(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 October 2006 (19.10.2006)

PCT

(10) International Publication Number
WO 2006/109186 A2

(51) International Patent Classification:
A61K 39/39 (2006.01) *A61P 37/00* (2006.01)

(21) International Application Number:
PCT/IB2006/001296

(22) International Filing Date: 13 April 2006 (13.04.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0507672.4 15 April 2005 (15.04.2005) GB

(71) Applicant: **BARNABA, Vincenzo** [IT/IT]; Fondazione "Andrea Cesalpino", Dipartimento Medicina Interna, Universita degli Studi di Roma "La Sapienza", Policlinico Umberto 1, viale del Policlinico 155, I-00161 Roma (IT).

(74) Agent: **MARSHALL, Cameron, John**; Carpmaels & Ransford, 43-45 Bloomsbury Square, London WC1 A2RA (GB).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/109186 A2

(54) Title: EARLY ENDOSOME NEUTRALISING COMPOUDS AS VACCINE ADJUVANTS

(57) Abstract: Early endosome neutralising compounds, such as chloroquine, can be used as vaccine adjuvants. Thus the invention provides an immunogenic composition comprising: (a) an adjuvant comprising an early endosome neutralising compound; and (b) an antigen. The invention further provides methods of treatment employing the compositions.

EARLY ENDOSOME NEUTRALISING COMPOUNDS AS VACCINE ADJUVANTS

All documents cited herein are incorporated by reference in their entirety.

TECHNICAL FIELD

This invention is in the field of vaccine adjuvants, particularly adjuvants which promote a cellular immune response and/or protective CD8 immunity.

BACKGROUND ART

Vaccination with purified antigens alone is typically insufficient to elicit a protective immune response, so vaccines almost always require formulation with adjuvants. Different adjuvants have different immunological profiles and there is an ongoing need for new adjuvants for inclusion in vaccines.

Currently, only aluminium salts and the MF59™ are approved for human use. However, aluminium salts are subject to safety concerns and are incompatible with some antigens. There is therefore a need to develop further adjuvants.

DISCLOSURE OF THE INVENTION

Surprisingly, it has now been found that early endosome neutralising compounds function as vaccine adjuvants when administered in combination with an antigen. Therefore, the invention provides an immunogenic composition comprising: (a) an adjuvant comprising an early endosome neutralising compound; and (b) an antigen.

The composition is preferably suitable for inducing a cellular immune response against the antigen, more preferably a protective CD8 response.

Generally, class I major histocompatibility complex (MHC) molecules present peptides derived from endogenous antigens (*i.e.*, viral, tumor-, or self-proteins), that have been synthesized and processed within cells. This selective pathway guarantees that effector CD8+ T cells kill unwanted cells, sparing cells which have taken up soluble pathogen or tumor-derived proteins via the endocytic route. However, some specialized antigen-presenting cells (APCs) are capable of presenting exogenous antigens to CD8+ T cells. This process, termed cross-presentation, is primarily carried out by dendritic cells (DCs) *in vivo*, and seems to be crucial for inducing both cytotoxic T lymphocyte (CTL) immunity (cross-priming) against allografts, tumors and pathogens that do not infect or functionally impair APCs, and tolerance (cross-tolerance) against self-antigens.

The inventor has found that the endosomal processing machinery of professional antigen presenting cells plays a critical role in the limitation of cross-presentation of soluble antigens. Treatment with early endosome neutralising compounds (*e.g.* chloroquine) abolishes endosomal antigen degradation which greatly improves cross-presentation efficiency and leads to a dramatic improvement of both antigen-accumulation in early endosomes, and translocation into cytosol. Inhibition of endocytic processing favours human cross-presentation *in vivo*.

The immune response

The adjuvant comprising an early endosome neutralising compound is preferably suitable for enhancing a cellular immune response against the antigen *e.g.* it can promote the activation of cytotoxic T cells and inflammatory T cells. Preferably, the cellular immune response is a protective CD8 response, such that the adjuvant promotes the formation and persistence of memory CD8+ T cells for long-term, vaccine-induced resistance against the antigen.

Early endosome neutralising compounds

The adjuvant included in the compositions of the invention may be any suitable early endosome neutralising compound, including those which are isolated from a naturally occurring source and those which are chemically synthesised.

The term early endosome neutralising compound refers to compounds that exert a pH neutralising effect or reduction in acidity of the early endosome compartment. This effect can conveniently be measured *in vitro* using dendritic cells (DC). The pH indicator LysoSensor Yellow/Blue DND-160 (L-7545 - Molecular Probe) increases in fluorescence intensity upon acidification, as indicated on the manufacturer's sheet. Briefly, DC are incubated with or without a given neutralizing compound for 30 min, and then pulsed with 5 μM LysoSensor for further 4 h. After washing, DC are fixed and then visualized by microscope. The dye emits yellow fluorescence when it accumulated in acidic vesicles, and green fluorescence in neutral vesicles.

The adjuvant may be selected from the group consisting of chloroquine, quinine, quinidine, cinchonine, mefloquine, halofantrine, DIDS (4,4′-diisothiocyanatostil- bene-2,2′-disulfonic acid), NPPB (5-nitro-2-(3-phenylpropylamino-benzoic acid), vacuolar-type H+-ATPase inhibitors such as bafilomycin A1 or folimycin and protonophores such as monensin and nigericin.

Preferably, the adjuvant is non toxic i.e. it has substantially no toxic effect when administered in pharmacologically useful amounts.

Preferably the early endosome neutralising compound is lysosomotrophic. More preferably the early endosome neutralising compound is chloroquine.

The adjuvant can be formulated in various ways within the composition.

The adjuvant is preferably present in a soluble aqueous form. If the composition is formulated as an oil-in-water emulsion then the adjuvant will usually be present in the aqueous phase.

The adjuvant will generally be used at a concentration of between 0.1 and 1 μg/ml

Preferably the early endosome neutralising compound adjuvant is chloroquine or a chloroquine related compound.

It will be appreciated that where endosome neutralising compounds exist as geometrical isomers and/or enantiomers or diastereomers then the invention extends to all such isomers of the compounds, and to mixtures thereof, including racemates.

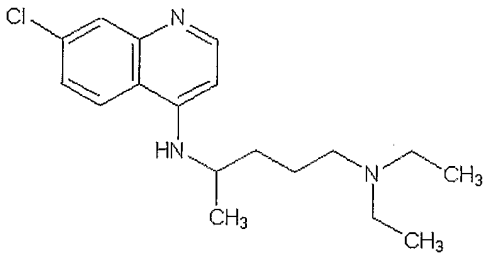
Chloroquine

Chloroquine is a lysosomotrophic amine which is used as an antimalarial and an amebicide. Chloroquine also has anti-inflammatory activity and is used in high doses to treat the auto-immune diseases rheumatoid arthritis, systemic lupus erythematosus, and discoid lupus erythematosus. Chloroquine reaches high concentration within the lysosomes of the malarial parasites. Chloroquine also inhibits the parasitic enzyme heme polymerase that converts the toxic heme into non-toxic hemazoin, thereby resulting in the accumulation of toxic heme within the parasite. It may also interfere with the biosynthesis of nucleic acids.

Chloroquine has been associated with the inhibition of antigen presentation to T-cells and was also found to reduce macrophage antigen catabolism [1]. Chloroquine has also been shown to suppress the T-cell response to minor histocompatibility antigens (MiHCs) and has been postulated to induce decreased T-cell viability at high concentrations [2]. Its activity as an adjuvant is therefore surprising.

Chloroquine offers many advantages when used as an adjuvant owing to its proven safety record in humans (including during pregnancy), low cost and ease of use. Furthermore, the chloroquine may have a further function within the compositions of the invention as a malarial treatment. As such, the immunogenic compositions of the invention may be particularly suitable in vaccines for use in malarial regions.

Preferably, the chloroquine adjuvant has the formula (1):



Analogues of chloroquine and chloroquine-related compounds (figure 3) are also suitable for use in the invention.

