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(54) Title: RAPID RESPONSES TO DELAYED BOOSTER IMMUNISATIONS

(57) **Abrégé/Abstract:**

Booster immunization can lead to rapid induction of protective immunity against pathogens (e.g. < 7 days after the booster dose). This rapid response means that booster immunization can be used at short notice prior to an event that might require an activated immune response. For instance, a subject can be primed at a young age against a pathogen that typically affects more elderly subjects under specific circumstances such that, when the subject is older, the immune response can be mobilized rapidly if those specific circumstances are expected. An example would be to prime a subject against infections that are typically acquired nosocomially and then, soon before a scheduled hospital appointment, to boost their immune response so that they enter hospital in an immune-alert state.



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(54) **Title**: RAPID RESPONSES TO DELAYED BOOSTER IMMUNISATIONS

(57) **Abstract**: Booster immunization can lead to rapid induction of protective immunity against pathogens (e.g. < 7 days after the booster dose). This rapid response means that booster immunization can be used at short notice prior to an event that might require an activated immune response. For instance, a subject can be primed at a young age against a pathogen that typically affects more elderly subjects under specific circumstances such that, when the subject is older, the immune response can be mobilized rapidly if those specific circumstances are expected. An example would be to prime a subject against infections that are typically acquired nosocomially and then, soon before a scheduled hospital appointment, to boost their immune response so that they enter hospital in an immune-alert state.



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RAPID RESPONSES TO DELAYED BOOSTER IMMUNISATIONS

This application claims the benefit of United States provisional patent application 61/133,140, filed 25th June 2008, the complete contents of which are incorporated herein by reference.

TECHNICAL FIELD

5 This invention is in the field of immunization.

BACKGROUND ART

The use of a prime-boost immunization schedule is well known. For example, children typically receive a variety of primary immunizations up to the age of 15 months (*e.g.* a DTPa vaccine) and then receive booster doses aged between 4-6 years and beyond (*e.g.* a Tdap vaccine). According to
10 current schedules, though, the immune response elicited by a booster dose does not lead to rapid re-establishment of immunity (*e.g.* of protective antibody titers). Furthermore, the primary immunizations aim to provide immediate protection against diseases that affect the pediatric population, and the aim of a booster is to prolong this immediate protection.

DISCLOSURE OF THE INVENTION

15 It has now been found that booster immunization can lead to rapid induction of protective immunity against pathogens (*e.g.* ≤ 7 days after the booster dose), even if priming doses had been given several years before and had not established protective immunity. This rapid response to delayed boosting means that booster immunization can be used at short notice prior to an event that might require an activated immune response, and the priming dose is used to prepare the immune system for providing
20 rapid protection when is needed later. Thus the aim of a boosting dose can be to conclude protection rather than prolong it. The rapid response means that booster immunization can be used at short notice prior to an event that might require an activated immune response. For instance, a subject can be primed at a young age against a pathogen that typically affects more elderly subjects under specific circumstances such that, when the subject is older, the immune response can be mobilized
25 rapidly if those specific circumstances are expected. An example would be to prime a subject against infections that are typically acquired nosocomially (*e.g.* MRSA, *Clostridium difficile*) and then, soon before a scheduled hospital appointment, to boost their immune response so that they enter hospital in an immune-alert state. Another example would be to prime a female during adolescence against infections that are typically passed on during childbirth (*e.g.* group B streptococcus, *S.agalactiae*)
30 and then, before birth, boost their immune response so that they have useful anti-GBS immunity at the time of delivery.

It has also been found that this prime/boost approach can be effective even if the pathogen has antigenically drifted since the priming immunization. Thus the priming immunization can be 'future proof', allowing it to be administered many years before the risk of infection.

Thus the invention provides a method for raising an immune response in a subject, comprising steps of: (i) administering at least one dose of a priming immunogenic composition to the subject, wherein the priming immunogenic composition elicits a primary immune response against a pathogen; and then (ii) administering a boosting immunogenic composition to the subject, wherein the boosting immunogenic composition elicits, within 21 days (or sooner) of its administration, a protective anamnestic immune response against the pathogen.

The invention also provides a method for priming a subject, comprising a step of administering a priming immunogenic composition to the subject, wherein the priming immunogenic composition elicits a primary immune response against a pathogen allowing the establishment of immunological memory, wherein the subject will later receive a boosting immunogenic composition that will, within 21 days of its administration (or sooner), elicit a protective anamnestic immune response against the pathogen.

The invention also provides a method for eliciting in a subject a protective anamnestic immune response against a pathogen, comprising a step of administering a boosting immunogenic composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 21 days of its administration (or sooner), a protective anamnestic immune response against the pathogen; and (ii) the subject previously received a priming immunogenic composition that elicited a primary immune response against the pathogen.

As explained below, inclusion of an adjuvant in a boosting composition is preferred.

