Liposome compositions comprising an adjuvant that activates or increases the activity of TLR2 and uses thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to United States Provisional Patent Application No. 61/544,020 filed on October 6, 2011~~, which is hereby incorporated by reference in its entirety~~.

FIELD OF THE INVENTION

The present application relates to vaccine compositions that enhance the production of antigen-specific antibodies in immunized subjects.

BACKGROUND OF THE INVENTION

Immune responses induced by vaccination can be categorized broadly into humoral or cellular types. A humoral response is typically desired to protect against viral or bacterial invaders, whereas immunity against virally infected cells and cancer cells typically involves a cell mediated response. Humoral immunity is typified by high levels of antibody production by B cells, whereas cellular immunity is characterized by increased activation of cytotoxic CD8 T lymphocytes.

The type of immunity induced by a vaccine largely depends on the type of adjuvant included in the vaccine. Adjuvants based on palmitic acid, such as dipalmitoyl-S-glyceryl-cysteine (PAM_2Cys) and tripalmitoyl-S-glyceryl-cysteine (PAM_3Cys) and variants thereof, have been reported to enhance humoral and cellular responses against a variety of antigens. For practical reasons, the solubility of such adjuvants has been typically improved with the addition of hydrophilic non-immunogenic amino acid residues (Lysines for example). Such adjuvants have been mixed with antigen but in many instances palmitic acid based adjuvants have been covalently linked to antigens before administering to a subject. Palmitic acid adjuvants have also been co-delivered with antigen using liposomes as carriers. Protein based and carbohydrate based antigens have been combined with palmitic acid adjuvants to produce antibody and T cell responses. The use of palmitic acid adjuvants for cancer applications is well documented, with activity mediated primarily by cellular responses.

herein as a "palmitic acid adjuvant". Palmitic acid is a low molecular weight lipid found in the immunologically reactive Braun's lipoprotein of *Escherichia coli*. Other common chemical names for palmitic acid include, for example, hexadecanoic acid in IUPAC nomenclature and 1-Pentadecanecarboxylic acid. The molecular formula of palmitic acid is $CH_3(CH_2)_{14}CO_2H$. As will be understood to those skilled in the art, it is possible that the lipid chain of palmitic acid may be altered. Exemplary compounds which may be used herein as palmitic acid adjuvants, and methods for their synthesis, are described for example in United States Patent Publications US 2008/0233143; US 2010/0129385; and US 2011/0200632~~, the disclosures of which are incorporated herein~~.

As described above for lipid moieties generally, a palmitic acid adjuvant contains at a minimum at least one palmitic acid moiety, which can be coupled onto an amino acid, an oligopeptide or other molecules. A palmitic acid moiety or a structure containing palmitic acid can be coupled covalently or non-covalently to an antigen to create antigenic compounds with built-in adjuvanting properties. The palmitic acid moiety or a chemical structure containing palmitic acid can be conjugated to a cysteine peptide (Cys) to allow for various structural configurations of the adjuvant, including linear and branched structures. The cysteine residue has been commonly extended by polar residues such as Serine (Ser) and/ or lysine (Lys) at the C terminus to create adjuvant compounds with improved solubility. Palmitic acid containing adjuvant compounds could be admixed with an antigen, associated with antigen through non-covalent interactions, or alternatively covalently linked to an antigen, either directly or with the use of a linker/spacer, to generate enhanced immune responses. Most commonly, two palmitic acid moieties are attached to a glyceryl backbone and a cysteine residue to create dipalmitoyl-S-glyceryl-cysteine (PAM_2Cys) or tripalmitoyl-S-glyceryl-cysteine (PAM_3Cys), which can also be used in multiple configurations as described above.

Palmitic acid adjuvants are known to activate B cells causing rapid proliferation and production of antibodies. B cells recognize the antigen co-delivered with the adjuvant in the vaccine formulation and through affinity maturation will proliferate with increasing specificity towards the antigen. Activated B cells are known to secrete large quantities of soluble immunoglobin antibodies that can bind to soluble targets, such as bacteria, present in the blood. Antibody effector functions are i) opsonization; ii) antibody-dependent cell-mediated cytotoxicity (ADCC); iii) complement activation; iv) neutralization.

