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(54) HUMAN PAPILLOMA VIRUS VACCINE FORMULATIONS
MENSCHLICHE PAPILLOMAVIRUS IMPFSTOFF-FORMULIERUNGEN
FORMULATIONS VACCINALES CONTRE LE VIRUS DU PAPILLOME HUMAIN

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BACKGROUND OF THE INVENTION

[0001] This invention relates to human papillomavirus (HPV) vaccine formulations which provide enhanced long-term storage stability.

FIELD OF THE INVENTION

[0002] Human Papillomavirus (HPV) infects the genital tract and has been associated with various dysplasias, cancers, and other diseases. These diseases are currently targets for vaccine development and vaccines containing virus-like particles (VLPs) which contain L1 or the combination of L1+L2 proteins are currently in clinical trials.

[0003] It has been found, however, that HPV VLPs are not stable during long-term storage, either in solution or when adsorbed onto aluminum adjuvant particles.

[0004] In order to develop a commercially useful vaccine, a stable formulation is needed.

[0005] A-9844944 (Merck & Co., Inc.) discloses alum adjuvanted HPV vaccines comprising sodium chloride, a non-ionic surfactant and a buffer which can be phosphate, citrate, acetate, succinate, Tris-HCl or MOPS to maintain a pH range of 6.0-8.0.

[0006] Example 39 of WO-A-9531532 (Merck & Co., Inc.) discloses compositions comprising HPV VLPs, vaccine stabilizers and vaccine adjuvants. The exemplary polyanion stabilizers listed do not include carboxymethyl cellulose (CMC).

BRIEF DESCRIPTION OF THE FIGURES

[0010] FIGURE 1A is a graph showing the effects of NaCl concentration and added excipients on the stability of HPV16 VLP-aluminum formulations at 2-8°C; FIGURE 1B shows the results at 15°C.

FIGURE 2 is a graph showing the effects of excipient on the accelerated stability of HPV16 VLP-aluminum formulations.

FIGURE 3 is a bar graph showing the effects of polyanion excipients on the accelerated stability of HPV16 VLP-aluminum formulations.

FIGURE 4 is a bar graph illustrating the effect of carboxymethyl cellulose (CMC) molecular weight and NaCl concentration on the accelerated stability of HPV16 VLP-aluminum formulations.

FIGURE 5 is a graph showing the effects of carboxymethyl cellulose (CMC) molecular weight and NaCl concentration on the accelerated stability of HPV16 VLP-aluminum formulations.

[0011] Under physiological solution conditions of salt and pH, the virus like particles (VLPs) of Human Papillomavirus (HPV) L1 protein are not stable during long term storage either in solution or after adsorption to aluminum adjuvant at 2-8°C. Thus this invention relates to new formulations of aluminum adjuvanted HPV VLP vaccines with improved storage stability. The formulations may be in aqueous solution.

[0012] In accordance with this invention, any type of HPV VLPs may be used as the antigenic portion of the vaccine. The VLPs may contain only L1 protein, or may be made of both L1 and L2 protein. The proteins may be of a wild-type amino acid composition, or they may contain mutations. VLPs containing only L1 protein which is of a wild-type amino acid composition are preferred.

[0013] The HPV vaccines which are particularly preferred are those associated with disease, including but not limited to: HPV 6a, HPV 6b, HPV 11, HPV16, and HPV18. In addition, the formulations of this invention are suited for combinations of HPV types, including multivalent vaccines containing a plurality of HPV antigens, such as a combination of HPV 6, 11, 16 and 18. It is preferred that the VLPs be made by recombinant techniques, as is known in the art. It is particularly preferred that the host cell used to make the VLPs is a yeast cell, although other cell types, such as bacterial, insect and mammalian cells.
are known and currently used as hosts.

[0014] The concentration of HPV VLPs which are adsorbed onto aluminum is from about 10-200 mcg/ml for each HPV VLP type. This may be adjusted, depending on such factors as antigenicity of the particular type of HPV, and the presence of multiple types of HPVs in a "cocktail"-type of vaccine.

[0015] Another embodiment of this invention is a formulation which omits the higher salt concentration, and comprises HPV VLPs adsorbed onto aluminum, a physiological concentration of salt (about 0.15M), and a polymeric polyanionic stabilizer in the presence or absence of buffer agents and nonionic detergents.

Salts

[0016] The ionic strength of the solution is maintained by the presence of salts. Almost any salt which can contribute to the control of the ionic strength may be used. Preferred salts which can be used to adjust ionic strength are: any physiologically acceptable salt, such as NaCl, KCl, Na₂SO₄, (NH₄)₂SO₄, sodium phosphate and sodium citrate. Particularly preferred salts are: NaCl, KCl, and Na₂SO₄. It has been found that increasing ionic strength dramatically enhances the stability of HPV VLPs against heat induced aggregation. For example, the stability of HPV VLP protein solution was analyzed for aggregate formation as a function of temperature by using an UV spectrophotometer (turbidity assay for cloud point determination). The cloud point data indicate that increasing ionic strength dramatically enhances the stability of HPV VLPs in solution against heat-induced aggregation with the temperature of the initiation of turbidity formation being raised approximately 7°C.

