the dermis. Cell mediated immunity appears to assist viral clearance and recovery from illness and may provide better cross protection between influenza strains than antibodies. It has also been described in the literature that intradermal administration allows for the induction of a mucosal immunity at the level of the mucosal surfaces. This offers a benefit compared to the parenteral route for a vaccine against a pathogen such as influenza where the portal of entry of the virus is through

the nasal route. Thus the mucosal surfaces, initially in the upper respiratory tract, offer the first line of defence.

Furthermore, it would be desirable to reduce the amount of antigen needed for a dose of influenza vaccine. Influenza vaccines are often in short supply.

Thus, the commercially available influenza vaccines remain the intramuscularly administered split or subunit injectable vaccines. These vaccines are prepared by disrupting the virus particle, generally with an organic solvent or a detergent, and separating or purifying the viral proteins to varying extents. Split vaccines are prepared by fragmentation of whole influenza virus, either infectious or inactivated, with solubilizing concentrations of organic solvents or detergents and subsequent removal of the solubilizing agent and some or most of the viral lipid material. Split vaccines generally contain contaminating matrix protein and nucleoprotein and sometimes lipid, as well as the membrane envelope proteins. Split vaccines will usually contain most or all of the virus structural proteins although not necessarily in the same proportions as they occur in the whole virus. Subunit vaccines on the other hand consist essentially of highly purified viral surface proteins, haemagglutinin and neuraminidase, which are the surface proteins responsible for eliciting the desired virus neutralising antibodies upon vaccination. Matrix and nucleoproteins are either not detectable or barely detectable in subunit vaccines.

Standards are applied internationally to measure the efficacy of influenza vaccines. The European Union official criteria for an effective vaccine against influenza are set out in the table below. Theoretically, to meet the European Union requirements, an influenza vaccine has to meet only one of the criteria in the table, for all strains of influenza included in the vaccine. However in practice, at least two or more probably all three of the criteria will need to be met for all strains, particularly for a new vaccine such as a new intradermal vaccine. Under some circumstances two criteria may be sufficient. For example, it may be acceptable for two of the three criteria to be met by all strains while the third criterion is met by some but not all strains (e.g. two out of three strains). The requirements are different for adult populations (18-60 years) and elderly populations (>60 years).

	18 – 60 years	> 60 years
Seroconversion rate*	>40%	>30%
Conversion factor**	>2.5	>2.0
Protection rate***	>70%	>60%

* Seroconversion rate is defined as the percentage of vaccinees who have at least a 4-fold increase in serum haemagglutinin inhibition (HI) titres after vaccination, for each vaccine strain.

** Conversion factor is defined as the fold increase in serum HI geometric mean titres (GMTs) after vaccination, for each vaccine strain.

*** Protection rate is defined as the percentage of vaccinees with a serum HI titre equal to or greater than 1:40 after vaccination (for each vaccine strain) and is normally accepted as indicating protection.

For an intradermal flu vaccine to be commercially useful it will not only need to meet those standards, but also in practice it will need to be at least as efficacious as the currently available intramuscular vaccines. It will also need to be produced by an acceptable process and will of course need to be commercially viable in terms of the amount of antigen and the number of administrations required. Furthermore, it will need to be administered using a procedure which is reliable and straightforward for medical staff to carry out.

20

Although intradermal flu vaccines based on inactivated virus have been studied in previous years, the fact that no intradermal flu vaccine is currently on the market reflects the difficulty to achieve effective vaccination via this route.

25 The present invention sets out to address the need for an intradermal flu vaccine.

In a first aspect the invention provides an intradermal influenza vaccine comprising an influenza antigen and an ADP-ribosylating toxin or a functional derivative thereof.

The invention also relates to a method for preparing an intradermal influenza vaccine comprising combining an influenza antigen with an ADP-ribosylating toxin or a functional derivative thereof.

5 The present invention also relates to a method for the prophylaxis of influenza infection or disease in an individual which method comprises administering to the individual intradermally an influenza vaccine comprising both an influenza antigen and an ADP-ribosylating toxin or a functional derivative thereof.

10 The present invention further relates to use of an influenza antigen and an ADP-ribosylating toxin or a functional derivative thereof in the preparation of an intradermal vaccine for the prophylaxis of influenza infection or disease.

The invention further relates to the use of an ADP-ribosylating toxin or a functional
15 derivative thereof, in the adjuvantation of an intradermal influenza vaccine.