Hirayama, and Harumi Sakai, Kindai Shuppan (2000); "Minimum Requirements for Biological Products", edited by the Association of Biologicals Manufacturers of Japan (1993).

The optimal amount of adjuvant and antigen to elicit an optimal immune response may depend on a number of factors including, without limitation, the composition, the disease, the subject, and may be readily ascertained by the skilled person using standard studies including, for example, observations of antibody titers and other immunogenic responses in the host.

The compositions as described herein may be effective when administered in a single application.

In another embodiment, the compositions as described herein may be used in combination, before or after, with other therapies.

Kits and Reagents

The present invention is optionally provided to a user as a kit. For example, a kit of the invention contains one or more of the compositions of the invention. The kit can further comprise one or more additional reagents, packaging material, containers for holding the components of the kit, and an instruction set or user manual detailing preferred methods of using the kit components.

~~*Embodiments of the Invention*~~

~~Particular embodiments of the invention include, without limitation, the following:~~

~~1. A composition comprising, consisting of, or consisting essentially of: liposomes; an antigen capable of inducing a humoral immune response; a carrier comprising a continuous phase of a hydrophobic substance; and an adjuvant that activates or increases the activity of TLR2, preferably a lipid-based adjuvant.~~

~~2. The composition of paragraph 1, wherein the adjuvant activates or increases the activity of toll-like receptor 2 (TLR2), or a TLR2 dimer such as TLR1/2 or TLR2/6.~~

3. The composition of paragraph 1, wherein the adjuvant only activates or increases the activity of a toll-like receptor (TLR) selected from TLR2, heterodimeric TLR1/2 and heterodimeric TLR2/6, but does not activate or increase the activity of other TLRs.

4. The composition of any one of paragraphs 1 to 3, wherein theadjuvant is a compound comprising, consisting of, or consisting essentially of at least one natural, synthetic or semi-synthetic lipid moiety, lipid component, or analog or derivative thereof, including for example a lipoamino acid, a lipoglycan, a lipopolysaccharide, a lipoteichoic acid or a cell wall component of a Gram-positive or Gram-negative bacteria, Rhodopseudomonas viridis or mycoplasma.

5. The composition of any one of paragraphs 1 to 4, wherein theadjuvant comprises, consists of, or consists essentially of PAM_2Cys-Ser-(Lys)4, PAM_3Cys-Ser-(Lys)4, PAM_3Cys-SKKKK(β-irradiated), *R*-PAM_3Cys-SKKKK, *S*-PAM_3Cys-SKKKK, PAM_3Cys-SKKKK(Biotin-Aca-Aca), PAM_3Cys-SKKKK(Fluorescein-Aca-Aca), PAM_3Cys-SKKKK (Rhodamine-Aca-Aca), PAM_3Cys-SKKKK-FLAG-tag, PHC-SKKKK, PHC-SKK-KK(Biotin-Aca-Aca), PAM_3Cys-SSNAKIDQLSSDVQT, PAM_3Cys-SSNKSTTGSGETTTA, PAM_3Cys-SSTKPVSQDTSPKPA, PAM_3Cys-SSGSKPSGGPLPDAK, PAM_3Cys-SSGNKSA PSSSASSS, PAM_3Cys-GSHQMKSEGHANMQL, PAM_3Cys-SSSNNDAAGNGAAQT, PAM_3Cys-KQNVSSLDEKNSVSV, PAM_3Cys-NNSGKDGNTSANSAD, PAM_3Cys-NNGGPE LKSDEVAKS, PAM_3Cys-SQEPAAPAAEATPAG, PAM_3Cys-SSSKSSDSSAPKAYG, PAM_3Cys-AQEKEAKSELDYDQT, PAM_2Cys-SKKKK (mixture of RR and RS stereoisomers), *R*-PAM_2Cys-SKKKK (RR-stereoisomer), *S*-PAM_2Cys-SKKKK (RS-stereoisomer), PAMCys(PAM)-SKKKK, PAM_2Cys-SKKKK(Biotin-Aca-Aca)-NH_2, PAM_2Cys-SKKKK (Fluorescein-Aca-Aca)-NH_2, PAM_2Cys-SKKKK(Rhodamine-Aca-Aca)-NH_2, PAM_2Cys-SKK KK-FLAG-tag, PAM-Dhc-SKKKK, PAM-CSKKKK, PAM-Dhc-GDPKHPKSF, PAM-CGDPKH PKSF, FSL-1 (Pam2CGDPKHPKSF), FSL-1-Ala, macrophage-activating lipopeptide-2 (MALP-2), lipoarabinomannan from M. smegmatis (LAM-MS), lipomannan from M. smegmatis (LM-MS), lipopolysaccharide from P. gingivalis (LPS-PG Ultrapure), lipoteichoic acid from B. subtilis (LTA-BS) or S. aureus (LTA-SA), or derivatives or analogs thereof, or is a heat-killed bacteria that comprises the cell wall component of a Gram-positive or Gram-negative bacteria.