[0017] The salts should be present in concentrations of from 0.10M to 1M. However, very high concentrations are not preferred due to the practical limitations of parenteral injection of high salt concentrations. Instead, more moderate salt concentrations, such as more physiological concentrations of 0.15M to 0.5M with 0.15M-0.32M NaCl are preferred.

Buffers

[0018] Some formulations of this invention also contain a buffer to maintain the pH range so that the vaccine is in the non-irritating pH range with optimal HPV VLP stability. The effect of pH on the stability of HPVs both in solution and adsorbed to aluminum formulation were also investigated in accordance with this invention. Results indicate that HPV VLPs are stable only within a relatively small range of pH 5.5-7.0, and that the preferred pH range is 6.0-6.5, and particularly 6.2 as measured by in vitro antigenicity.

[0019] The storage stability of HPV-aluminum formulations was further tested with the addition of a buffer and non-ionic surfactant. Better pH control of the aluminum adjuvanted HPV VLP vaccine during storage was observed when either histidine or imidazole was added as a buffer agent. In general, the concentration of the buffer should range from 2 mM to 100 mM, with 5 mM to about 20 mM being preferred, and 10 mM being another preferred concentration. Phosphate-containing buffers are generally not preferred, as they may interact with aluminum adjuvants. The interaction of phosphate buffer ions with aluminum adjuvant as well as the non interaction of histidine and imidazole buffers with aluminum adjuvant was demonstrated by zeta potential measurements of the surface charge of the aluminum adjuvant.

Non-ionic surfactant

[0020] A further component in some of formulation of this invention is a non-ionic surfactant. The surfactant may be selected from the group consisting of: polyoxyethylene sorbitol fatty acid esters (Polysorbates) such as Polysorbate 80 (e.g., TWEEN 80®), Polysorbate 20, (e.g., TWEEN 20®), polyoxyethylene alkyl ethers (e.g., Brij 35®, and Brij 58®), as well as others, including Triton X-100®, Triton X-114®, NP-40®, Span 85 and the Pluronics series of non-ionic surfactants (e.g., Pluronic 121), with Polysorbate 80 being particularly preferred. The surfactant is generally present in a concentration of from 0.0005% to 0.5% (wt/vol). Polysorbate 80 was found to protect HPV VLP-aluminum from inactivation during simulated shipping stress (i.e., shaking or stirring). For Polysorbate 80, a preferred concentration is about 0.01%.

Polymeric polyanion stabilizer

[0021] A further significant enhancement of the stability of the HPV-VLP formulation may also accomplished by the addition of polyanionic, polymeric excipients, such as a stabilizer. As used throughout the specification and claims, the term "polyanionic polymer" is meant to refer to compounds which have either a single long chain, or those with multiple cross linked chains; either type possessing multiple negative charges along the chain(s) when in solution. Examples of polyanionic polymers include: proteins, polyanions, peptides and poly-nucleic acids. Specific stabilizers may be selected from the group consisting of:

carboxymethyl cellulose (particularly 10-800cps),
heparin (6-30 kDa),
poly-amino acids (2-100 kDa) such as poly(Glu), Poly(Asp), and Poly(Glu, Phe),
oxidized glutathione [Glu-Cys-Gly]₂ (613 Da),
poly-nucleotides such as polycytidylic acid (200-700 kDa), and polyadenylic acid(200-700 kDa),
RNA, DNA, and serum albumins.
The concentration of the stabilizer, when present, is from 0.01% to 0.5%, particularly 0.05-0.1% (by weight), although the addition of even a ten fold lower amount of polyvanionic excipients (for example, 0.01% albumin, DNA or heparin) still provides enhanced stability to HPV VLP-aluminum formulations, although the stabilizing effect is relatively less significant perhaps due to the lower concentrations. As described in more detail in the Examples, polyvanions provided a dramatic stabilization of HPV-aluminum formulations. The stabilizing mechanisms of these classes of excipients may vary from directly binding to HPV VLP molecule (such as occurs with DNA), inhibiting HPV VLP or HPV VLP-aluminum adsorption on surfaces, increasing solution viscosity, neutralizing surface charge, reformation of HPV VLP disulfide bonds or interfering with amino acid side chain oxidation, and/or increasing the conformational rigidity of HPV VLPs. It is important to note that HPV VLP L1 protein contains a polyvanion binding site of multiple positively charged amino acids in the C-terminal region of the protein.

These polyvanions were further tested under accelerated stability study conditions at 37°C. Polyvanion containing formulations of HPV-aluminum retained about 80% of in vitro antigenicity while the control HPV formulation (without addition of polyvanions) had almost no in vitro antigenicity after one week of incubation at 37°C. The same set of samples after two weeks of incubation at 37°C show no significant difference from the one-week data indicating the strong stabilizing effect of these polyvanionic excipients.