Any pharmaceutically acceptable salt or ester of chloroquine may be used as an adjuvant according to the invention. Examples of suitable salts are chloroquine hydrochloride, chloroquine phosphate and chloroquine diphosphate. Preferably, the salt used in the immunogenic composition of the invention is chloroquine diphosphate. The invention extends to all isomers of the compounds of formula (1), and to mixtures thereof, including racemates.

Chloroquine-related compounds

Quinine, quinidine (cinchona alkaloids), cinchonine, mefloquine and halofantrine are aryl amino alcohols with similar structures and are predicted to have similar mechanisms of action. Plasmodium cross resistance for more than one compound of this class has been reported.

Quinine acts as a blood schizonticide although it also has gametocytocidal activity against *P. vivax* and *P. malariae*. Because it is a weak base, it is concentrated in the food vacuoles of *P. falciparum*. It is said to act by inhibiting heme polymerase, thereby allowing accumulation of its cytotoxic substrate, heme. Quinidine is an antiarrhythmic drug related to quinine.

Mefloquine has been found to produce swelling of *P. falciparum* food vacuoles. It may act by forming toxic complexes with free heme that damage membranes and interact with other plasmodial components. It is effective against the blood forms of falciparum malaria, including the chloroquine resistant types.

Halofantrine is a phenanthrene methanol structurally related to quinine. Its mechanism of action may be similar to that of chloroquine, quinine, and mefloquine; by forming toxic complexes with ferritoporphyrin IX that damage the membrane of the parasite. This synthetic anti malarial is effective against multi drug resistant (including mefloquine resistant) *P. falciparum* malaria.

As with chloroquine, quinine, quinidine (cinchona alkaloids), cinchonine, mefloquine and halofantrine further function within the compositions of the invention as malarial treatments. As such, the immunogenic compositions of the invention may be particularly suitable in vaccines for use in malarial regions.

NPPB (5- nitro-2-(3-phenylpropylamino) benzoic acid) and DIDS (4,4'diisothio-cyanatostilbene-2,2'-disulfonicacid)

NPPB is a Cl- channel inhibitor and, like DIDS, reduces endosomal acidification due to the inhibition of chloride channel activity (Biochim Biophys Acta. 1996 Oct 23;1284(2):171-80). Via the same mechanism, NPPB inhibits the transport of a wide range of solutes into human erythrocytes infected *in vitro* with *P. falciparum*. This generally increases human erythrocyte permeability to a variety of low molecular weight solutes (Biol Chem. 1994 Feb;269(5):3339-47). In addition, DIDS is known to specifically bind to band 3-related adhesin and to inhibit the binding of Plasmodium falciparum-infected erythrocytes (PE) to endothelial cells (mediated by the band 3-related adhesin).

Monensin and nigericin, which are protonophores, are known to neutralize the endosome lumen [3].These compounds are also suitable for use in the present invention.

Antigens

The compositions of the invention are preferably immunogenic *e.g.* vaccines. Vaccines according to the invention may either be prophylactic (*i.e.* to prevent infection) or therapeutic (*i.e.* to treat disease after infection), but will typically be prophylactic.

The compositions of the invention therefore comprise an immunologically effective amount of at least one antigen. By 'immunologically effective amount', it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (*e.g.* non-human primate, primate,

etc.), the capacity of the individual's immune system to synthesise antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

Antigens suitable for use in the compositions of the invention may be bacterial or viral antigens. Suitable antigens may be further classified as protein antigens, carbohydrate antigens or glycoconjugate antigens. The compositions of the invention may include one or more antigens. Examples of suitable antigens are:

- an outer-membrane vesicle (OMV) preparation from *N.meningitidis* serogroup B, such as those disclosed in refs. 4-7 *etc.*
- a saccharide antigen from *N.meningitidis* serogroup A, C, W135 and/or Y, such as the oligosaccharide disclosed in ref. 8 from serogroup C [see also ref. 9] or the oligosaccharides of ref. 10.
- a protein antigen from *N.meningitidis* serogroup B, such as those disclosed in refs. 11-19, *etc.*
- antigens from *Helicobacter pylori* such as CagA [20 to 23], VacA [24, 25], NAP [26, 27, 28], HopX [*e.g.* 29], HopY [*e.g.* 29] and/or urease.
- a saccharide antigen from *Streptococcus pneumoniae* [*e.g.* 30, 31, 32].
- a protein antigen from *S.pneumoniae* (*e.g.* from PhtA, PhtD, PhtB, PhtE, SpsA, LytB, LytC, LytA, Sp125, Sp101, Sp128, Sp130 and Sp133, as disclosed in reference 33.)
- an antigen from hepatitis A virus, such as inactivated virus [*e.g.* 34, 35].
- an antigen from hepatitis B virus, such as the surface and/or core antigens [*e.g.* 35, 36].
- an antigen from hepatitis C virus [*e.g.* 37].
- a diphtheria antigen, such as a diphtheria toxoid [*e.g.* chapter 3 of ref. 38].
- a tetanus antigen, such as a tetanus toxoid [*e.g.* chapter 4 of ref. 38].
- an antigen from *Bordetella pertussis*, such as pertussis holotoxin (PT) and filamentous haemagglutinin (FHA) from *B.pertussis*, optionally also in combination with pertactin and/or agglutinogens 2 and 3 [*e.g.* refs. 39 & 40].
- a saccharide antigen from *Haemophilus influenzae* type b [*e.g.* 9].
- polio antigen(s) [*e.g.* 41, 42] such as IPV.
- an antigen from *Neisseria gonorrhoeae* [*e.g.* 11,13, 43].
- an antigen from *Chlamydia pneumoniae* [*e.g.* refs. 44 to 50].
- an antigen from *Chlamydia trachomatis* [*e.g.* 51].
- an antigen from *Porphyromonas gingivalis* [*e.g.* 52].
- rabies antigen(s) [*e.g.* 53] such as lyophilised inactivated virus [*e.g.* 54, RabAvert™].
- measles, mumps and/or rubella antigens [*e.g.* chapters 9, 10 & 11 of ref. 38].

- influenza antigen(s) [*e.g.* chapter 19 of ref. 38], such as the haemagglutinin and/or neuraminidase surface proteins. The flu antigen may be selected from a pandemic strain.
- antigen(s) from a paramyxovirus such as respiratory syncytial virus (RSV [55, 56]) and/or parainfluenza virus (PIV3 [57]).
- an antigen from *Moraxella catarrhalis* [*e.g.* 58].
- an antigen from *Streptococcus pyogenes* (group A streptococcus) [*e.g.* 59, 60, 61].
- an antigen from *Streptococcus agalactiae* (group B streptococcus) [*e.g.* 62, 63, 64].
- an antigen from *Staphylococcus aureus* [*e.g.* 65].
- an antigen from *Bacillus anthracis* [*e.g.* 66, 67, 68].
- an antigen from a virus in the flaviviridae family (genus flavivirus), such as from yellow fever virus, Japanese encephalitis virus, four serotypes of Dengue viruses, tick-borne encephalitis virus, West Nile virus.
- a pestivirus antigen, such as from classical porcine fever virus, bovine viral diarrhoea virus, and/or border disease virus.
- a parvovirus antigen *e.g.* from parvovirus B19.
- a prion protein (*e.g.* the CJD prion protein)
- an amyloid protein, such as a beta peptide [69]
- a cancer antigen, such as those listed in Table 1 of ref. 70 or in tables 3 & 4 of ref. 71.

Where a saccharide antigen is used, it is preferably conjugated to a carrier in order to enhance immunogenicity. Conjugation of *H.influenzae* B, meningococcal and pneumococcal saccharide antigens is well known in the art. Toxic protein antigens may be detoxified where necessary (*e.g.* detoxification of pertussis toxin by chemical and/or genetic means).

Where a diphtheria antigen is included in the composition it is preferred also to include tetanus antigen and pertussis antigens. Similarly, where a tetanus antigen is included it is preferred also to include diphtheria and pertussis antigens. Similarly, where a pertussis antigen is included it is preferred also to include diphtheria and tetanus antigens. DTP combinations are thus preferred.