6. The composition of any one of paragraphs 1 to 5, wherein theadjuvant is a palmitic acid adjuvant.

~~7. The composition of any one of paragraphs 1 to 6, wherein the adjuvant comprises, consists of, or consists essentially of dipalmitoyl-S-glyceryl-cysteine (PAM_2Cys) or tripalmitoyl-S-glyceryl-cysteine (PAM_3Cys).~~

~~8. The composition of any one of paragraphs 1 to 6, wherein the adjuvant is PAM_2Cys-Ser-(Lys)4, PAM_3Cys-Ser-(Lys)4, FSL-1 or MALP-2.~~

~~9. The composition of paragraph 8, wherein the adjuvant is PAM_2Cys-Ser-(Lys)4.~~

~~10. The composition of paragraph 8, wherein theadjuvant is PAM_3Cys-Ser-(Lys)4.~~

~~11. The composition of any one of paragraphs 1 to 10 which further comprises at least one other suitable adjuvant in addition to the adjuvant that activates or increases the activity of TLR2.~~

~~12. The composition of any one of paragraphs 1 to 11, wherein the antigen is a polypeptide or a carbohydrate.~~

~~13. The composition of any one of paragraphs 1 to 12, wherein the antigen comprises, consists of, or consists essentially of a B cell epitope, or a plurality of B cell epitopes.~~

~~14. The composition of paragraph 13, wherein the B cell epitope is derived from a virus or bacteria.~~

~~15. The composition of paragraph 14, wherein the B cell epitope is derived from influenza virus, Bordetella pertussis or Bacillus anthracis.~~

~~16. The composition of paragraph 15, wherein the B cell epitope is an epitope of a hemagglutinin protein of H5N1 influenza virus, an epitope of pertussis toxoid protein, or an epitope of an anthrax recombinant protective antigen.~~

~~17. The composition of any one of paragraphs 1 to 13, wherein the antigen is: an antigen associated with an infectious disease; a membrane surface-bound cancer~~

~~antigen; a toxin; an allergen such as pollen; or an antigen associated with a disease where it is desirable to sequester the antigen in circulation, such as for example an amyloid protein.~~

~~18. The composition of paragraph 17, wherein the infectious disease is influenza, a respiratory tract infection caused by human respiratory syncytial virus, pertussis, anthrax or malaria.~~

~~19. The composition of paragraph 17, wherein the toxin is a drug substance, such as cocaine.~~

~~20. The composition of any one of paragraphs 1 to 13, wherein the antigen is a hapten carrier adduct.~~

~~21. The composition of any one of paragraphs 1 to 20, wherein the liposome comprises, consists of, or consists essentially of a phospholipid or unesterified cholesterol.~~