The present invention also relates to a method for the prophylaxis of influenza infection or disease in a subject which method comprises sequential administration or co-administration of an intradermal influenza vaccine and an ADP-ribosylating toxin
20 or a functional derivative thereof.

The present invention also relates to an intradermal delivery device containing an influenza vaccine in combination with an ADP-ribosylating toxin or a functional
25 derivative thereof.

The present invention also relates to a kit comprising an influenza vaccine, suitably comprised within an intradermal delivery device, and a delivery means for an ADP-ribosylating toxin or a functional derivative thereof.

30 As used herein, the term "intradermal delivery" means delivery of the vaccine to the region of the dermis in the skin. However, the vaccine will not necessarily be located exclusively in the dermis. The dermis is the layer in the skin located between about 1.0 and about 2.0 mm from the surface in human skin, but there is a certain amount of variation between individuals and in different parts of the body. In general, it can be

expected to reach the dermis by going 1.5 mm below the surface of the skin. The dermis is located between the stratum corneum and the epidermis at the surface and the subcutaneous layer below. Depending on the mode of delivery, the vaccine may ultimately be located solely or primarily within the dermis, or it may ultimately be distributed within the epidermis and the dermis.

Preferably the vaccines according to the invention are administered to a location between about 1.0 and 2.0 mm below the surface of the skin. More preferably the vaccine is delivered to a distance of about 1.5 mm below the surface of the skin.

10

The influenza antigen can be in the form of a whole live or inactivated virus, split influenza virus (grown in any suitable substrate such as eggs or cells such as MDCK cells, for example), whole flu virosomes (for example, as described by R. Gluck, Vaccine, 1992, 10, 915-920, and Stegmann et al 1987, EMBO Journal 6, 2651 – 2659, the whole contents of which are hereby incorporated) or purified or recombinant proteins thereof, such as HA, NP, NA, or M proteins, or combinations thereof. Virosomes are especially preferred.

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 according to the invention suitably meets some or all of the EU criteria for influenza vaccines as set out hereinabove, such that the vaccine is approvable in Europe. Preferably, at least two out of the three EU criteria are met, for the or all strains of influenza represented in the vaccine. More preferably, at least two criteria are met for all strains and the third criterion is met by all strains or at least by all but one of the strains. Most preferably, all strains present meet all three of the criteria.

The vaccine according to the invention suitably has a lower quantity of haemagglutinin than conventional vaccines and is administered in a lower volume. Preferably the quantity of haemagglutinin per strain of influenza is about 1-7.5 µg or 1-5 µg, more preferably approximately 3 µg or approximately 5 µg, which is about one fifth or one third, respectively, of the dose of haemagglutinin used in conventional vaccines for intramuscular administration. 6 µg of haemagglutinin per strain of influenza is also strongly preferred, thus 2-6.5 µg is also a preferred range.

Preferably the volume of a dose of vaccine according to the invention is between 0.025 ml and 2.5 ml, more preferably approximately 0.1 ml or approximately 0.2 ml. A 50 µl dose volume might also be considered. A 0.1 ml dose is approximately one fifth of the volume of a conventional intramuscular flu vaccine dose. The volume of liquid that can be administered intradermally depends in part upon the site of the injection. For example, for an injection in the deltoid region, 0.1 ml is the maximum preferred volume whereas in the lumbar region a large volume e.g. about 0.2 ml can be given.

The present invention combines delivery of an intradermal influenza vaccine with an ADP-ribosylating toxin or a functional derivative thereof, wherein the toxin serves to adjuvant the effect of the influenza antigen(s). The term 'functional derivative' as used herein refers to the adjuvant activity of the ADP-ribosylating toxin, and suitable toxin functional derivatives have an adjuvant activity for intradermal influenza vaccines.

The ADP-ribosylating toxin of the invention is preferably the *E. Coli* heat labile enterotoxin (LT), cholera toxin (CT) or a functional derivative thereof.

Both CT and LT are heterodimers consisting of a pentameric ring of -subunits, cradling a toxic A subunit. Their structure and biological activity are disclosed in Clements and Finklestein, 1979, *Infection and Immunity*, 24:760-769; Clements *et al.*, 1980, *Infection and Immunity*, 24:91-97.

Non toxic derivatives of ADP-ribosylating toxins, such as non-toxic LT and CT derivatives are preferred.