The pathogen

The invention can be used to immunize against a variety of pathogens. It is ideally suited for immunizing against diseases that, in general, occur in adult life rather than in childhood. Similarly, it is well suited for immunizing against pathogens that are not covered by current childhood immunization schedules (*C.diphtheriae*, *C.tetani*, *B.pertussis*, poliovirus, hepatitis B virus, *H.influenzae* type B, *N.meningitidis*, *S.pneumoniae*, measles virus, mumps virus, rubella virus, rotavirus, H3N2 and H1N1 influenza A viruses, influenza B virus, varicella zoster virus, hepatitis A virus, human papillomavirus). The invention is very useful against pathogens that, in general, are transmitted nosocomially. The invention is also useful against pathogens that, in general, are transmitted during birth. In some embodiments of the invention the pathogen is not an influenza A virus having a H5 hemagglutinin.

Thus the invention can be used to immunize against pathogens such as, but not limited to: *Staphylococcus aureus* (including MRSA strains), *Clostridium difficile*, Coagulase-negative *Staphylococcus* species ('CoNS', including *S.haemolyticus* and *S.epidermidis*), *Candida* strains (such as *C.albicans*), *Enterococci*, *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, and extraintestinal pathogenic *Escherichia coli* ('ExPEC'). Immunogens from these pathogens are known e.g. references 1 to 18.

The pathogen may exist in multiple different pathogenic variants. Depending on the particular pathogen then such variants may be *e.g.* serogroups, serotypes, immunotypes, serovars, biovars, strains, clades, or species.

The human subject

5 A subject receives at least one dose of priming composition and at least one dose of boosting composition. In some embodiments the criteria for administering the priming composition are wide, but the criteria for administering the boosting composition are narrower *e.g.* the priming composition might be given to substantially all children in a given population, whereas the boosting composition is given only to adults at specific risk of infection by from the relevant pathogen. In other
10 embodiments the priming and boosting compositions are administered at substantially the same breadth as each other.

The subject (particularly at the time of the boosting dose) may be at increased risk (*e.g.* higher risk than the general population) of hospitalization, and thus of catching a nosocomial infection. Such subjects include, but are not limited to: subjects with increased risk of burns, such as firefighters or
15 members of armed forces; subjects with increased risk of wounds, such as members of armed forces or police services; healthcare workers, such as physicians, surgeons or nurses; veterinarians; nursing home inhabitants; patients preparing for or undergoing invasive medical procedures; subjects preparing for cosmetic surgery; subjects preparing for, receiving or with prosthetic implants *e.g.* heart valves, joints, chronic vascular catheters, cosmetic implants; hemodialysis patients *e.g.* with
20 end-stage renal disease; patients with continuous ambulatory peritoneal dialysis; diabetic patients; patients aged at least 65 *e.g.* ≥ 70 , ≥ 75 , ≥ 80 , ≥ 85 , ≥ 90 ; patients with dermatologic conditions; users of illicit intravenous drugs; HIV positive patients; cancer patients; coronary artery bypass patients; and transplant patients.

The subject may be female, particularly where the pathogen is *S.agalactiae*.

25 ***Priming and boosting doses***

A subject receives at least one dose of priming composition and at least one dose of boosting composition. The priming and boosting compositions will typically be administered more than 1 year apart, *e.g.* ≥ 14 months apart, ≥ 16 months apart, ≥ 18 months apart, ≥ 2 years apart, ≥ 5 years apart, ≥ 10 years apart, ≥ 15 years apart, ≥ 20 years apart, ≥ 25 years apart, ≥ 30 years apart, ≥ 35 years apart, ≥ 40
30 years apart, ≥ 45 years apart, ≥ 50 years apart, *etc.*

The priming composition elicits a primary immune response against a pathogen. Thus it will typically elicit both an antibody response and memory B cells against the pathogen. Its effect is to prepare the immune system for later exposure to the boosting composition. More than one dose of priming composition may be given *e.g.* two doses. In some embodiments the priming composition
35 protects (at least temporarily) the subject against infection and/or disease caused by the pathogen. In

other embodiments the priming composition does not protect the subject against infection and/or disease caused by the pathogen, and such protection will be provided by the boosting composition.