~~22. The composition of any one of paragraphs 1 to 21, wherein the antigen is encapsulated in the liposomes or both the antigen and the adjuvant are encapsulated in the liposomes.~~

~~23. The composition of any one of paragraphs 1 to 22, which is a water free liposome suspension.~~

~~24. The composition of any one of paragraphs 1 to 23, wherein the composition is capable of inducing a humoral immune response with a single dose.~~

~~25. The composition of paragraph 24, wherein the humoral immune response is characterized by antigen specific antibody production.~~

~~26. The composition of paragraph 25 which is capable of generating the antigen specific antibody at an antibody titer of up to about 1/2,048,000 by about three weeks post vaccination of a subject.~~

~~27. The composition of paragraph 25 which is capable of generating the antigen specific antibody at an antibody titer of up to about 1/8,192,000 by about eight weeks post vaccination of a subject.~~

28. The composition of any one of paragraphs 24 to 27, wherein the humoral immune response is associated with the activation or generation of T-helper 2 (Th2) cells or T-helper 17 (Th17) cells.

29. The composition of any one of paragraphs 1 to 28 for the treatment or prevention of a disease or disorder ameliorated by a humoral immune response.

30. The composition of any one of paragraphs 1 to 28 for the treatment or prevention of: an infectious disease; a cancer involving a membrane surface-bound cancer antigen; or a disease or disorder where it is desirable to sequester antigen in circulation, such as Alzheimer's disease.

31. The composition of any one of paragraphs 1 to 28 for neutralizing a toxin, virus, bacterium or allergen, with an antibody.

32. A method for treating or preventing a disease or disorder ameliorated by a humoral immune response, said method comprising, consisting of, or consisting essentially of administering the composition of any one of paragraphs 1 to 28 to a subject.

33. A method for treating or preventing an infectious disease; a cancer involving a membrane surface-bound cancer antigen; or a disease or disorder where it is desirable to sequester antigen in circulation, such as Alzheimer's disease, said method comprising, consisting of, or consisting essentially of administering the composition of any one of paragraphs 1 to 28 to a subject.

34. The method of paragraph 33, wherein the infectious disease is influenza, a respiratory tract infection caused by human respiratory syncytial virus, pertussis, anthrax or malaria.

35. A method for neutralizing a toxin, virus, bacterium or allergen, with an antibody, said method comprising, consisting of, or consisting essentially of administering the composition of any one of paragraphs 1 to 28 to a subject.

36. The method of paragraph 35, wherein the toxin is a drug substance, such as cocaine.

~~37. The method of any one of paragraphs 32 to 36, wherein the subject is a mammal, preferably a human.~~

~~38. A kit useful for treating or preventing a disease or disorder ameliorated by a humoral immune response; or useful for treating or preventing an infectious disease; a cancer involving a membrane surface-bound cancer antigen; or a disease or disorder where it is desirable to sequester antigen in circulation, such as Alzheimer's disease; or useful for neutralizing a toxin, virus, bacterium or allergen, with an antibody, wherein the kit comprises, consists of, or consists essentially of the composition of any one of paragraphs 1 to 28, and instructions for its use thereof.~~

~~39. A method of preparing the composition of paragraph 1, comprising, consisting of, or consisting essentially of: hydrating a homogenous mixture of S100 lecithin and cholesterol in the presence of the antigen to form a liposome preparation with encapsulated antigen; extruding and then mixing the liposome preparation with the adjuvant; lyophilizing and reconstituting the resultant product in a carrier comprising a continuous phase of a hydrophobic substance. In an alternate embodiment, the hydrating step may be performed in the presence of a homogenous mixture of dioleoyl phosphatidylcholine (DOPC) and cholesterol.~~

~~40. A method of preparing the composition of paragraph 1, comprising, consisting of, or consisting essentially of: hydrating a homogenous mixture of dioleoyl-phosphatidylcholine (DOPC) and cholesterol in the presence of the antigen and the adjuvant to form a liposome preparation with encapsulated antigen and adjuvant; lyophilizing and reconstituting the liposome preparation in a carrier comprising a continuous phase of a hydrophobic substance. In an alternate embodiment, the hydrating step may be performed in the presence of a homogenous mixture of S100 lecithin and cholesterol.~~

The invention is further illustrated by the following non-limiting examples.