Non-toxic derivatives of LT have been developed which lack the proteolytic site required to enable the non-toxic form of LT to be “switched on” into its toxic form, once released from the cell. One form of LT (termed mLT(R192G)) is rendered resistant to proteolytic cleavage by a substitution of the amino acid arginine with glycine at position 192, and has been shown to have a greatly reduced toxicity whilst retaining its potent adjuvant activity. mLT(R192G) is, therefore, termed a proteolytic site mutant. Methods for the manufacture of mLT(R192G) are disclosed in the patent application WO 96/06627. Other derivatives of LT include the active site mutants such as mLT(A69G) which contain a substitution of an glycine for an alanine in position 69 of the LTA sequence, and the LTK63 (serine to lysine at position 63) and LTR72 (alanine to arginine) mutants (see Pizza et al. Vaccine, 19, 2534 – 2541, 2001). The use of mLT(R192G) as a mucosal vaccine is described in patent application WO 96/06627.

Also preferred is LTII, including LTIIA and LTIIB (as disclosed in Pickett, CL, Twiddy EM, Coker C, Holmes RK. 1989. Cloning, nucleotide sequence, and hybridization studies of the type IIb heat-labile enterotoxin gene of Eschericia coli. J. Bacteriol. 171:4945-52; and Martin, M, Metzger DJ, Michalek SM, Connell TD, and Russell MW. 2000. Comparative analysis of the mucosal adjuvanticity of the type II heat-labile enterotoxins LT-IIa and LT-IIb. Infect. Immun. 68:281-7)

Suitable functional derivatives of derivatives of LT and CT are those which retain adjuvant activity, and include the LT B subunit LTB and detoxified versions of LT such as mLT discussed above. Preferred LT derivatives include derivatives having the mutations discussed above, either alone or in combination. Generally preferred are LT mutants that have a proteolytic site and/or an active site mutation.

Preferably the LT derivative is sufficiently detoxified to be suitable for use in humans.

Suitable safety levels for toxicity, for example for LT derivatives, may be assessed using the criteria as outlined in Tamura et al. (Jpn. J. Infect. Disease, 53, 98 – 106, 2000).

- 5 Reference to ADP-ribosylating toxins herein is taken to refer to all suitable functional derivatives of ADP-ribosylating toxins unless otherwise apparent from the context.

Identification of toxin derivatives with suitable adjuvant activity can be assayed using standard techniques in suitable models, such as mouse models for example.

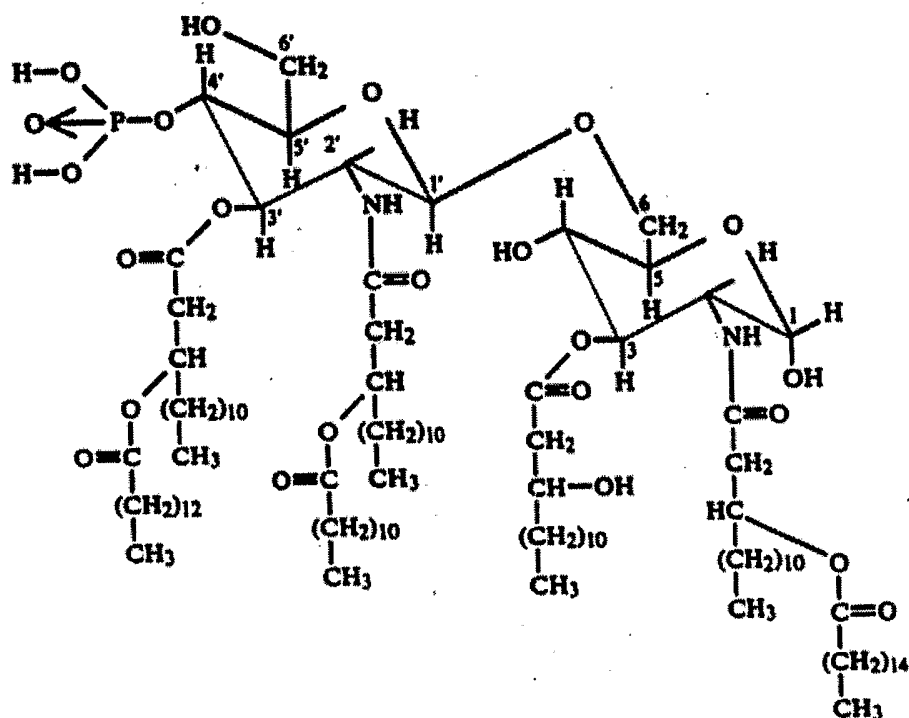
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Preferably the influenza antigen is formulated with an ADP-ribosylating toxin or a functional derivative thereof to form a mixed vaccine combination.