Saccharide antigens are preferably in the form of conjugates. Preferred carrier proteins for conjugates are bacterial toxins or toxoids, such as diphtheria toxoid or tetanus toxoid. The CRM197 mutant of diphtheria toxin [72-74] is a particularly preferred carrier for, as is a diphtheria toxoid. Other suitable carrier proteins include the *N.meningitidis* outer membrane protein [75], synthetic peptides [76,77], heat shock proteins [78,79], pertussis proteins [80,81], cytokines [82], lymphokines [82], hormones [82], growth factors [82], artificial proteins comprising multiple human $CD4^+$ T cell epitopes from various pathogen-derived antigens [83] such as the N19 protein [84], protein D from *H.influenzae* [85,86], pneumococcal surface protein PspA [87], pneumolysin [88], iron-uptake proteins [89], toxin A or B from *C.difficile* [90], *etc.*

Antigens in the composition will typically be present at a concentration of at least 1μg/ml each. In general, the concentration of any given antigen will be sufficient to elicit an immune response against that antigen.

As an alternative to using proteins antigens in the mixture, nucleic acid encoding the antigen may be used. Protein components of the mixture may thus be replaced by nucleic acid (preferably DNA *e.g.* in the form of a plasmid) that encodes the protein. Similarly, compositions of the invention may comprise proteins which mimic saccharide antigens *e.g.* mimotopes [91] or anti-idiotype antibodies.

Pharmaceutical compositions

The composition of the invention will typically, in addition to the components mentioned above, comprise one or more 'pharmaceutically acceptable carriers', which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The vaccines may also contain diluents, such as water, saline, glycerol, *etc.* Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier. A thorough discussion of pharmaceutically acceptable excipients is available in reference 92.

Compositions of the invention are generally presented in aqueous form (*e.g.* solutions or suspensions). In some embodiments of the invention the compositions are in aqueous form from the packaging stage to the administration stage ("full liquid vaccine"). In this way the composition can be administered direct from their packaged form, without the need for reconstitution in an aqueous medium. In other embodiments, however, one or more components of the compositions may be packaged in a lyophilised form, and a vaccine for actual administration may be reconstituted when necessary. Thus compositions of the invention may be prepared at a packaging stage, or may be prepared extemporaneously prior to use.

Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect. Direct delivery of the compositions will generally be parenteral (*e.g.* by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue). The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*e.g.* see ref. 93), needles, and hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule (*e.g.* including booster doses).

Compositions may be presented in vials or in ready-filled syringes. The syringes may be supplied with or without needles. A syringe will include a single dose of the composition, whereas a vial may include a single dose or multiple doses.

Compositions of the invention may be packaged in unit dose form or in multiple dose form. For multiple dose forms, vials are preferred to pre-filled syringes. Effective dosage volumes can be routinely established, but a typical human dose of a composition for injection has a volume of about 0.5ml. Similar doses may be used for other delivery routes *e.g.* an intranasal vaccine for atomisation may have a volume of about 125µl per spray, with four sprays administered to give a total dose of about 0.5ml.

The pH of the composition (including lyophilised compositions, after reconstitution) is preferably between 6 and 8, preferably about 7. Stable pH may be maintained by the use of a buffer. The composition may be sterile and/or pyrogen-free. Compositions of the invention may be isotonic with respect to humans.

Compositions of the invention may include an antimicrobial and/or a preservative, particularly when packaged in multiple dose format.

Compositions of the invention may comprise detergent *e.g.* a Tween (polysorbate), such as Tween 80. Detergents are generally present at low levels *e.g.* <0.01%.

Compositions of the invention may include sodium salts (*e.g.* sodium chloride) to give tonicity.

Compositions of the invention will generally include a buffer. A phosphate or histidine buffer is typical.

Methods of treatment

The invention also provides the use of: (a) an early endosome neutralising compound adjuvant; and (b) at least one antigen, in the manufacture of a medicament for administration to a mammal to induce an immune response, preferably a cellular immune response, more preferably protective CD8 immunity.

The invention also provides a method for raising an immune response against at least one antigen, comprising the step of administering at least one antigen to a patient with an early endosome neutralising compound adjuvant.

The adjuvant and the antigen(s) may be administered simultaneously, sequentially or separately. For example, the adjuvant may be administered to prime the mammal before administration of the antigen or after the administration of the antigen to boost the mammal's immune response to that antigen.

The invention also provides the use of at least one antigen in the manufacture of a medicament for raising an immune response in a patient, wherein the medicament is administered with early endosome neutralising compound adjuvant. Similarly, the invention provides the use of an adjuvant

comprising an early endosome neutralising compound in the manufacture of a medicament for raising an immune response in a patient, wherein the medicament is administered with at least one antigen. The inclusion of chloroquine in the medicament preferably increases the immune response against the antigen.

The invention also provides the use of at least one antigen in the manufacture of a medicament for raising an immune response in a patient, where the patient has been pre-treated with an early endosome neutralising compound adjuvant. The invention also provides the use of an adjuvant comprising an early endosome neutralising compound in the manufacture of a medicament for raising an immune response in a patient, where the patient has been pre-treated with at least one antigen. The inclusion of chloroquine in the medicament preferably increases the immune response against the antigen.

The invention also provides a composition of the invention for use in medicine.

The invention also provides (a) an early endosome neutralising compound adjuvant; and (b) at least one antigen, for simultaneous separate of sequential administration.

The mammal is preferably a human. Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.* a toddler or infant) or a teenager; where the vaccine is for therapeutic use, the human is preferably an adult. A vaccine intended for children may also be administered to adults *e.g.* to assess safety, dosage, immunogenicity, *etc.*

The invention may be used to elicit systemic and/or mucosal immunity. For example, the invention may be used to elicit the production of specific IgA, IgG and/or IgM antibodies.

Dosage treatment can be a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. A primary dose schedule may be followed by a booster dose schedule. Suitable timing between priming doses (*e.g.* between 4-16 weeks), and between priming and boosting, can be routinely determined.

Further adjuvants

Chloroquine can act as an adjuvant within the compositions of the invention. It is also possible to include one or more further adjuvants. Such adjuvants include, but are not limited to:

A. Mineral-containing compositions

Mineral containing compositions suitable for use as adjuvants in the invention include mineral salts, such as aluminium salts and calcium salts. The invention includes mineral salts such as hydroxides (*e.g.* oxyhydroxides), phosphates (*e.g.* hydroxyphosphates, orthophosphates), sulphates, *etc.* [*e.g.* see chapters 8 & 9 of ref. 94], or mixtures of different mineral compounds, with the compounds taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*), and with adsorption being preferred. The mineral containing compositions may also be formulated as a particle of metal salt [95].

A typical aluminium phosphate adjuvant is amorphous aluminium hydroxyphosphate with PO_4/Al molar ratio between 0.84 and 0.92, included at 0.6mg Al^{3+}/ml. Adsorption with a low dose of aluminium phosphate may be used *e.g.* between 50 and 100μg Al^{3+} per conjugate per dose. Where an aluminium phosphate it used and it is desired not to adsorb an antigen to the adjuvant, this is favoured by including free phosphate ions in solution (*e.g.* by the use of a phosphate buffer).

B. Oil Emulsions

Oil emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as MF59 [Chapter 10 of ref. 94; see also ref. 96] (5% Squalene, 0.5% Tween 80, and 0.5% Span 85, formulated into submicron particles using a microfluidizer). Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) may also be used.

C. Saponin formulations [chapter 22 of ref. 94]

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

Saponin compositions have been purified using HPLC and RP-HPLC. Specific purified fractions using these techniques have been identified, including QS7, QS17, QS18, QS21, QH-A, QH-B and QH-C. Preferably, the saponin is QS21. A method of production of QS21 is disclosed in ref. 97. Saponin formulations may also comprise a sterol, such as cholesterol [98].