The priming composition(s) may be administered with one or more other vaccine(s). For example, a priming composition may be administered to a subject at substantially the same time as (*e.g.* during the same medical consultation or visit to a healthcare professional or vaccination centre) a measles vaccine, a mumps vaccine, a rubella vaccine, a MMR vaccine, a varicella vaccine, a MMRV vaccine, a diphtheria vaccine, a tetanus vaccine, a pertussis vaccine, a DTP vaccine, a conjugated *H.influenzae* type b vaccine, an inactivated poliovirus vaccine, a hepatitis B virus vaccine, a meningococcal conjugate vaccine (such as a tetravalent A-C-W135-Y vaccine), a pneumococcal conjugate vaccine, a rotavirus vaccine, an influenza virus vaccine, a hepatitis A virus vaccine, a herpes simplex virus vaccine, and/or a human papillomavirus virus (HPV) vaccine. For instance, a priming immunization against *S.agalactiae* may be given between 11-18 years *e.g.* at the same time as a Tdap vaccine, a HPV vaccine, a meningococcal conjugate vaccine, *etc.*

Because of administration of the previous priming composition, the boosting composition elicits a secondary immune response against the pathogen. Thus the memory B cells that were elicited by the priming composition are activated, and they can rapidly proliferate and differentiate into plasma cells that secrete high-affinity serum antibodies. The result is that the subject mounts an anamnestic immune response that is protective against infection and/or disease caused by the pathogen.

The protective anamnestic immune response will generally comprise at least an antibody response, and the antibody response may include neutralizing and/or opsonizing antibodies.

The anamnestic immune response is mounted by the subject within 21 days of receiving the boosting composition *e.g.* ≤ 20 days, ≤ 19 days, ≤ 18 days, ≤ 17 days, ≤ 16 days, ≤ 15 days, ≤ 14 days, ≤ 13 days, ≤ 12 days, ≤ 11 days, ≤ 10 days, ≤ 9 days, ≤ 8 days, ≤ 7 days, ≤ 6 days, ≤ 5 days, ≤ 4 days, ≤ 3 days, ≤ 2 days, or even ≤ 1 day. In preferred embodiments the subject mounts a protective anamnestic immune response within ≤ 7 days of receiving the boosting composition.

In some embodiments the boosting composition is administered prior to a specific event *e.g.* before a hospital appointment, before a trip abroad, before a tour of duty, before battle, before giving birth, before entering a nursing home as an inhabitant, before an invasive medical procedure or operation, before receiving a prosthetic implant, before hemodialysis, before receiving chemotherapy, before receiving radiotherapy, *etc.* In such embodiments the boosting composition is administered at least as far prior to the event as the duration required for the protective anamnestic immune response *e.g.* if it takes 7 days to achieve a protective anamnestic immune response then the boosting composition will be administered at least 7 days in advance of the event. Typically, however, the boosting composition will not be administered too far in advance *e.g.* no more than a year prior to the event.

In other embodiments the boosting composition is administered when planning for a specific event. For instance, if pregnancy is planned then the boosting composition can be administered to a female

before conception, or after conception, to provide the protective anamnestic immune response ready for birth.

In other embodiments the boosting composition is administered at an arbitrary time unrelated to a particular event *e.g.* at a particular age.

- 5 The priming composition and boosting composition each contain an immunogen that elicits an immune response against the same pathogen. The priming immunogen and boosting immunogen will each typically comprise a T-dependent antigen. They may include the same immunogen or different immunogens. The immunogen can take any suitable form *e.g.* it may comprise a protein, a conjugated saccharide, outer membrane vesicles, inactivated whole cells, *etc.*
- 10 If the pathogen exists in multiple different pathogenic variants then the priming immunogen and the boosting immunogen may be from the same variant or from different variants. For example, the priming and boosting immunogen may be from different serogroups, different serotypes, different immunotypes, different serovars, different biovars, different strains, different clades, or different species of the same pathogen. With H5 influenza, for instance, a boosting hemagglutinin may be
15 from a different clade from a priming hemagglutinin.

The priming composition and/or the boosting composition may include an immunological adjuvant.

The priming composition and/or the boosting composition may be administered by the same route or by different routes. Suitable routes include, but are not limited to, intramuscular injection (*e.g.* into the arm or leg), subcutaneous injection, intranasal, oral, intradermal, transcutaneous and transdermal.

20 ***Natural priming***

- As an alternative to administering a priming dose, a natural infection may serve to prime a subject's immune response. The infection may be with the same pathogen or an immunologically related pathogen (*e.g.* as in the cowpox/smallpox immunological relationship). Where the infection is with the same pathogen species, but the pathogen exists in multiple different pathogenic variants, the
25 booster dose may be such a variant *e.g.* from a different serogroup, serotype, immunotype, serovar, biovar, strain, or clade of the species which previously caused the natural infection.

- The boosting composition will typically be administered more than 1 year after the natural infection, *e.g.* ≥ 14 months later, ≥ 16 months later, ≥ 18 months later, ≥ 2 years later, ≥ 5 years later, ≥ 10 years later, ≥ 15 years later, ≥ 20 years later, ≥ 25 years later, ≥ 30 years later, ≥ 35 years later, ≥ 40 years
30 later, ≥ 45 years later, ≥ 50 years later, *etc.*

Because of the previous infection, the boosting composition elicits a secondary immune response against the pathogen. Thus the memory B cells that were elicited by the infection are activated, and they can rapidly proliferate and differentiate into plasma cells as described above.