- 15 In an alternative aspect of the invention the intradermal influenza vaccine may be delivered sequentially or co-administered with an ADP-ribosylating toxin. There is no requirement for the ADP-ribosylating toxin to be delivered intradermally and suitably the toxin component is delivered transdermally, preferably via a patch at the injection site post injection.

- 20 The vaccine according to the invention may comprise further adjuvants or immunostimulants such as, but not limited to, detoxified lipid A from any source and non-toxic derivatives of lipid A, saponins and other reagents capable of stimulating a TH1 type response.

- 25 It has long been known that enterobacterial lipopolysaccharide (LPS) is a potent stimulator of the immune system, although its use in adjuvants has been curtailed by its toxic effects. A non-toxic derivative of LPS, monophosphoryl lipid A (MPL), produced by removal of the core carbohydrate group and the phosphate from the reducing-end glucosamine, has been described by Ribi et al (1986, Immunology and
30 Immunopharmacology of bacterial endotoxins, Plenum Publ. Corp., NY, p407-419) and has the following structure:



A further detoxified version of MPL results from the removal of the acyl chain from the 3-position of the disaccharide backbone, and is called 3-O-Deacylated monophosphoryl lipid A (3D-MPL). It can be purified and prepared by the methods taught in GB 2122204B, which reference also discloses the preparation of diphosphoryl lipid A, and 3-O-deacylated variants thereof.

Other purified and synthetic lipopolysaccharides have been described (US 6,005,099 and EP 0 729 473 B1; Hilgers *et al.*, 1986, *Int.Arch.Allergy.Immunol.*, 79(4):392-6; Hilgers *et al.*, 1987, *Immunology*, 60(1):141-6; and EP 0 549 074 B1).

A preferred form of 3D-MPL is in the form of an emulsion having a small particle size less than 0.2µm in diameter, and its method of manufacture is disclosed in WO 94/21292. Aqueous formulations comprising monophosphoryl lipid A and a surfactant have been described in WO9843670A2.

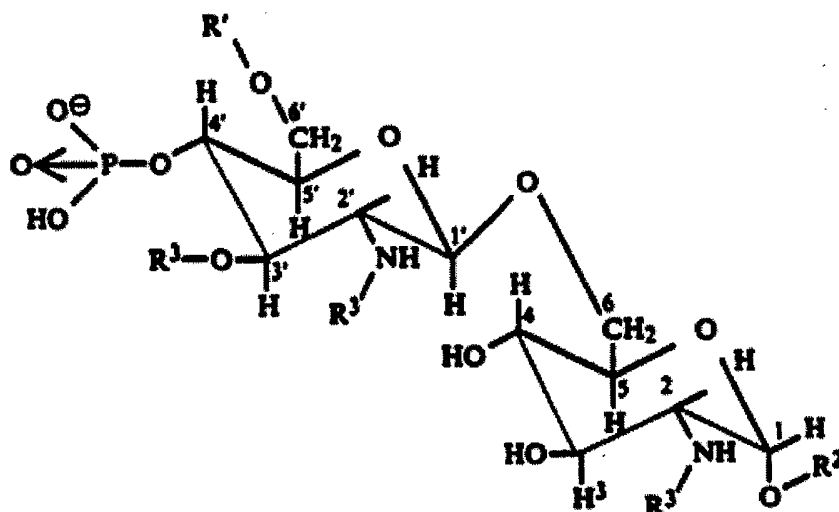
The bacterial lipopolysaccharide derived adjuvants may be purified and processed from bacterial sources, or alternatively they may be synthetic. For example, purified monophosphoryl lipid A is described in Ribi et al 1986 (supra), and 3-O-Deacylated

monophosphoryl or diphosphoryl lipid A derived from *Salmonella sp.* is described in GB 2220211 and US 4912094.

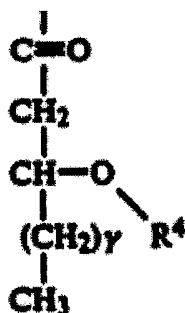
Particularly preferred bacterial lipopolysaccharide adjuvants are 3D-MPL and the $\beta(1-6)$ glucosamine disaccharides described in US 6,005,099 and EP 0 729 473 B1.

Accordingly, LPS derivatives that may be used in the present invention are those immunostimulants that are similar in structure to that of LPS or MPL or 3D-MPL. The LPS derivative may be an acylated monosaccharide, which is a sub-portion to the above structure of MPL.