Combinations of saponins and cholesterols can be used to form unique particles called immunostimulating complexs (ISCOMs) [chapter 23 of ref. 94]. ISCOMs typically also include a phospholipid such as phosphatidylethanolamine or phosphatidylcholine. Any known saponin can be used in ISCOMs. Preferably, the ISCOM includes one or more of QuilA, QHA and QHC. ISCOMs are further described in refs. 98-100. Optionally, the ISCOMS may be devoid of additional detergent [101].

A review of the development of saponin based adjuvants can be found in refs. 102 & 103.

D. Virosomes and virus-like particles

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

Hepatitis E virus, measles virus, Sindbis virus, Rotavirus, Foot-and-Mouth Disease virus, Retrovirus, Norwalk virus, human Papilloma virus, HIV, RNA-phages, Qß-phage (such as coat proteins), GA-phage, fr-phage, AP205 phage, and Ty (such as retrotransposon Ty protein p1). VLPs are discussed further in refs. 104-109. Virosomes are discussed further in, for example, ref. 110

E. Bacterial or microbial derivatives

Adjuvants suitable for use in the invention include bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof.

Non-toxic derivatives of LPS include monophosphoryl lipid A (MPL) and 3-O-deacylated MPL (3dMPL). 3dMPL is a mixture of 3 de-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains. A preferred "small particle" form of 3 De-O-acylated monophosphoryl lipid A is disclosed in ref. 111. Such "small particles" of 3dMPL are small enough to be sterile filtered through a 0.22μm membrane [111]. Other non-toxic LPS derivatives include monophosphoryl lipid A mimics, such as aminoalkyl glucosaminide phosphate derivatives *e.g.* RC-529 [112,113].

Lipid A derivatives include derivatives of lipid A from *Escherichia coli* such as OM-174. OM-174 is described for example in refs. 114 & 115.

Immunostimulatory oligonucleotides suitable for use as adjuvants in the invention include nucleotide sequences containing a CpG motif (a dinucleotide sequence containing an unmethylated cytosine linked by a phosphate bond to a guanosine). Double-stranded RNAs and oligonucleotides containing palindromic or poly(dG) sequences have also been shown to be immunostimulatory.

The CpG's can include nucleotide modifications/analogs such as phosphorothioate modifications and can be double-stranded or single-stranded. References 116, 117 and 118 disclose possible analog substitutions *e.g.* replacement of guanosine with 2'-deoxy-7-deazaguanosine. The adjuvant effect of CpG oligonucleotides is further discussed in refs. 119-124.

The CpG sequence may be directed to TLR9, such as the motif GTCGTT or TTCGTT [125]. The CpG sequence may be specific for inducing a Th1 immune response, such as a CpG-A ODN, or it may be more specific for inducing a B cell response, such a CpG-B ODN. CpG-A and CpG-B ODNs are discussed in refs. 126-128. Preferably, the CpG is a CpG-A ODN.

Preferably, the CpG oligonucleotide is constructed so that the 5' end is accessible for receptor recognition. Optionally, two CpG oligonucleotide sequences may be attached at their 3' ends to form "immunomers". See, for example, refs. 125 & 129-131.

Bacterial ADP-ribosylating toxins and detoxified derivatives thereof may be used as adjuvants in the invention. Preferably, the protein is derived from *E.coli* (*E.coli* heat labile enterotoxin "LT"), cholera ("CT"), or pertussis ("PT"). The use of detoxified ADP-ribosylating toxins as mucosal adjuvants is described in ref. 132 and as parenteral adjuvants in ref. 133. The toxin or toxoid is preferably in the form of a holotoxin, comprising both A and B subunits. Preferably, the A subunit contains a

detoxifying mutation; preferably the B subunit is not mutated. Preferably, the adjuvant is a detoxified LT mutant such as LT-K63, LT-R72, and LT-G192. The use of ADP-ribosylating toxins and detoxified derivaties thereof, particularly LT-K63 and LT-R72, as adjuvants can be found in refs. 134-141. Numerical reference for amino acid substitutions is preferably based on the alignments of the A and B subunits of ADP-ribosylating toxins set forth in ref. 142, specifically incorporated herein by reference in its entirety.

F. Human immunomodulators

Human immunomodulators suitable for use as adjuvants in the invention include cytokines, such as interleukins (*e.g.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 [143], *etc.*) [144], interferons (*e.g.* interferon-γ), macrophage colony stimulating factor, and tumor necrosis factor.

G. Bioadhesives and Mucoadhesives

Bioadhesives and mucoadhesives may also be used as adjuvants in the invention. Suitable bioadhesives include esterified hyaluronic acid microspheres [145] or mucoadhesives such as cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. Chitosan and derivatives thereof may also be used as adjuvants in the invention [146].

H. Microparticles

Microparticles may also be used as adjuvants in the invention. Microparticles (*i.e.* a particle of ~100nm to ~150μm in diameter, more preferably ~200nm to ~30μm in diameter, and most preferably ~500nm to ~10μm in diameter) formed from materials that are biodegradable and non-toxic (*e.g.* a poly(α-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, *etc.*), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (*e.g.* with SDS) or a positively-charged surface (*e.g.* with a cationic detergent, such as CTAB).

I. Liposomes (Chapters 13 & 14 of ref. 94)

Examples of liposome formulations suitable for use as adjuvants are described in refs. 147-149.

J. Polyoxyethylene ether and polyoxyethylene ester formulations

Adjuvants suitable for use in the invention include polyoxyethylene ethers and polyoxyethylene esters [150]. Such formulations further include polyoxyethylene sorbitan ester surfactants in combination with an octoxynol [151] as well as polyoxyethylene alkyl ethers or ester surfactants in combination with at least one additional non-ionic surfactant such as an octoxynol [152]. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether (laureth 9), polyoxyethylene-9-steoryl ether, polyoxytheylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether.

K. Polyphosphazene (PCPP)

PCPP formulations are described, for example, in refs. 153 and 154.

L. Muramyl peptides

Examples of muramyl peptides suitable for use as adjuvants in the invention include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE).

M. Imidazoquinolone Compounds.

Examples of imidazoquinolone compounds suitable for use adjuvants in the invention include Imiquamod and its homologues (*e,g.* "Resiquimod 3M"), described further in refs. 155 and 156.

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention: (1) a saponin and an oil-in-water emulsion [157]; (2) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) [158]; (3) a saponin (*e.g.* QS21) + a non-toxic LPS derivative (*e.g.* 3dMPL) + a cholesterol; (4) a saponin (*e.g.* QS21) + 3dMPL + IL-12 (optionally + a sterol) [159]; (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions [160]; (6) SAF, containing 10% squalane, 0.4% Tween 80™, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion. (7) RibiTM adjuvant system (RAS), (Ribi Immunochem) containing 2% squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); and (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dMPL).

Other substances that act as immunostimulating agents are disclosed in chapter 7 of ref. 94.

Preparation of compositions of the invention

The early endosome neutralising compound adjuvant of the invention is particularly suited to inclusion in immunogenic compositions and vaccines. A process of the invention may therefore include the step of mixing the adjuvant with an antigen. The invention provides a composition or vaccine obtainable in this way. Where a composition of the invention includes antigens from more than one organism, the antigens are preferably prepared separately and then admixed with the early endosome neutralising compound adjuvant to give a composition of the invention.

A composition of the invention may thus be prepared from a kit comprising: (a) early endosome neutralising compound adjuvant and (b) at least one antigen. The early endosome neutralising compounds and/or the at least one antigen may be present in lyophilised form. The invention also provides a method for preparing a composition of the invention, comprising mixing an early endosome neutralising compound adjuvant with one or more antigens (*e.g.* 1, 2, 3), wherein said one or more antigens are in liquid form.

Compositions of the invention may be formed by adding antigen to bulk adjuvant, or adding adjuvant to bulk antigen. Where the composition includes more than one antigen and/or more than one adjuvant, antigen(s) and adjuvant(s) may be mixed in any suitable order.