- Thus the invention provides a method for eliciting in a subject a protective anamnestic immune
35 response against a pathogen, comprising a step of administering a boosting immunogenic

composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 21 days of its administration (or sooner), a protective anamnestic immune response against the pathogen; and (ii) previous infection had caused the subject to mount a primary immune response against the pathogen.

5 *Medical uses*

The invention also provides an immunogen for use in a method of the invention.

10 The invention also provides the use of an immunogen in the manufacture of a vaccine for protecting a subject against disease and/or infection caused by a pathogen, wherein (i) the subject previously received a priming immunogenic composition that elicited a primary immune response against the pathogen, and (ii) the vaccine elicits, within 21 days of its administration, a protective anamnestic immune response against the pathogen.

15 The invention also provides the use of an immunogen in the manufacture of a vaccine for priming a subject's immune system for later boosting, wherein the boosting elicits, within 21 days of its administration, a protective anamnestic immune response against a pathogen, and the priming elicits a primary immune response against the pathogen.

20 The invention also provides an immunogen for use in a method for raising a primary immune response against a pathogen in a subject comprising a step of administering a priming immunogenic composition to the subject, wherein the subject will later receive a boosting immunogenic composition that will, within 21 days of its administration, elicit a protective anamnestic immune response against the pathogen.

25 The invention also provides an immunogen for use in a method for raising a booster immune response against a pathogen in a subject comprising a step of administering a boosting immunogenic composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 21 days of its administration, a protective anamnestic immune response against the pathogen; and (ii) the subject previously received a priming immunogenic composition that elicited a primary immune response against the pathogen.

30 The invention also provides the use of an immunogen in the manufacture of a vaccine for protecting a subject against disease and/or infection caused by a pathogen, wherein (i) previous infection of the subject had caused it to mount a primary immune response against the pathogen, and (ii) the vaccine elicits, within 21 days of its administration, a protective anamnestic immune response against the pathogen.

The invention also provides an immunogen for use in a method for raising a booster immune response against a pathogen in a subject comprising a step of administering a boosting immunogenic composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 21

days of its administration, a protective anamnestic immune response against the pathogen; and (ii) previous infection had caused the subject to mount a primary immune response against the pathogen.

Adjuvant(s)

The priming composition and/or the boosting composition may include an immunological adjuvant to enhance the immune responses (humoral and/or cellular) elicited in a subject. If both of the compositions include an adjuvant, they may include the same adjuvant or different adjuvants. If only one of the two vaccines is adjuvanted then preferably it is the second. The use of an adjuvanted booster dose is shown below (study I) to be effective, and the use of adjuvanted priming and boosting doses gave the best protective anamnestic immune responses.

Suitable adjuvants for use with the invention include, but are not limited to, mineral-containing compositions and oil-in-water emulsions.

Mineral-containing compositions can include calcium salts, aluminum salts, or mixtures thereof. Suitable calcium salts include calcium phosphate (*e.g.* the “CAP” particles disclosed in ref. 19). Suitable aluminum salts include hydroxides, phosphates, sulfates, *etc.*, with the salts taking any suitable form (*e.g.* gel, crystalline, amorphous, *etc.*). Adsorption to these salts is preferred. The mineral containing compositions may also be formulated as a particle of metal salt [20].

The adjuvants known as aluminum hydroxide and aluminum phosphate may be used. These names are conventional, but are used for convenience only, as neither is a precise description of the actual chemical compound which is present (*e.g.* see chapter 9 of reference 24). The invention can use any of the “hydroxide” or “phosphate” adjuvants that are in general use as adjuvants.

The concentration of Al^{+++} in a composition for administration to a subject is preferably less than 10mg/ml *e.g.* ≤ 5 mg/ml, ≤ 4 mg/ml, ≤ 3 mg/ml, ≤ 2 mg/ml, ≤ 1 mg/ml, *etc.* A useful range is between 0.3 and 1mg/ml. A maximum of 0.85mg/dose is preferred.

Oil-in-water emulsions have been found to be particularly suitable for adjuvanting vaccines. Various such emulsions are known, and they typically include at least one oil (*e.g.* squalene) and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5 μ m in diameter, and may even have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220nm are preferred as they can be subjected to filter sterilization.

Specific oil-in-water emulsion adjuvants useful with the invention include, but are not limited to:

- A submicron emulsion of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85.

This adjuvant is known as ‘MF59’ [21-23], as described in more detail in Chapter 10 of ref. 24

and chapter 12 of ref. 25. The MF59 emulsion advantageously includes citrate ions *e.g.* 10mM sodium citrate buffer.