Untreated "naïve" mice were terminated and spleens collected. A single cell suspension was prepared from the splenocytes and red blood cells lysed using ACK lysis buffer. B cells were isolated using a negative selection magnetic isolation kit from Miltenyi (Auburn, CA, USA). Cells were resuspended in complete RPMI media containing 10% FBS, 1% penicillin-streptomycin, 1% L-glutamine and 0.1% b-mercaptoethanol (c-RPMI) at a final concentration of 2×10^6 cells/ mL. B cells were added to wells of a 96-well plate (2×10^5 cells/ well) with anti-Ig (2.5ug/ mL; BD Biosciences, Mississauga, Canada) and anti-CD40 (1ug/ mL; BD Biosciences). B cells were stimulated with the following adjuvants in triplicate: Pam2Cys (EMC Microcollections, Tuebingen, Germany), Pam3Cys (EMC Microcollections), Poly I:C (Thermo Fisher, MI, USA) and LPS (Sigma-Aldrich, Oakville, Canada), or no adjuvant. Each adjuvant was dosed at 10 ug/mL, 1 ug/mL and 0.1 ug/mL, except LPS which was dosed at 100 ng/mL, 10 ng/mL and 1 ng/mL. Cells were incubated at 37°C/ 5% CO_2 for 3 days. Eighteen hours before the end of the experiment, [^{3}H]-thymidine was added to each well at a final concentration of 0.2 uCi/ well. Plates were harvested using Titertek Cell Harvester (Skatron Instruments, Sterling, VA, USA) onto filter membranes which were then counted with Beckman LS6000IC liquid scintillation counter (Beckman Coulter Inc., Mississauga, ON, Canada). Proliferation was quantified by averaging the triplicate counts per minute (CPM) representing the incorporation of [3H]-thymidine.

Results of adjuvant stimulation of B cells show that while Pam3Cys and Pam2Cys can induce potent proliferation of B cells, Poly I:C does not (see Figure 7). LPS, included as a positive control, is known to induce B cell proliferation of low concentrations. Based on these results, it is reasonable to expect that vaccines containing Pam2Cys or Pam3Cys would have similar effects on B cells *in vivo*, and would be capable of facilitating the production of similar levels of antigen specific antibodies.

~~All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.~~ The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

~~Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to~~

~~those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.~~

~~It must be noted that as used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.~~ Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, *i.e.*, "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

~~As used herein in the specification and in the claims, "or" should be understood to encompass the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, *i.e.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items.~~

As used herein, whether in the specification or the appended claims, the transitional terms "comprising", "including", "carrying", "having", "containing", "involving", and the like are to be understood as being inclusive or open-ended (*i.e.*, to mean including but not limited to), and they do not exclude unrecited elements, materials or method steps. Only the transitional phrases "consisting of" and "consisting essentially of", respectively, are closed or semi-closed transitional phrases with respect to claims. The transitional phrase "consisting of" excludes any element, step, or ingredient which is not specifically recited. The

CLAIMS:

1. A composition comprising:

liposomes;

an antigen that comprises a B cell epitope and is capable of inducing a humoral immune response;

a carrier comprising a continuous phase of a hydrophobic substance; and

an adjuvant that activates or increases the activity of toll-like receptor 2 (TLR2) .

2. The composition of claim 1, wherein the adjuvant, preferably a lipid-based adjuvant, activates or increases the activity of TLR2, or a TLR2 dimer such as TLR1/2 or TLR2/6.

3. The composition of claim 1 or 2, wherein the adjuvant is a compound comprising at least one natural, synthetic or semi-synthetic lipid moiety, lipid component, or analog or derivative thereof, including for example a lipoamino acid, a lipoglycan, a lipopolysaccharide, a lipoteichoic acid or a cell-wall component of a Gram-positive or Gram-negative bacteria, Rhodopseudomonas viridis or mycoplasma.