Definitions

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

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

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. Cross-presentation efficiency is dependent on inhibition of antigen degradation.

a-d. Representative flow cytometry analysis of HLA-A2-restricted $NS3_{1406-1415}$-specific $CD8^+$ T cell clone producing IFN-γ in response to NS3Ag cross-presented by HLA-$A2^+$ DCs, in the presence or absence of the indicated compounds. Dot plots show double CD8 and IFN-γ staining. Results are expressed as percentage of cells, indicated in each quadrant. **e.** NS3Ag cross-presentation after 12 hr pulsing by six independent HLA-$A2^+$ DC populations to $NS3_{1406-1415}$-specific CD8 clone (mean SD), in the absence (diamond) or presence of chloroquine (triangle) (presence versus absence of chloroquine: *$p<0.0001$), leupeptin (square), or lactacystin (circle). **f.** Chase time of NS3Ag (50 μg/ml) cross-presentation after 30 min of pulsing by six independent DC populations (mean±SD), in the presence (triangle) or absence (diamond) of chloroquine. Presence versus absence of chloroquine: *$p=0.001$; **$p<0.0001$. **g.** NS3Ag (filled diamond), NS3Ag + chloroquine (triangle), or peptide (empty diamond) presentation by three independent HLA-$A2^+$ EBV-B cells to $NS3_{1406-1415}$-specific CD8 clone (mean±SD) **h.** Presentation of NS3Ag (presence versus absence of chloroquine: *$p<0.0001$) by four independent DC populations to $NS3_{1241-1260}$-specific CD4 T cell clone (mean±SD), in the presence (triangle) or absence (diamond) of chloroquine. **i-j.** Presentation of the indicated peptides to the $NS3_{1406-1415}$-specific CD8 clone (**i**) or the $NS3_{1241-1260}$-specific CD4 T cell clone (**j**) in the absence (diamond) or presence of chloroquine (triangle), or lactacystin (circle).

Figure 2. Chloroquine-boosted cross-presentation elicits antigen-specific $CD8^+$ T cells in vivo.

(a-b). Representative flow cytometry experiments on PBMCs from either a subject, submitted to both chloroquine-treatment (300 mg for two consecutive days) and anti-hepatitis B virus (HBV) vaccine boost (**a**), or a subject only submitted to the anti-HBV vaccine boost (**b**). Fresh cells were tested for their capacity to promptly produce IFN-γ within 4-6 hrs of contact with the indicated antigen or peptide. Dot plot are gated on CD8 cells and show IFN-γ staining. Results are expressed as percentage of IFN-γ^+ cells. **c-d.** Histograms reporting percentages of functional HBenvAg-specific CD8 T cells *in vivo* from all individuals studied [either chloroquine-treated (**c**) or untreated (**d**)],

submitted to the anti-HBV vaccination boost. The responses both to increasing HBenvAg concentrations and to a single dose of the indicated HLA-A2-binder peptide are reported.

Figure 3. Chemical structure of chloroquine-related compounds

MODES FOR CARRYING OUT THE INVENTION

Cross presentation efficiency is dependent on inhibition of antigen degradation

Dendritic cells (DCs) or EBV-transformed (EBV-)B cells, as antigen-presenting cells (APCs), were pulsed with increasing concentrations of soluble recombinant NS3Ag(1187-1465) , or relevant NS3 peptides in the presence or absence of 10 mg/ml chloroquine, 10 mg/ml leupeptin, or 80 mM lactacystin, fixed with 0.05% glutaraldehyde, and co-cultured ($2\text{-}3x10^4$/well) with HLA-matched NS3(1406-1415)-specific CD8+, or NS3(1241-1260)-specific CD4+ T cell clones ($2\text{-}3x10^4$/well) for 6 hours at 37°C.

After 2 hours, 10 mg/ml Brefeldin-A was added to the culture. Cells were washed, stained with tricolor (TC)-labelled anti-CD8, fixed and permeabilized using Cytofix/Cytoperm solution at 4°C for 20 min. Cells were then re-washed with Perm Wash Buffer, and stained for detection of intracytoplasmic IFN-γ with FITC-labelled anti-IFN-γ for 30 min at 4°C. Cells were washed, acquired with a FACScanTM flow cytometer and analyzed using CellQuest™ software.

Negative controls were obtained by staining cells with an irrelevant isotype-matched mAb. The results of this experiment are presented in figure 1.

Mouse model

C57BL/6 mice were immunized intraperitoneally with syngeneic dendritic cells (DCs) that had previously been pulsed with high concentrations of soluble ovalbumin (0.5-1 mg) in the presence or absence of 10-20 µg/ml of chloroquine. The group of mice that were immunized with DC+OVA+chloro, but not the group immunized with DC+OVA alone, also received 800 µg/mouse of chloroquine intraperitoneally.

After 15-30 days, mice were sacrificed and their spleen or lymph node cells were tested in a cytotoxicity assay using syngeneic target cells pulsed with $OVA_{257\text{-}264}$ peptide. Results of one representative experiment are below:

Group	% lysis		
	50	**25**	**12.5**
DC+OVA	35	20	3
DC+OVA+cq	76	44	12

Thus chloroquine increases the ability of DCs to lyse target cells.

Chloroquine-boosted cross-presentation elicits antigen-specific CD8$^+$ T cells <u>in vivo</u>

Six volunteers (four HLA-A2+), who had been vaccinated against hepatitis B virus (HBV) 8-10 years before, were administered 500 mg Chloroquine Bayer (Bayer AG, Leverkusen, Germany) per os, corresponding to 300 mg base chloroquine. This was followed by a booster dose of anti-HBV vaccine (Engerix-B,GlaxoSmithKline, UK) on the 2nd day. As a control, eight healthy individuals (six HLA-A2+), who only received the booster dose of their regular procedure of anti-HBV vaccination, were used.

In both groups, PBMCs were collected before and after 15 days from the vaccination booster, and each sample was stored in at least three independent vials in liquid nitrogen, in order to perform the functional analyses at least twice for each time point.

PBMC samples were stimulated for 6 hr with autologous irradiated PBMCs, which had been previously pulsed with different concentrations of recombinant HBenvAg (GlaxoSmithKline Biologicals Rixensart, Belgium) or peptide in the presence of chloroquine, and stained with FITC-labelled anti-CD8 mAb. Finally, the induction IFN-g production was determined, as described above.

The results for this experiment are presented in figure 2.

Effect of chloroquine on CD8+ T cell responses in an animal model of disease

The effect of chloroquine in CD8+ T cell responses is investigated in a model of tumour vaccination. OT-I cells (naïve ovalbumin (OVA)-specific CD8 T cells) are transferred into mice carriers. Mice are then vaccinated with soluble OVA with or without intraperitoneal administration of a chloroquine adjuvant. Tumour progression (size/growth) is monitored to determine the effect of the chloqoquine adjuvant in relation to priming, expansion and effector differentiation of the OT-I cells.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

REFERENCES (the contents of which are hereby incorporated in full)