- An emulsion of squalene, a tocopherol, and Tween 80. The emulsion may include phosphate buffered saline. It may also include Span 85 (*e.g.* at 1%) and/or lecithin. These emulsions may have from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% Tween 80, and the weight ratio of squalene:tocopherol is preferably ≤ 1 as this provides a more stable emulsion. Squalene and Tween 80 may be present volume ratio of about 5:2. One such emulsion can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90ml of this solution with a mixture of (5g of DL- α -tocopherol and 5ml squalene), then microfluidising the mixture. The resulting emulsion may have submicron oil droplets *e.g.* with an average diameter of between 100 and 250nm, preferably about 180nm. An adjuvant containing (per 0.25ml or per dose) 10.68mg squalene, 11.86mg DL- α -tocopherol and 4.86mg polysorbate 80 is ideal.
- An emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (*e.g.* polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (*e.g.* a sorbitan ester or mannide ester, such as sorbitan monoleate or 'Span 80'). The emulsion is preferably thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm [26]. The emulsion may also include one or more of: alditol; a cryoprotective agent (*e.g.* a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. Such emulsions may be lyophilized.

20 **General**

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 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.

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

MODES FOR CARRYING OUT THE INVENTION

Human study I [27,28]

Subjects were primed with an influenza vaccine prepared from H5N3 strain A/duck/Singapore/1997 (clade 0). The vaccine was either unadjuvanted (group 2) or was adjuvanted with the MF59 squalene-in-water emulsion (group 1). A third group of subjects (group 3) did not receive the H5N3 vaccine.

After 6 to 8 years, and at a time of heightened risk of a pandemic H5N1 outbreak, subjects were immunized with an influenza vaccine prepared from a H5N1 strain (clade 1 or 2). Two doses were administered, at days 0 & 21, both with MF59 adjuvant. Thus groups 1 and 2 received a boosting composition.

Results are shown in Table I.

Geometric mean antibody titers and sero-responses were significantly higher in primed subjects than in unprimed subjects. By day 7 after one dose of vaccine, $\geq 80\%$ of MF59-H5 primed recipients achieved seroprotective HAI titers of $\geq 1:40$ to all clade 1, 2.1, 2.2, and 2.3 avian H5 virus variants tested as well as the original A/duck/Singapore/1997 clade 0 antigen.

Subjects who had previously been primed by the clade 0 vaccine mounted a better and more rapid immune response against the new clade than the un-primed subjects. Among primed subjects, protective cross-reacting antibody titers to diverse H5N1 virus variants were seen by day 7 after a single boosting dose. Within the primed group, subjects who had received an adjuvanted priming dose mounted a better immune response than subjects who had received an unadjuvanted prime.

Although these results were obtained using influenza virus, they offer proof of principle that a priming dose of a vaccine can be administered to subjects at a time of low risk of infection by the relevant pathogen, and then their immune system can be boosted several years later at a time of heightened risk to provide protective levels of immunity in less than a week. This finding is applicable not only to H5 influenza but to a wide range of pathogens, particularly those that are often caught in hospitals.

Human study II [30]

A phase II clinical study was performed to evaluate the immunogenicity in adults aged 19-61 of one or two booster administrations of a monovalent AS03-adjuvanted vaccine containing 3.75 μ g HA from H5N1 A/Indonesia/5/05. The patients had previously (about 14-months earlier) been vaccinated with two doses of a pandemic candidate vaccine containing 3.8, 7.5, 15 or 30 μ g HA from H5N1 A/Vietnam/1194/2004, with or without AS03 adjuvant (*i.e.* 8 groups in total). This study mimics an outbreak which occurs after priming subjects with a pandemic vaccine based on a H5N1 strain heterologous to the emerging pandemic strain.

Analysis of the priming study showed that two administrations of non-adjuvanted vaccine was not sufficient to elicit an immune response considered as protective according to currently used regulatory criteria (from the CHMP and CBER). These subjects (4 groups) received two administrations of the A/Indonesia/5/05 vaccine; other subjects received a single booster vaccine; a control group of unprimed subjects received two doses of the A/Indonesia/5/05 vaccine.

A proportion of subjects who had been immunized 14 months previously (whether the priming vaccines were adjuvanted or unadjuvanted) became seropositive 7 days after receiving one dose of the booster vaccine, compared to the control subjects. Seropositivity rates were higher in subjects who had received adjuvanted priming vaccine. 21 days after the last booster vaccination, a majority of subjects in all groups became seropositive.

The $\geq 40\%$ seroconversion rate threshold required by the European Committee for Medicinal Products for Human Use (CHMP) for adults aged 18-60 years was exceeded 7 days after boosting in subjects who had received adjuvanted priming vaccine, and the seroconversion rate remained high 21 days after vaccination. In subjects whose priming vaccines had been unadjuvanted, the $\geq 40\%$ seroconversion rate was also exceeded 7 days after the first vaccination, although the lower limit of the 95% confidence interval for seroconversion was inferior to the threshold for the 7.5 and 15 μg group.