4. The composition of any one of claims 1 to 3, wherein the adjuvant is PAM_2Cys-Ser-(Lys)4, PAM_3Cys-Ser-(Lys)4, PAM_3Cys-SKKKK(β-irradiated), *R*-PAM_3Cys-SKKKK, *S*-PAM_3Cys-SKKKK, PAM_3Cys-SKKKK(Biotin-Aca-Aca), PAM_3Cys-SKKKK (Fluorescein-Aca-Aca), PAM_3Cys-SKKKK (Rhodamine-Aca-Aca), PAM_3Cys-SKKKK-FLAG-tag, PHC-SKKKK, PHC-SKKKK(Biotin-Aca-Aca), PAM_3Cys-SSNAKIDQLSSDVQT, PAM_3Cys-SSNKSTTGSGETTTA, PAM_3Cys-SSTKPVSQDTSPKPA, PAM_3Cys-SSGSKPSGG PLPDAK, PAM_3Cys-SSGNKSAPSSSASSS, PAM_3Cys-GSHQMKSEGHANMQL, PAM_3Cys-SSSNNDAAGNGAAQT, PAM_3Cys-KQNVSSLDEKNSVSV, PAM_3Cys-NNSGKDGNTS ANSAD, PAM_3Cys-NNGGPELKSDEVAKS, PAM_3Cys-SQEPAAPAAEATPAG, PAM_3Cys-SSSKSSDSSAPKAYG, PAM_3Cys-AQEKEAKSELDYDQT, PAM_2Cys-SKKKK (mixture of RR and RS stereoisomers), *R*-PAM_2Cys-SKKKK (RR stereoisomer), *S*-PAM_2Cys-SKKKK (RS stereoisomer), PAMCys(PAM)-SKKKK, PAM_2Cys-SKKKK(Biotin-Aca-Aca)-NH_2, PAM_2Cys-SKKKK (Fluorescein-Aca-Aca)-NH_2, PAM_2Cys-SKKKK(Rhodamine-Aca-Aca)-NH_2, PAM_2Cys-SKKKK-FLAG-tag, PAM-Dhc-SKKKK, PAM-CSKKKK, PAM-Dhc-GDPKHPKSF, PAM-

CGDPKHPKSF, FSL-1 (Pam2CGDPKHPKSF), FSL-1-Ala, macrophage activating lipopeptide-2 (MALP-2), lipoarabinomannan from M. smegmatis (LAM-MS), lipomannan from M. smegmatis (LM-MS), lipopolysaccharide from P. gingivalis (LPS-PG Ultrapure), lipoteichoic acid from B. subtilis (LTA-BS) or S. aureus (LTA-SA), or derivatives or analogs thereof, or is a heat-killed bacteria that comprises the cell-wall component of a Gram-positive or Gram-negative bacteria.

5. The composition of any one of claims 1 to 4, wherein the adjuvant is a palmitic acid adjuvant.

6. The composition of any one of claims 1 to 5, wherein the adjuvant comprises dipalmitoyl-S-glyceryl-cysteine (PAM_2Cys) or tripalmitoyl-S-glyceryl-cysteine (PAM_3Cys).

7. The composition of any one of claims 1 to 6, wherein the adjuvant is PAM_2Cys-Ser-(Lys)4 or PAM_3Cys-Ser-(Lys)4.

8. The composition of any one of claims 1 to 7, wherein the antigen is a polypeptide or a carbohydrate.

9. The composition of any one of claims 1 to 8, wherein the antigen comprises a plurality of B cell epitopes.

10. The composition of any one of claims 1 to 8, wherein the B cell epitope is derived from a virus or bacteria, including for example influenza virus, Bordetella pertussis, or Bacillus anthracis.