[1] Ziegler H.K. & Unanue E.R. (1982) PNAS January 1, 79(1):175-178
[2] Schultz K.R. et al. (1997) Transplantation Oct 15:64(7):1055-65
[3] Alami *et al.* (1998) *Mol Biol Cell* 9(2):387-402.
[4] Bjune *et al.* (1991) *Lancet* 338(8775):1093-96
[5] WO01/52885.
[6] Fukasawa *et al.* (1999) *Vaccine* 17:2951-2958.
[7] Rosenqvist *et al.* (1998) *Dev. Biol. Stand.* 92:323-333.
[8] Costantino *et al.* (1992) *Vaccine* 10:691-698.
[9] Costantino *et al.* (1999) *Vaccine* 17:1251-1263.
[10] International patent application WO03/007985.
[11] WO99/24578
[12] WO99/36544
[13] WO99/57280
[14] WO00/66791
[15] WO01/64922
[16] WO01/64920
[17] WO03/020756
[18] WO2004/032958
[19] WO2004/048404.
[20] Covacci & Rappuoli (2000) *J. Exp. Med.* 19:587-592.
[21] WO93/18150.
[22] Covacci *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90: 5791-5795.
[23] Tummuru *et al.* (1994) *Infect. Immun.* 61:1799-1809.
[24] Marchetti *et al.* (1998) *Vaccine* 16:33-37.
[25] Telford *et al.* (1994) *J. Exp. Med.* 179:1653-1658.
[26] Evans *et al.* (1995) *Gene* 153:123-127.
[27] WO96/01272 & WO96/01273, especially SEQ ID NO:6.
[28] WO97/25429.
[29] WO98/04702.
[30] Watson (2000) *Pediatr Infect Dis J* 19:331-332.
[31] Rubin (2000) *Pediatr Clin North Am* 47:269-285, v.
[32] Jedrzejas (2001) *Microbiol Mol Biol Rev* 65:187-207.
[33] WO02/22167.
[34] Bell (2000) *Pediatr Infect Dis J* 19:1187-1188.
[35] Iwarson (1995) *APMIS* 103:321-326.
[36] Gerlich *et al.* (1990) *Vaccine* 8 Suppl:S63-68 & 79-80.
[37] Hsu *et al.* (1999) *Clin Liver Dis* 3:901-915.
[38] *Vaccines* (1988) eds. Plotkin & Mortimer. ISBN 0-7216-1946-0.
[39] Gustafsson *et al.* (1996) *N. Engl. J. Med.* 334:349-355.
[40] Rappuoli *et al.* (1991) *TIBTECH* 9:232-238.
[41] Sutter *et al.* (2000) *Pediatr Clin North Am* 47:287-308.
[42] Zimmerman & Spann (1999) *Am Fam Physician* 59:113-118, 125-126.
[43] International patent application WO02/079243.
[44] International patent application WO02/02606.

[45] Kalman *et al.* (1999) *Nature Genetics* 21:385-389.
[46] Read *et al.* (2000) *Nucleic Acids Res* 28:1397-406.
[47] Shirai *et al.* (2000) *J. Infect. Dis.* 181(Suppl 3):S524-S527.
[48] International patent application WO99/27105.
[49] International patent application WO00/27994.
[50] International patent application WO00/37494.
[51] International patent application WO99/28475.
[52] Ross *et al.* (2001) *Vaccine* 19:4135-4142.
[53] Dreesen (1997) *Vaccine* 15 Suppl:S2-6.
[54] *MMWR Morb Mortal Wkly Rep* 1998 Jan 16;47(1):12, 19.
[55] Anderson (2000) *Vaccine* 19 Suppl 1:S59-65.
[56] Kahn (2000) *Curr Opin Pediatr* 12:257-262.
[57] Crowe (1995) *Vaccine* 13:415-421.
[58] McMichael (2000) *Vaccine* 19 Suppl 1:S101-107.
[59] International patent application WO02/34771.
[60] Dale (1999) *Infect Dis Clin North Am* 13:227-43, viii.
[61] Ferretti *et al.* (2001) *PNAS USA* 98: 4658-4663.
[62] Paoletti *et al.*, (1990) *J Biol Chem* 265:18278-83.
[63] Wessels *et al.*, (1990) *J Clin Invest* 86:1428-33.
[64] Baker *et al.*, (2004) *J Infect Dis* 171:879-84.
[65] Kuroda *et al.* (2001) *Lancet* 357(9264):1225-1240; see also pages 1218-1219.
[66] *J Toxicol Clin Toxicol* (2001) 39:85-100.
[67] Demicheli *et al.* (1998) *Vaccine* 16:880-884.
[68] Stepanov *et al.* (1996) *J Biotechnol* 44:155-160.
[69] Ingram (2001) *Trends Neurosci* 24:305-307.
[70] Rosenberg (2001) *Nature* 411:380-384.
[71] Moingeon (2001) *Vaccine* 19:1305-1326.
[72] Anonymous (Jan 2002) *Research Disclosure*, 453077.
[73] Anderson (1983) *Infect Immun* 39(1):233-238.
[74] Anderson *et al.* (1985) *J Clin Invest* 76(1):52-59.
[75] EP-A-0372501.
[76] EP-A-0378881.
[77] EP-A-0427347.
[78] WO93/17712
[79] WO94/03208.
[80] WO98/58668.
[81] EP-A-0471177.
[82] WO91/01146
[83] Falugi *et al.* (2001) *Eur J Immunol* 31:3816-3824.
[84] Baraldo *et al*, (2004) *Infect Immun.*72:4884-7
[85] EP-A-0594610.
[86] WO00/56360.
[87] WO02/091998.
[88] Kuo *et al.* (1995) *Infect Immun* 63:2706-13.
[89] WO01/72337
[90] WO00/61761.

[91] Charalambous & Feavers (2001) *J Med Microbiol* 50:937-939.
[92] Gennaro (2000) *Remington: The Science and Practice of Pharmacy.* 20th edition, ISBN: 0683306472.
[93] WO98/20734.
[94] *Vaccine Design...* (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum.
[95] WO00/23105.
[96] WO90/14837.
[97] US patent 5,057,540.
[98] WO96/33739.
[99] EP-A-0109942.
[100] WO96/11711.
[101] WO00/07621.
[102] Barr *et al.* (1998) *Advanced Drug Delivery Reviews* 32:247-271.
[103] Sjolanderet *et al.* (1998) *Advanced Drug Delivery Reviews* 32:321-338.
[104] Niikura *et al.* (2002) *Virology* 293:273-280.
[105] Lenz *et al.* (2001) *J Immunol* 166:5346-5355.
[106] Pinto *et al.* (2003) *J Infect Dis* 188:327-338.
[107] Gerber *et al.* (2001) *Virol* 75:4752-4760.
[108] WO03/024480
[109] WO03/024481
[110] Gluck *et al.* (2002) *Vaccine* 20:B10-B16.
[111] EP-A-0689454.
[112] Johnson *et al.* (1999) *Bioorg Med Chem Lett* 9:2273-2278.
[113] Evans *et al.* (2003) *Expert Rev Vaccines* 2:219-229.
[114] Meraldi *et al.* (2003) *Vaccine* 21:2485-2491.
[115] Pajak *et al.* (2003) *Vaccine* 21:836-842.
[116] Kandimalla *et al.* (2003) *Nucleic Acids Research* 31:2393-2400.
[117] WO02/26757.
[118] WO99/62923.
[119] Krieg (2003) *Nature Medicine* 9:831-835.
[120] McCluskie *et al.* (2002) *FEMS Immunology and Medical Microbiology* 32:179-185.
[121] WO98/40100.
[122] US patent 6,207,646.
[123] US patent 6,239,116.
[124] US patent 6,429,199.
[125] Kandimalla *et al.* (2003) *Biochemical Society Transactions* 31 (part 3):654-658.
[126] Blackwell *et al.* (2003) *J Immunol* 170:4061-4068.
[127] Krieg (2002) *Trends Immunol* 23:64-65.
[128] WO01/95935.
[129] Kandimalla *et al.* (2003) *BBRC* 306:948-953.
[130] Bhagat *et al.* (2003) *BBRC* 300:853-861.
[131] WO03/035836.
[132] WO95/17211.
[133] WO98/42375.
[134] Beignon *et al.* (2002) *Infect Immun* 70:3012-3019.

[135] Pizza *et al.* (2001) *Vaccine* 19:2534-2541.
[136] Pizza *et al.* (2000) *Int J Med Microbiol* 290:455-461.
[137] Scharton-Kersten *et al.* (2000) *Infect Immun* 68:5306-5313.
[138] Ryan *et al.* (1999) *Infect Immun* 67:6270-6280.
[139] Partidos *et al.* (1999) *Immunol Lett* 67:209-216.
[140] Peppoloni *et al.* (2003) *Expert Rev Vaccines* 2:285-293.
[141] Pine *et al.* (2002) *J Control Release* 85:263-270.
[142] Domenighini *et al.* (1995) *Mol Microbiol* 15:1165-1167.
[143] WO99/40936.
[144] WO99/44636.
[145] Singh *et al.* (2001) *J Cont Release* 70:267-276.
[146] WO99/27960.
[147] US patent 6,090,406
[148] US patent 5,916,588
[149] EP-A-0626169.
[150] WO99/52549.
[151] WO01/21207.
[152] WO01/21152.
[153] Andrianov *et al.* (1998) *Biomaterials* 19:109-115.
[154] Payne *et al.* (1998) *Adv Drug Delivery Review* 31:185-196.
[155] Stanley (2002) *Clin Exp Dermatol* 27:571-577.
[156] Jones (2003) *Curr Opin Investig Drugs* 4:214-218.
[157] WO99/11241.
[158] WO94/00153.
[159] WO98/57659.
[160] European patent applications 0835318, 0735898 and 0761231.