When primed with an adjuvanted vaccine from a strain different from the boosting strain, subjects develop adequate seroprotective humoral responses directed against the boosting strain. The capacity to rapidly (as soon as within 7 days) develop a seroprotective response against a strain drifted from the strain used in the primary vaccination is sustained as long as 14 months, when subjects have been primed with an adjuvanted vaccine and are boosted with adjuvanted vaccine. HI antibodies levels obtained after boosting with an adjuvanted vaccine at either 6 or 14 months after primary vaccination with an adjuvanted vaccine are similar, when using a vaccine strain drifted from the primary strain for boosting and when testing against the strain used for boosting.

Persistent cross-clade CD4-positive and memory B cell responses were detected 14 months after primary vaccination with adjuvanted vaccine. These responses were higher as compared to subjects whose priming dose was unadjuvanted. Increases in cross-clade CD4-positive and memory B cell responses were observed in all groups, but were higher in subjects whose priming vaccines were adjuvanted.

Human study III [29]

Healthy volunteers aged 18 to 59 years receive a single priming vaccination of a whole virion Vero cell-derived H5N1 (A/Vietnam/1203/2004) influenza vaccine at day zero, and a single boosting dose (A/Indonesia/05/2005) is given a year later.

The primary measure of the study is the number of subjects with an antibody response to the vaccine strain associated with protection 21 days after the booster vaccination, defined as the titer measured by microneutralization test $\geq 1:20$. The secondary measure is the number of subjects with an antibody response associated with protection 21, 42, 180 and 360 days after the priming vaccination and 21 days after the booster vaccination.

Human study IV [30]

Healthy adults aged 18-60 years received two doses of monovalent adjuvanted egg-derived split H5N1 vaccine, spaced by either 6 months or 12 months. The adjuvant was "AS03", an oil-in-water emulsion comprising squalene. Antigen dose was 3/75 μg HA per dose. Two different vaccines were used; "VT", containing antigen from the A/VietNam/1194/2005 strain (clade 1); and "IN", containing antigen from the A/Indonesia/5/2005 strain (clade 2.1). Four groups of patients (about 65 patients per group) were immunized as follows:

Group	First vaccine	Time of second vaccine	Second vaccine
A	VT	6 months	VT
B	VT	12 months	VT
C	VT	6 months	IN
D	VT	12 months	IN

Seropositivity rates and GMTs were measured at time zero, 3 weeks later, at the time of the second vaccine, one week after the second vaccine, and three weeks after the second vaccine. Data from groups B and D are not yet available; data from groups A and C were as follows:

	A		C	
	SP%	GMT	SP%	GMT
<i>Antibodies against A/Indonesia/5/2005</i>				
Zero	0%	5.0	0%	5.0
+ 3 weeks	12.7%	5.6	14.3%	6.3
Second dose	4.1%	5.3	7.3%	5.5
+ 1 week	78.7%	64.6	98.1%	152.9
+ 3 weeks	85.4%	92.4	98.1%	303.4
<i>Antibodies against A/Vietnam/1194/2005</i>				
Zero	3.6%	5.3	0%	5.0
+ 3 weeks	50.9%	16.3	55.4%	20.9
Second dose	36.7%	9.7	45.5%	12.0
+ 1 week	89.4%	202.6	98.1%	226.3
+ 3 weeks	89.6%	287.2	98.1%	434.7

5

All groups who received a booster dose after 6 months mounted a rapid and effective immune response, showing a significant increase in GMTs for HI antibodies against the A/Indonesia/5/2005 and the A/Vietnam/1194/2004 strains at both post-booster time points. Moreover, within 7 days of the booster dose, all groups met the CHMP seroconversion threshold ($\geq 40\%$ seroconversion, using day 0 as a baseline) for both strains, and the response was sustained until at least 14 days later.

10

The vaccine was shown to confer broad cross-clade immunity which was maintained even when (i) the second dose was given many months after the first dose, and (ii) the second dose was from a different H5N1 strain. In comparison with further data, two vaccination doses, whether given 21 days or six months apart, were shown to elicit a comparable immune response against the vaccine strain, confirming that the timing of administration of the second dose can be flexible and undertaken up to six months after first immunization while maintaining the quality of the immune response.

15

A one-dose priming administration followed 6 months later by a booster dose containing a heterologous vaccine strain meets or exceeds all relevant CHMP and FDA criteria for these vaccines. Professor Albert Osterhaus, Head Department of Virology, Institute of Virology Erasmus Medical

Centre Rotterdam, confirmed that the vaccine used in this study “provides broad and persistent immunity, also against drifted H5N1 strains” and that “this level of immunity can be maintained when the second dose is given many months after the first, even with a different H5N1 strain.”