11. The composition of claim 10, wherein the B cell epitope is an epitope of a hemagglutinin protein of H5N1 influenza virus, an epitope of pertussis toxoid protein, or an epitope of an anthrax recombinant protective antigen.

12. The composition of any one of claims 1 to 9, wherein the antigen is: an antigen associated with an infectious disease; a membrane surface-bound cancer antigen; a toxin; an allergen such as pollen; or an antigen associated with a disease where it is desirable to sequester the antigen in circulation, such as for example an amyloid protein.

13. The composition of any one of claims 1 to 12, wherein the antigen is encapsulated in the liposomes or both the antigen and the adjuvant are encapsulated in the liposomes.

14. The composition of any one of claims 1 to 13, wherein the composition is capable of inducing a humoral immune response with a single dose.

15. Use of the composition of any one of claims 1 to 14 in the manufacture of a medicament for the treatment or prevention of a disease or disorder ameliorated by a humoral immune response.

16. Use of the composition of any one of claims 1 to 14 in the manufacture of a medicament for the treatment or prevention of: an infectious disease; a cancer involving a membrane surface-bound cancer antigen; or a disease or disorder where it is desirable to sequester antigen in circulation, such as Alzheimer's disease.

17. Use of the composition of any one of claims 1 to 14 in the manufacture of a medicament for neutralizing a toxin, virus, bacterium or allergen, with an antibody.

CLAIMS:

1. A composition comprising:

liposomes;

an antigen that comprises a B cell epitope and is capable of inducing a humoral immune response;

a carrier comprising a continuous phase of a hydrophobic substance; and

an adjuvant that activates or increases the activity of toll-like receptor 2 (TLR2) .

2. The composition of claim 1, wherein the adjuvant activates or increases the activity of TLR2 by a TLR2 dimer of TLR1/2 or TLR2/6.

3. The composition of claim 2, wherein the adjuvant is a lipid-based adjuvant.

4. The composition of any one of claims 1 to 3, wherein the adjuvant is a compound comprising at least one natural, synthetic or semi-synthetic lipid moiety, lipid component, or analog or derivative thereof.

5. The composition of claim 4, wherein the adjuvant comprises a lipoamino acid, a lipoglycan, a lipopolysaccharide, a lipoteichoic acid or a cell-wall component of a Gram-positive or Gram-negative bacteria, Rhodopseudomonas viridis or mycoplasma.

6. The composition of any one of claims 1 to 3, wherein the adjuvant is dipalmitoyl-S-glyceryl (PAM_2)-Cys-SKKKK, tripalmitoyl-S-glyceryl (PAM_3)-Cys-SKKKK, β-irradiated PAM_3-Cys-SKKKK, *R*-PAM_3-Cys-SKKKK, *S*-PAM_3-Cys-SKKKK, PAM_3-Cys-SKKKK-Biotin-aminocaproic acid (Aca)-Aca, PAM_3-Cys-SKKKK-Fluorescein-Aca-Aca, PAM_3-Cys-SKKKK-Rhodamine-Aca-Aca, PAM_3-Cys-SKKKK-FLAG-tag, PAM_3-Cys-SSNAKIDQLSSDVQT, PAM_3-Cys-SSNKSTTGSGETTTA, PAM_3-Cys-SSTKPVSQDTSPKPA, PAM_3-Cys-SSGSKPSGGPLPDAK, PAM_3-Cys-SSGNKSAPSSSASSS, PAM_3-Cys-GSHQMKSEGHANMQL, PAM_3-Cys-SSSNNDAAGNGAAQT, PAM_3-Cys-KQNVSSLDEKNSVSV, PAM_3-Cys-NNSGKDGNTSANSAD, PAM_3-Cys-NNGGPELKSDEVAKS, PAM_3-Cys-SQEPAAPAAEATPAG, PAM_3-Cys-SSSKSSDSSAPKAYG, PAM_3-Cys-AQEKEAKSELDYDQT, PAM_2-Cys-SKKKK (mixture of RR and RS stereoisomers), *R*-PAM_2-Cys-SKKKK (RR stereoisomer), *S*-PAM_2-Cys-SKKKK (RS stereoisomer), palmitoyl-S-glyceryl (PAM)-Cys-PAM-SKKKK, PAM_2-Cys-SKKKK-