CLAIMS

1. An immunogenic composition comprising: (a) an adjuvant comprising an early endosome neutralising compound; and (b) an antigen.

2. The immunogenic composition of claim 1, wherein the adjuvant induces a cellular immune response against the antigen.

3. The immunogenic composition of claim 2, wherein the cellular immune response is a protective CD8 response.

4. The immunogenic composition of claim 1, wherein the adjuvant is selected from the group consisting of: chloroquine; quinine; quinidine; cinchonine; mefloquine; halofantrine; 4,4'-diisothiocyanatostil-bene-2,2'-disulfonic acid; 5-nitro-2-(3-phenylpropylamino-benzoic acid); bafilomycin A1; folimycin; monensin; nigericin; a compound of Figure 3; and pharmaceutically acceptable salts and esters thereof.

5. The immunogenic composition of claim 4, wherein the adjuvant is chloroquine or a chloroquine-related compound.

6. Use of: (a) an early endosome neutralising compound adjuvant; and (b) an antigen, in the manufacture of a medicament for administration to a mammal to induce a cellular immune response.

7. Use of at least one antigen in the manufacture of a medicament for raising an immune response in a patient, wherein the medicament is administered with early endosome neutralising compound adjuvant.

8. The use of claim 6 or 7 wherein the cellular immune response is a protective CD8 immunity.

9. The use of claim 8, wherein the adjuvant is selected from the group consisting of: chloroquine; quinine; quinidine; cinchonine; mefloquine; halofantrine; 4,4'-diisothiocyanatostil-bene-2,2'-disulfonic acid; 5-nitro-2-(3-phenylpropylamino-benzoic acid); bafilomycin A1; folimycin; monensin; nigericin; a compound of Figure 3; and pharmaceutically acceptable salts and esters thereof.

10. The use of claim 9, wherein the adjuvant is chloroquine or a chloroquine-related compound.

11. A method for raising an immune response against an antigen, comprising the step of administering the antigen to a patient with an early endosome neutralising compound adjuvant.

12. The method of claim 11 wherein the adjuvant and the antigen are administered simultaneously sequentially or separately.

13. The use of an antigen in the manufacture of a medicament for raising an immune response in a patient, where the patient has been pre-treated with an early endosome neutralising compound adjuvant.

Figure 1

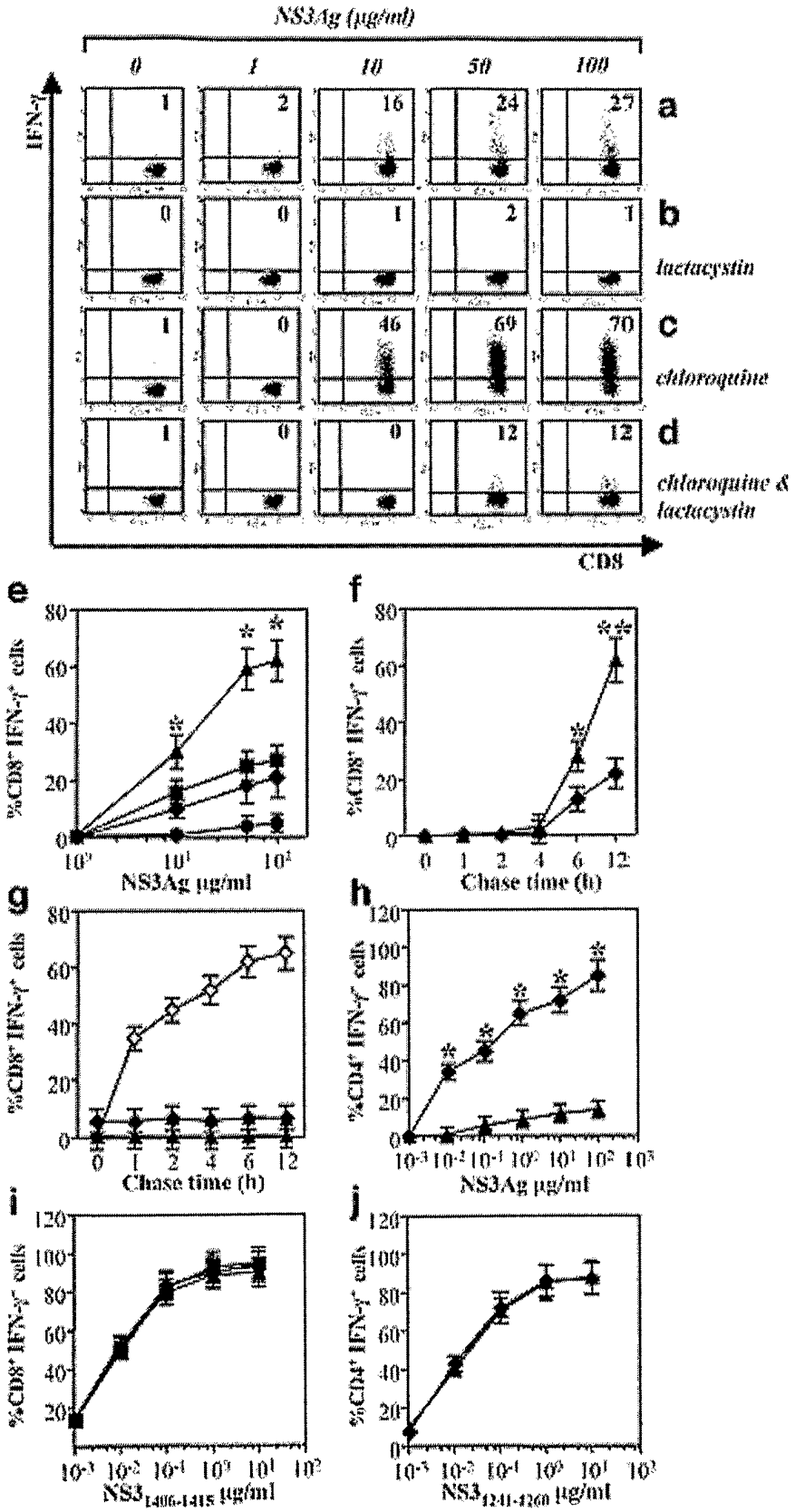

NS3Ag (µg/ml)
0
1
10
50
100
IFN-γ
CD8
a
b
lactacystin
c
chloroquine
d
chloroquine & lactacystin
e
%CD8+ IFN-γ+ cells
NS3Ag µg/ml
f
Chase time (h)
g
h
%CD4+ IFN-γ+ cells
i
NS3 1406-1415 µg/ml
j
NS3 1241-1260 µg/ml