Human study V

- 5 Subjects received an adjuvanted whole virion Vero-derived H5N1 (A/Vietnam/1203/2004) influenza vaccine for immunological priming. Six different formulations were used for priming (4 adjuvanted HA doses of 3.75µg, 7.5µg, 15µg or 30µg per dose; 2 unadjuvanted HA doses of 7.5µg or 15µg). 12-17 months later, 77 patients received a boosting dose containing 7.5µg HA from a different H5N1 strain (A/Indonesia/05/2005). Immune responses were assessed using a microneutralisation test
10 against wild-type A/Indonesia/05/2005, using sera collected at days 0 (priming), 21, 42 and 180, and then at days 0 (booster dose), 7 and 21. GMT values were in the range 0-20 in all groups after priming, up to receipt of the booster dose. The boosting dose then caused GMTs to rise sharply: 7 days later GMT values were in the range of 50-100, and within 21 days of the booster dose GMTs were in the range 80-220. The percentage of subjects with neutralizing antibody responses (MN titer
15 ≥ 20) in the booster phase were as follows:

HA dose:	3.75µg	7.5µg	7.5µg	15µg	15µg	30µg
Adjuvant:	+	+	-	+	-	+
MN against A/Indonesia/05/2005						
Day 0	0	7	0	0	0	8
Day 7	81	93	91	92	100	100
Day 21	94	100	100	100	88	100
MN against A/Vietnam/1203/2004						
Day 0	12	13	25	15	50	25
Day 7	81	93	91	92	100	92
Day 21	94	93	92	100	100	100

- Overall, the results show that the vaccine induces memory B cells which can provide an immediate antibody response to later H5N1 infection. A single heterologous boost 12–17 months after the primary immunization resulted in the rapid induction of very high titers against both the initial
20 vaccine and the booster strain, providing 90-100% seroprotection 7 days after the booster vaccination. Moreover, the rapid induction of a protective immune response was achieved not only against the clade 1 priming strain and the clade 2 boosting strain, but also against representative viruses of clade 2.2 (turkey/Turkey) and 2.3 (Anhui).

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

Table I

Measure	G 1	G 2	G 3
% of subjects with HI titer ≥ 40 at day 0	0	0	3
at day 8	75	56	12
at day 15	90	60	9
at day 22	75	58	13
at day 43	75	58	45
% of subjects with seroconversion at day 8	75	56	8
at day 15	90	60	10
at day 22	75	58	14
at day 43	75	58	45
Geometric mean HI at day 1	4	4	4.9
day 8	72	51	5.9
day 15	256	79	7.3
day 22	112	52	8.0
day 43	95	44	26
Ratio of HI relative to day 1 at day 8	18	13	1.3
at day 15	64	20	1.56
at day 22	28	13	1.8
at day 43	24	11	5.9
% of subjects with MN titer ≥ 80 at day 0	0	0	0
at day 8	9	5	1
at day 15	9	9	1
at day 22	12	9	1
at day 43	12	10	5
Geometric mean MN titer at day 1	10	10	10
day 8	219	151	11
day 15	1145	473	12
day 22	375	324	12
day 43	415	241	33
Ratio of MN relative to day 1 at day 8	22	15	1.1
at day 15	115	47	1.2
at day 22	37	32	1.2
at day 43	41	24	3.3

REFERENCES

- [1] Shinefield (2006) *Vaccine* 24 Suppl 2:S2-65-9.
- [2] Halsey (2008) *Am J Health Syst Pharm.* 65(8):705-15.
- [3] Wang & Barrett (2007) *Methods Mol Biol* 391:209-25.
- [4] Maira-Litran *et al.* (2004) *Vaccine.* 22(7):872-9.
- [5] WO2007/113222.
- [6] WO2006/032500.
- [7] WO2003/097091.
- [8] Koch *et al.* (2004) *Vaccine* 22(7):822-30.
- [9] Egorova *et al.* (1997) *Zh Mikrobiol Epidemiol Immunobiol.* (6):96-101.
- [10] Szostak *et al.* (1994) *J Biotechnol.* 44(1-3):161-70.
- [11] Solov'ev *et al.* (2006) *Zh Mikrobiol Epidemiol Immunobiol.* (1):20-5
- [12] Kurupati *et al.* (2006) *Proteomics* 6(3):836-44.
- [13] US patent 5955090.
- [14] Döring & Pier (2008) *Vaccine* 26(8):1011-24.
- [15] WO02/34771.
- [16] WO2006/089264.
- [17] WO2006/091517.
- [18] WO2008/020330.
- [19] US patent 6355271.
- [20] WO00/23105.
- [21] WO90/14837.
- [22] Podda & Del Giudice (2003) *Expert Rev Vaccines* 2:197-203.
- [23] Podda (2001) *Vaccine* 19: 2673-2680.
- [24] *Vaccine Design: The Subunit and Adjuvant Approach* (eds. Powell & Newman) Plenum Press 1995 (ISBN 0-306-44867-X).
- [25] *Vaccine Adjuvants: Preparation Methods and Research Protocols* (Volume 42 of *Methods in Molecular Medicine* series). ISBN: 1-59259-083-7. Ed. O'Hagan.
- [26] US-2007/014805.
- [27] Stephenson *et al.* (2008) *NEJM* 359:1631-33.
- [28] Galli *et al.* (2009) *PNAS USA* 106:7962-7.
- [29] Clinical trial NCT00895544.
- [30] US-2009/0028903.