A preferred disaccharide adjuvant is a purified or synthetic lipid A of the following formula:



wherein R^2 may be H or PO_3H_2 ; R^3 may be an acyl chain or β -hydroxymyristoyl or a 3-acyloxyacyl residue having the formula:



and wherein X and Y have a value of from 0 up to about 20.

Further preferred adjuvants include those disclosed in WO00/00462.

5

Saponins are taught in: Lacaille-Dubois, M and Wagner H. (1996. A review of the biological and pharmacological activities of saponins. *Phytomedicine* vol 2 pp 363-386). Saponins are steroid or triterpene glycosides widely distributed in the plant and marine animal kingdoms. Saponins are noted for forming colloidal solutions in water which foam on shaking, and for precipitating cholesterol. When saponins are near cell membranes they create pore-like structures in the membrane which cause the membrane to burst. Haemolysis of erythrocytes is an example of this phenomenon, which is a property of certain, but not all, saponins.

10

15 Saponins are known as adjuvants in vaccines for systemic administration. The adjuvant and haemolytic activity of individual saponins has been extensively studied in the art (Lacaille-Dubois and Wagner, *supra*). For example, Quil A (derived from the bark of the South American tree *Quillaja Saponaria* Molina), and fractions thereof, are described in US 5,057,540 and "Saponins as vaccine adjuvants", Kensil, C. R., *Crit Rev Ther Drug Carrier Syst*, 1996, 12 (1-2):1-55; and EP 0 362 279 B1.

20

Particulate structures, termed Immune Stimulating Complexes (ISCOMS), comprising fractions of Quil A are haemolytic and have been used in the manufacture of vaccines (Morein, B., EP 0 109 942 B1; WO 96/11711; WO 96/33739). The haemolytic saponins QS21 and QS17 (HPLC purified fractions of Quil A) have been described as

potent systemic adjuvants, and the method of their production is disclosed in US Patent No.5,057,540 and EP 0 362 279 B1. Other saponins which have been used in systemic vaccination studies include those derived from other plant species such as *Gypsophila* and *Saponaria* (Bomford *et al.*, *Vaccine*, 10(9):572-577, 1992).

5

An enhanced adjuvant system involves the combination of a non-toxic lipid A derivative and a saponin derivative, particularly the combination of QS21 and 3D-MPL as disclosed in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol as disclosed in WO 96/33739.

10

A particularly potent adjuvant formulation involving QS21 and 3D-MPL in an oil in water emulsion is described in WO 95/17210 and is a preferred formulation.

Preferably the formulation additionally comprises an oil in water emulsion.

15

Additional components that are preferably present in an adjuvanted vaccine formulation according to the invention include non-ionic detergents such as the octoxynols and polyoxyethylene esters as described herein, particularly *t*-octylphenoxy polyethoxyethanol (Triton X-100) and polyoxyethylene sorbitan monooleate (Tween 80); and bile salts or cholic acid derivatives as described herein, in particular sodium deoxycholate or taurodeoxycholate. Thus, a particularly preferred formulation comprises 3D-MPL, Triton X-100, Tween 80 and sodium deoxycholate, which may be combined with an influenza virus antigen preparation to provide a vaccine suitable for intradermal application.

25

In one preferred embodiment of the present invention, the intradermal influenza vaccines comprise a vesicular adjuvant formulation comprising cholesterol, a saponin and an LPS derivative. In this regard the preferred adjuvant formulation comprises a unilamellar vesicle comprising cholesterol, having a lipid bilayer preferably comprising dioleoyl phosphatidyl choline, wherein the saponin and the LPS derivative are associated with, or embedded within, the lipid bilayer. More preferably, these adjuvant formulations comprise QS21 as the saponin, and 3D-MPL as the LPS derivative, wherein the ratio of QS21:cholesterol is from 1:1 to 1:100 weight/weight,

30

and most preferably 1:5 weight/weight. Such adjuvant formulations are described in EP 0 822 831 B, the disclosure of which is incorporated herein by reference.

The invention provides in a further aspect a pharmaceutical kit comprising an intradermal administration device and an influenza vaccine formulation comprising an ADP-ribosylating toxin, as described herein. The device is preferably supplied already filled with the vaccine. Preferably the vaccine is in a liquid volume smaller than for conventional intramuscular vaccines as described herein, particularly a volume of between about 0.05 ml and 0.2 ml. Preferably the device is a short needle delivery device for administering the vaccine to the dermis.

Suitable devices for use with the intradermal vaccines described herein include short needle devices such as those described in US 4,886,499, US5,190,521, US 5,328,483, US 5,527,288, US 4,270,537, US 5,015,235, US 5,141,496, US 5,417,662.