Figure 2

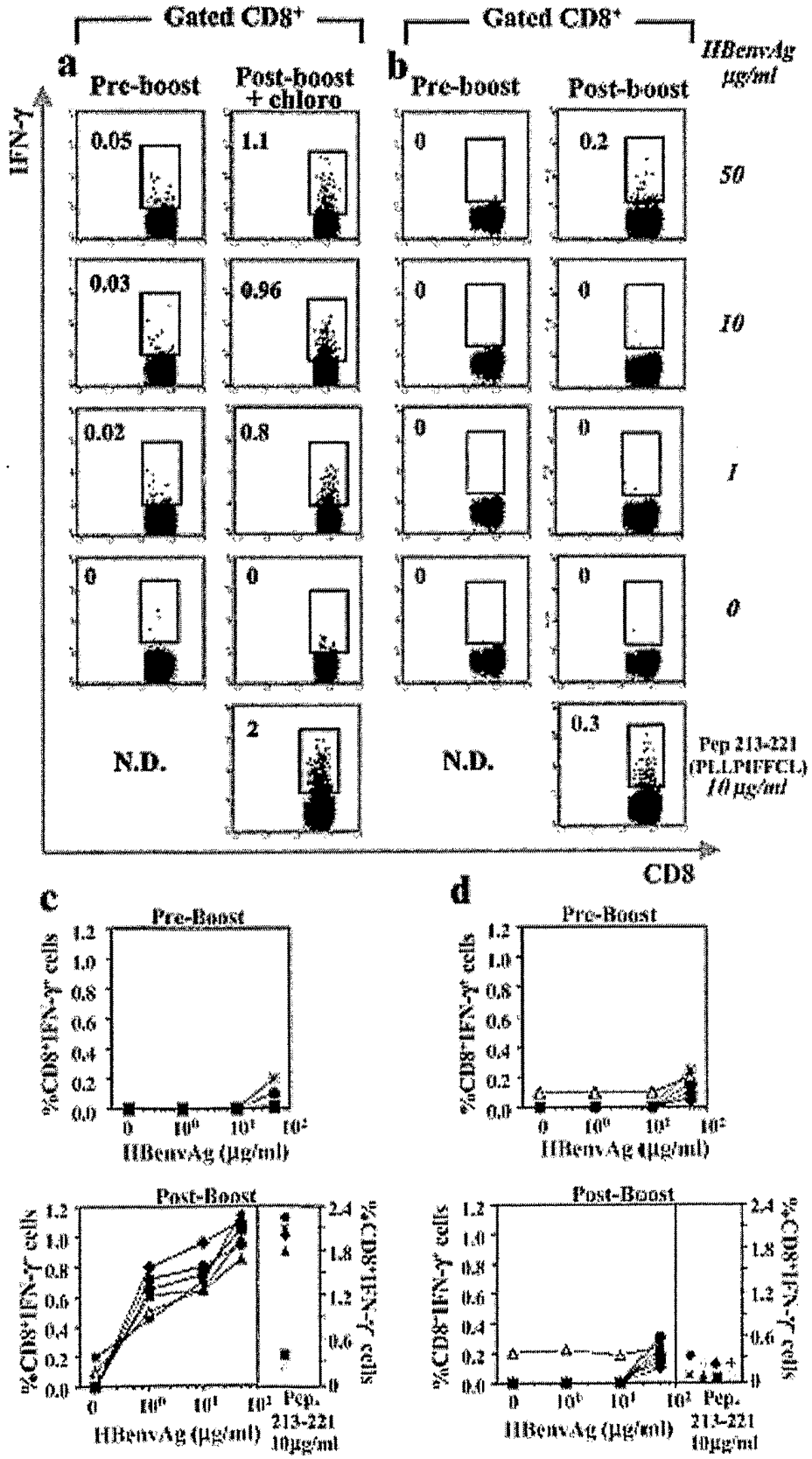
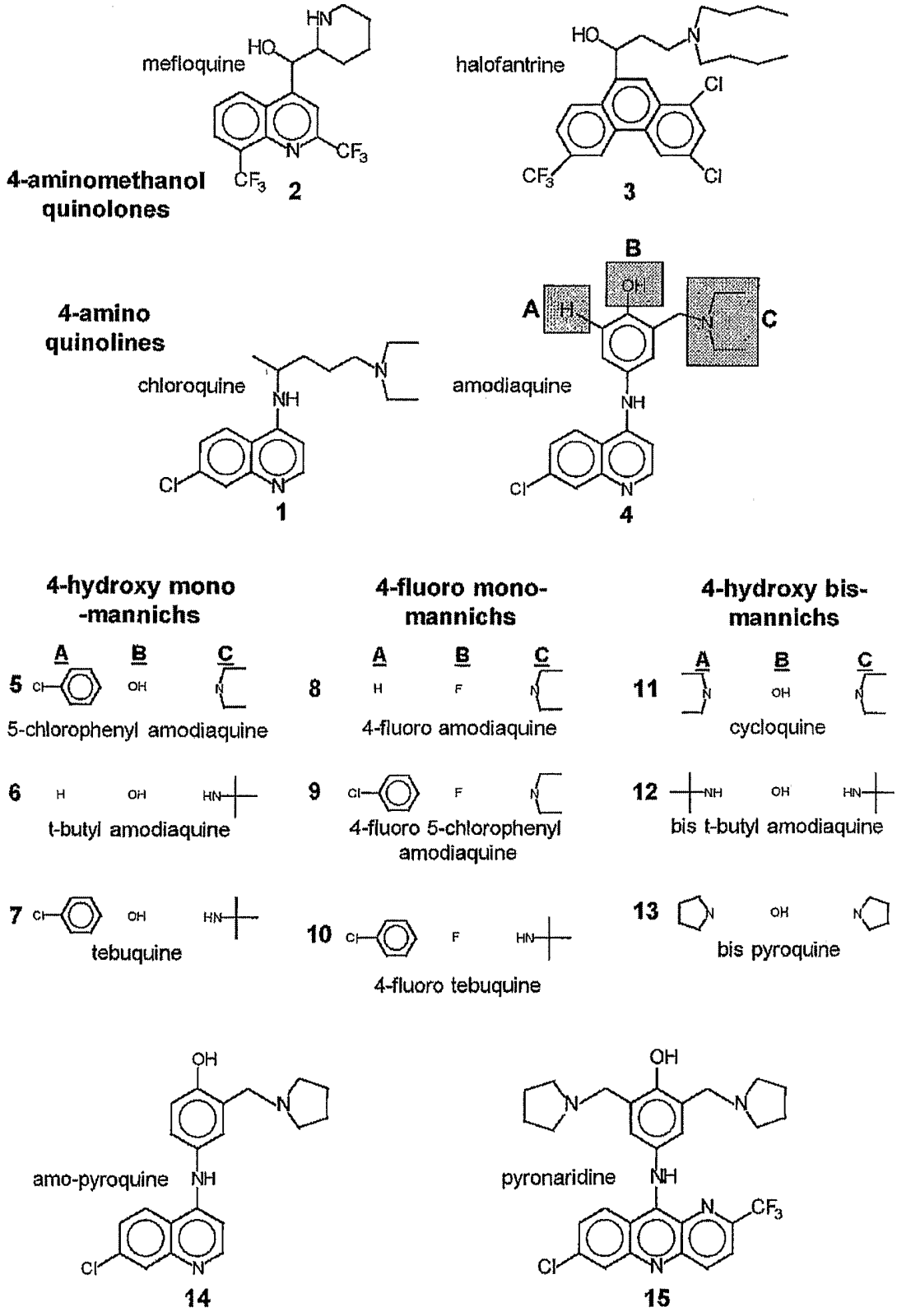

Gated CD8+
Gated CD8+
a
b
Pre-boost
Post-boost + chloro
Pre-boost
Post-boost
HBenvAg µg/ml
IFN-γ
0.05
1.1
0
0.2
50
0.03
0.96
0
0
10
0.02
0.8
0
0
1
0
0
0
0
0
N.D.
2
N.D.
0.3
Pep 213-221 (PLLPIFFCL) 10 µg/ml
CD8
c
d
Pre-Boost
Pre-Boost
%CD8+IFN-γ+ cells
HBenvAg (µg/ml)
Post-Boost
Post-Boost
%CD8+IFN-γ+ cells
HBenvAg (µg/ml)
Pep. 213-221 10µg/ml

Figure 3


4-aminomethanol quinolones
mefloquine
2
halofantrine
3
4-amino quinolines
chloroquine
1
amodiaquine
4
4-hydroxy mono -mannichs
4-fluoro mono-mannichs
4-hydroxy bis-mannichs
5-chlorophenyl amodiaquine
4-fluoro amodiaquine
cycloquine
t-butyl amodiaquine
4-fluoro 5-chlorophenyl amodiaquine
bis t-butyl amodiaquine
tebuquine
4-fluoro tebuquine
bis pyroquine
amo-pyroquine
14
pyronaridine
15