Biotin-Aca-Aca-NH_2, PAM_2-Cys-SKKKK-Fluorescein-Aca-Aca-NH_2, PAM_2-Cys-SKKKK-Rhodamine-Aca-Aca-NH_2, PAM_2-Cys-SKKKK-FLAG-tag, PAM-dihydroxypropylcystein (Dhc)-SKKKK, PAM-CSKKKK, PAM-Dhc-GDPKHPKSF, PAM-CGDPKHPKSF, PAM_2-CGDPKHPKSF (FSL-1), FSL-1-Ala, macrophage activating lipopeptide-2 (MALP-2), lipoarabinomannan from M. smegmatis (LAM-MS), lipomannan from M. smegmatis (LM-MS), lipopolysaccharide from P. gingivalis (LPS-PG), lipoteichoic acid from B. subtilis (LTA-BS) or S. aureus (LTA-SA), or derivatives or analogs thereof, or is a heat-killed bacteria that comprises the cell-wall component of a Gram-positive or Gram-negative bacteria.

7. The composition of any one of claims 1 to 6, wherein the adjuvant is a palmitic acid adjuvant.

8. The composition of any one of claims 1 to 7, wherein the adjuvant comprises dipalmitoyl-S-glyceryl-cysteine (PAM_2-Cys) or tripalmitoyl-S-glyceryl-cysteine (PAM_3-Cys).

9. The composition of any one of claims 1 to 8, wherein the adjuvant is dipalmitoyl-S-glyceryl (PAM_2)-Cys-SKKKK or tripalmitoyl-S-glyceryl (PAM_3)-Cys-SKKKK.

10. The composition of any one of claims 1 to 9, wherein the antigen is a polypeptide or a carbohydrate.

11. The composition of any one of claims 1 to 10, wherein the antigen comprises a plurality of B cell epitopes.

12. The composition of any one of claims 1 to 10, wherein the B cell epitope is derived from a virus or bacteria.

13. The composition of claim 12, wherein the virus or bacteria is influenza virus, Bordetella pertussis, or Bacillus anthracis.

14. The composition of claim 12 or 13, wherein the B cell epitope is an epitope of a hemagglutinin protein of H5N1 influenza virus, an epitope of pertussis toxoid protein, or an epitope of an anthrax recombinant protective antigen.

15. The composition of any one of claims 1 to 11, wherein the antigen is: an antigen associated with an infectious disease; a membrane surface-bound cancer antigen; a toxin; an

allergen; or an antigen associated with a disease where it is desirable to sequester the antigen in circulation.

16. The composition of claim 15, wherein the allergen is pollen.

17. The composition of claim 15, wherein the antigen associated with a disease where it is desirable to sequester the antigen in circulation is an antigen of an amyloid protein.

18. The composition of any one of claims 1 to 17, wherein the antigen is encapsulated in the liposomes or both the antigen and the adjuvant are encapsulated in the liposomes.

19. The composition of any one of claims 1 to 18, wherein the composition is capable of inducing a humoral immune response with a single dose.

20. Use of the composition of any one of claims 1 to 19 in the manufacture of a medicament for the treatment or prevention of a disease or disorder ameliorated by a humoral immune response.

21. Use of the composition of any one of claims 1 to 19 in the manufacture of a medicament for the treatment or prevention of: an infectious disease; a cancer involving a membrane surface-bound cancer antigen; or a disease or disorder where it is desirable to sequester antigen in circulation.

22. The use of claim 21, wherein the disease or disorder where it is desirable to sequester antigen in circulation is Alzheimer's disease.

23. Use of the composition of any one of claims 1 to 19 in the manufacture of a medicament for neutralizing a toxin, virus, bacterium or allergen, with an antibody.