Intradermal vaccines may also be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in WO99/34850, incorporated herein by reference, and functional equivalents thereof such as that disclosed in EP1092444. Also suitable are jet injection devices which deliver liquid vaccines to the dermis via a liquid jet injector or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis. Jet injection devices are described for example in US 5,480,381, US 5,599,302, US 5,334,144, US 5,993,412, US 5,649,912, US 5,569,189, US 5,704,911, US 5,383,851, US 5,893,397, US 5,466,220, US 5,339,163, US 5,312,335, US 5,503,627, US 5,064,413, US 5,520, 639, US 4,596,556, US 4,790,824, US 4,941,880, US 4,940,460, WO 97/37705 and WO 97/13537. Also suitable are ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis. Additionally, conventional syringes may be used in the classical mantoux method of intradermal administration. However, the use of conventional syringes requires highly skilled operators and thus devices which are capable of accurate delivery without a highly skilled user are preferred.

Suitable kits also comprise a prefilled intradermal delivery device comprising an influenza vaccine and a separate delivery means for an ADP-ribosylating toxin. Suitable delivery means includes a transdermal patch.

For the avoidance of doubt, the teaching of all references cited herein is hereby incorporated into the present invention.

- 5 The present invention is illustrated herein by reference to the following non-limiting Example and Figures, wherein:

Fig 1 illustrates Anti-A/New Caledonia/20/99 HI titers against 6 different vaccine formulations;

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Fig 2 illustrates Anti-A/Panama/2007/99 HI titers against 6 different vaccine formulations; and

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Fig 3 illustrates Anti-B/Shangdong/7/97 HI titers against 6 different vaccine formulations.

Example 1

20

6 vaccine combinations were tested on a sample of 5 pigs using the following regime:

Vaccine combinations

25

'Plain IM' = FlurarixTM (split flu) 15 µg delivered IM

'Plain ID' = FlurarixTM 3 µg delivered ID

'Berna ID' = flu virosomes 3µg delivered ID

'Berna ID' + LT = flu virosomes 3 µg delivered ID plus LT adjuvant at differing concentrations : 0.1µg, 0.5µg and 2.5 µg.

30

FlurarixTM (GSK) is a commercially available trivalent split vaccine, see for example WO02067983 for split influenza production. Virosomes were obtained commercially (Inflexal V, Berna Biotech, Berne Switzerland). LT was obtained from John Clements at Tulane University, New Orleans, LA, USA.

Protocol

Priming was carried out with trivalent whole inactivated flu at 25 µg hemagglutinin per strain using whole trivalent A/New Caledonia/20/99, A/Panama/2007/99 ,

5 B/Shangdong/7/97 - 25 µg IN at Day 0.

Vaccination was carried out with trivalent split flu, either 15 or 3 µg IM / ID as explained above or 3µg Borna virosomes ID (trivalent A/New Caledonia/20/99, A/Moscow/10/99, B/Hong Kong/330/2001) on Day 28.

10

Bleeding was carried out at Day 0, pre-vaccination at Day 28 and post-vaccination at Day 48.

The results are shown in figures 1-3. HI titres were measured using standard techniques (Dowdle et al., 1979. Influenza Viruses. In: Diagnostic procedures for viral, rickettsial, and chlamydial infections. American Public Health Association, Washington, D.C. pp 585-609).

15

The results demonstrate that LT is a highly effective adjuvant for an influenza vaccine delivered by the ID route, and is able to stimulate HI titres to levels seen with IM vaccination of a trivalent split influenza vaccine (Fluarix™).

20

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Claims

- 1 An intradermal influenza vaccine comprising an influenza antigen and an
ADP-ribosylating toxin or a functional derivative thereof.
- 5 2 A kit comprising an intradermal influenza vaccine, an ADP-ribosylating toxin
or a functional derivative thereof and a delivery means for said toxin or
derivative.
- 10 3 A method for preparing an intradermal influenza vaccine comprising
combining an influenza antigen with an ADP-ribosylating toxin or a functional
derivative thereof.
- 15 4 A method for the prophylaxis of influenza infection or disease in an individual
which method comprises administering to the individual intradermally an
influenza vaccine comprising both an influenza antigen and an ADP-
ribosylating toxin or a functional derivative thereof.
- 20 5 A method for the prophylaxis of influenza infection or disease in a subject
which method comprises sequential administration or co-administration of an
intradermal influenza vaccine and an ADP-ribosylating toxin or a functional
derivative thereof.
- 25 6 A method according to claim 6 wherein the ADP-ribosylating toxin is
delivered by a route which is not intradermal.
- 7 An intradermal delivery device containing an influenza vaccine in
combination with an ADP-ribosylating toxin or a functional derivative thereof.
- 30 8 Use of an ADP-ribosylating toxin or a functional derivative thereof
in the adjuvantation of an intradermal influenza vaccine.
- 9 A vaccine, use, kit, method or device according to claims 1-8 wherein the
ADP-ribosylating toxin is LT.