CLAIMS:

1. A method for raising an immune response in a subject, comprising steps of: (i) administering a priming immunogenic composition to the subject, wherein the priming immunogenic composition elicits a primary immune response against a pathogen; and
5 then (ii) administering a boosting immunogenic composition to the subject, wherein the boosting immunogenic composition elicits, within 7 days of its administration, a protective anamnestic immune response against the pathogen.
2. A method for priming a subject, comprising a step of administering a priming immunogenic composition to the subject, wherein the priming immunogenic
10 composition elicits a primary immune response against a pathogen, wherein the subject will later receive a boosting immunogenic composition that will, within 7 days of its administration, elicit a protective anamnestic immune response against the pathogen.
3. A method for eliciting in a subject a protective anamnestic immune response against a pathogen, comprising a step of administering a boosting immunogenic composition to
15 the subject, wherein: (i) the boosting immunogenic composition elicits, within 7 days of its administration, a protective anamnestic immune response against the pathogen; and (ii) the subject previously received a priming immunogenic composition that elicited a primary immune response against the pathogen.
4. Use of an immunogen in the manufacture of a vaccine for protecting a subject against
20 disease and/or infection caused by a pathogen, wherein (i) the subject previously received a priming immunogenic composition that elicited a primary immune response against the pathogen, and (ii) the vaccine elicits, within 7 days of its administration, a protective anamnestic immune response against the pathogen.
5. Use of an immunogen in the manufacture of a vaccine for priming a subject's immune
25 system for later boosting, wherein the boosting elicits, within 7 days of its administration, a protective anamnestic immune response against a pathogen, and the priming elicits a primary immune response against the pathogen.
6. An immunogen for use in a method for raising a primary immune response against a pathogen in a subject comprising a step of administering a priming immunogenic
30 composition to the subject, wherein the subject will later receive a boosting immunogenic composition that will, within 7 days of its administration, elicit a protective anamnestic immune response against the pathogen.
7. An immunogen for use in a method for raising a booster immune response against a pathogen in a subject comprising a step of administering a boosting immunogenic

composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 7 days of its administration, a protective anamnestic immune response against the pathogen; and (ii) the subject previously received a priming immunogenic composition that elicited a primary immune response against the pathogen.

- 5 8. A method for eliciting in a subject a protective anamnestic immune response against a pathogen, comprising a step of administering a boosting immunogenic composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 7 days of its administration (or sooner), a protective anamnestic immune response against the pathogen; and (ii) previous infection had caused the subject to mount a primary immune response against the pathogen.
- 10
9. An immunogen for use in a method for raising a booster immune response against a pathogen in a subject comprising a step of administering a boosting immunogenic composition to the subject, wherein: (i) the boosting immunogenic composition elicits, within 7 days of its administration, a protective anamnestic immune response against the pathogen; and (ii) previous infection had caused the subject to mount a primary immune response against the pathogen.
- 15
10. Use of an immunogen in the manufacture of a vaccine for protecting a subject against disease and/or infection caused by a pathogen, wherein (i) previous infection of the subject had caused it to mount a primary immune response against the pathogen, and (ii) the vaccine elicits, within 7 days of its administration, a protective anamnestic immune response against the pathogen.
- 20
11. The method, use or immunogen of any preceding claim, wherein the pathogen is selected from the group consisting of: *Staphylococcus aureus*, *Clostridium difficile*, Coagulase-negative *Staphylococcus* species, *Candida*, *Enterococci*, *Klebsiella pneumoniae*, *Acinetobacter*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, and extraintestinal pathogenic *Escherichia coli*.
- 25
12. The method, use or immunogen of any preceding claim, wherein the priming composition and boosting composition are administered more than 5 years apart.
13. The method, use or immunogen of any preceding claim, wherein the protective anamnestic immune response comprises a neutralizing antibody response and/or opsonizing antibody response.
- 30
14. The method, use or immunogen of any preceding claim, wherein the priming composition and/or the boosting composition includes an immunological adjuvant.

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15. The method, use or immunogen of claim 14, wherein the adjuvant is an oil-in-water emulsion comprising squalene and at least one surfactant.
16. The method, use or immunogen of any preceding claim, wherein the pathogen is not an influenza A virus having a H5 hemagglutinin.

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