- 10 A vaccine according to claims 1-8 wherein the ADP-ribosylating toxin is
LTIIb
- 5 11 A vaccine, use, kit or method according to claims 1-6 wherein the vaccine
comprises influenza virosomes.

Fig 1

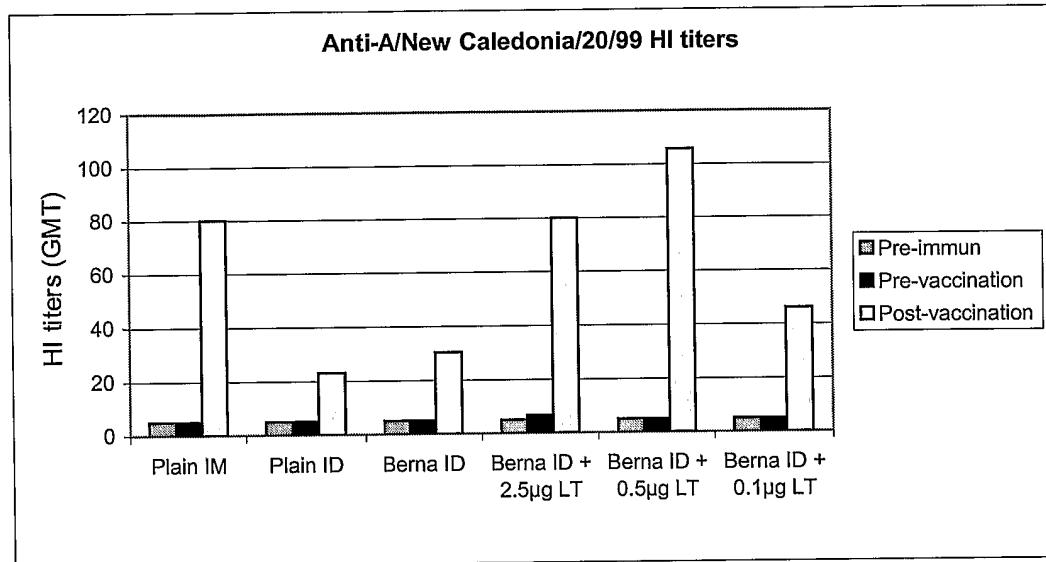


Fig 2

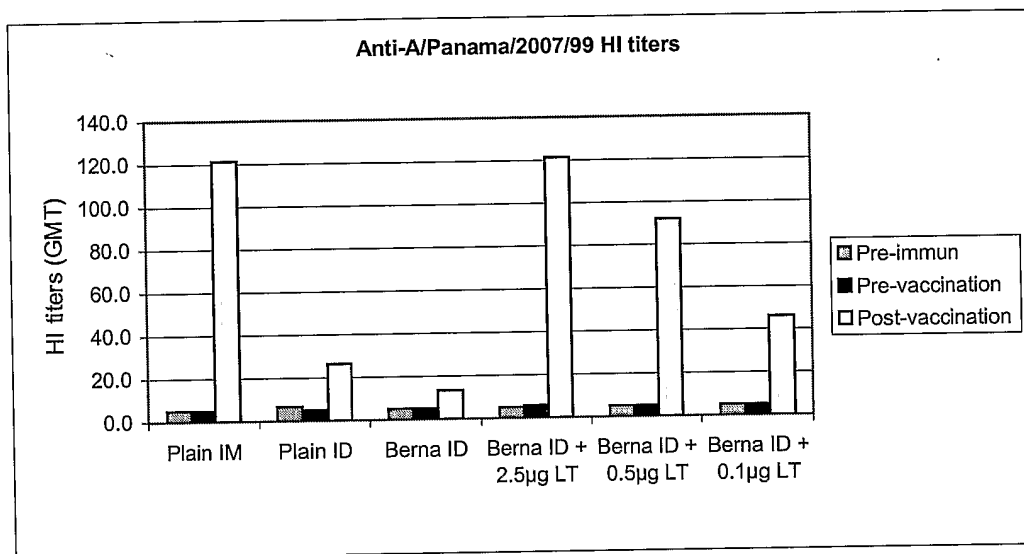
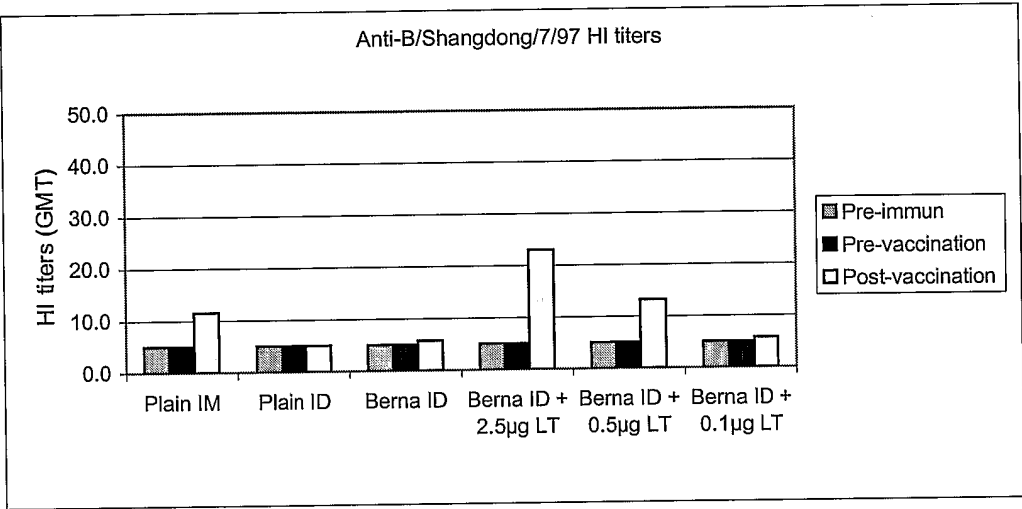


Fig 3



INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/09011

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K39/145 A61K39/39 C07K14/245

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01 34185 A (POWDERJECT VACCINES INC) 17 May 2001 (2001-05-17)	1-8
Y	examples 3-5,7	9-11
X	WO 00 50078 A (CHIRON CORP ;HAGAN DEREK O (US); SINGH MANMOHAN (US)) 31 August 2000 (2000-08-31) page 24 example 1 claims 8,10	1,3,8,9

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

° Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

15 October 2003

Date of mailing of the international search report

28/10/2003

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Authorized officer

Brouns, G

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/09011

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TUMPEY TERRENCE M ET AL: "Mucosal delivery of inactivated influenza vaccine induces B-cell-dependent heterosubtypic cross-protection against lethal influenza A H5N1 virus infection" JOURNAL OF VIROLOGY, vol. 75, no. 11, June 2001 (2001-06), pages 5141-5150, XP002257842 ISSN: 0022-538X the whole document	1,3,8,9
Y	MARTIN MICHAEL ET AL: "Comparative analysis of the mucosal adjuvant activity of the type II heat-labile enterotoxins LT-IIa and LT-IIb" INFECTION AND IMMUNITY, vol. 68, no. 1, January 2000 (2000-01), pages 281-287, XP002178228 ISSN: 0019-9567 cited in the application page 286; figures 2-6	9,10
Y	GLUCK R ET AL: "Immunogenicity of new virosome influenza vaccine in elderly people" LANCET (NORTH AMERICAN EDITION), vol. 344, no. 8916, 1994, pages 160-163, XP002257843 ISSN: 0099-5355 the whole document	11
Y	SCHAAD U B ET AL: "Comparison of immunogenicity and safety of a virosome influenza vaccine with those of a subunit influenza vaccine in pediatric patients with cystic fibrosis" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 44, no. 5, May 2000 (2000-05), pages 1163-1167, XP002257844 ISSN: 0066-4804 figures 1,2	11
A	WO 98 20734 A (ALVING CARL R ;US ARMY (US); GLENN GREGORY M (US)) 22 May 1998 (1998-05-22) examples 21,22	1-11
P,A	WO 02 074244 A (GLENN GREGORY M ;IOMAI CORP (US); SIX HOWARD R (US)) 26 September 2002 (2002-09-26) examples 1,1-4	6

INTERNATIONAL SEARCH REPORT

International application No.
 PCT/EP 03/09011

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 4-6 are directed to a method of prophylactic treatment of the human or animal body, the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/09011

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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WO 02074244	A	26-09-2002	WO 02074244 A2	26-09-2002