The polyanion stabilization effect was also investigated for HPV VLPs in solution under accelerated conditions at 37°C. The data indicate that for polynucleotide poly(A) or poly(C), a concentration of 0.001% (by weight or 10 mcg/ml) is required for HPV VLP (at 80 mcg/ml protein) to maintain maximum stability against heat induced inactivation as measured by in vitro antigenicity. Sedimentation and UV analysis indicates that polyvanions bind to the VLP directly.

In addition, HPV VLP adsorption to aluminum adjuvant may be inhibited by prebinding of polyvanions to the HPV VLP. For example, carboxymethyl cellulose can inhibit HPV VLP binding to aluminum at certain concentrations of the polyvanion. The addition of carboxymethyl cellulose after HPV VLP adsorption to aluminum adjuvant, however, results in virtually no detectable release of HPV VLP from aluminum while providing a dramatic stability enhancement in a concentration dependent manner.

Especially preferred among the polyvanions (0.001 - 0.25% by weight) is carboxymethyl cellulose, (10-800 cps) with an approximate typical molecular weight of 50,000-700,000 Da, with lower viscosity 10-200 cps carboxymethyl cellulose being especially preferred.

Preferred formulations of this invention include the following:

I. a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof; b) 0.32 M NaCl; c) 10 mM histidine buffer, pH 6.2; and d) 0.01% Polysorbate 80.

II. a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof; b) 0.32 M NaCl; c) 10 mM histidine buffer, pH 6.2; d) 0.01% Polysorbate 80, and 0.05% carboxymethyl cellulose.

III. a) 10-200 mcg/ml of each HPV VLP type adsorbed onto aluminum, wherein the VLPs are selected from the group consisting of: HPV 6a, HPV 6b, HPV 11, HPV 16, HPV 18, and mixtures thereof; b) 0.15 M NaCl; and c) 0.05% carboxymethyl cellulose; and d) optionally 10 mM histidine, pH 6.2 and 0.01% polysorbate 80.

EXAMPLE 1

General Methods

A frozen solution of yeast derived recombinant L1 protein HPV16 VLP (with greater than 95% purity) at a protein concentration of 870 mcg/ml in 0.5M NaCl, 0.003% polysorbate 80, pH of approximately 6.2 was used for most of these experiments. Aluminum adjuvant was manufactured at Merck. Excipients were purchased commercially. All the vaccine formulations were prepared as follows: The HPV VLP solution with or without dilution was added to aluminum adjuvant for HPV VLP alum adsorption, then the excipients were added. All final desired concentrations for all excipients, HPV VLP and aluminum adjuvant in the formulation could be approached by either direct mixing with designed volume, or by adjusting with a settle/decant process.

In vitro antigenicity assays.

To release HPV from the aluminum adjuvant, an aluminum dissolution method was developed which included dilution of HPV-aluminum formulation into a high salt solution containing citrate and polysorbate 80. The HPV VLP samples from the aluminum dissolution method are directly subjected to an in vitro antigenicity assay using Biacore analysis (utilizing an HPV VLP type specific neutralizing antibody). The HPV VLP samples from the aluminum adjuvanted stability studies are directly compared to a frozen stock solution of the same HPV VLP to determine in vitro antigenicity.

pH measurement.

The pH measurements of HPV-aluminum for-
mulations were performed at ambient temperature using an Orion pH meter of model 420A. All samples incubated at various temperatures were equilibrated at room temperature for 30 minutes before pH determination. The pH meter was calibrated manually using two standard buffers that bracket the expected sample pH range, with a correlation coefficient between 95%-100%. Temperature effect on the pH of histidine buffered solutions was determined by varying temperature gradually from 4°C to 37°C.

Protein concentration determination (UV spectroscopy)

[0031] The protein concentration of HPV in both bulk solutions and formulated samples (after aluminum dissolution) were determined by UV absorbance spectra measurement at ambient temperature using a HP 8452A Diode Array spectrophotometer and a cuvette with a path length of 1 cm. The sample volumes used were approximately 200 to 250 microliters. The protein concentration was calculated using a multicomponent second derivative analysis technique developed for use with HPV VLPs.

Other analyses

[0032] Sedimentation velocity experiments were performed using Beckman XL I analytical ultracentrifuge.

[0033] Turbidity assays were carried out using a HP 8452A Diode Array spectrophotometer equipped with a HP 845X UV-Visible system software and a temperature control system. The light scattering of the solutions were followed at 320-350 nm under the kinetic mode of the program by increasing the temperature from about 25°C to 80°C.

[0034] Zeta potential of aluminum particles and HPV VLPs were determined using a Malvern Zetasizer 3000 System with varying the solution pH from 4 to 9 or the excipient concentrations.

Accelerated and Real Time Stability Studies.

[0035] HPV-aluminum formulation stability studies were carried out under both accelerated and real time conditions. The temperature of accelerated stability studies varied from 15°C to 37°C. The temperature of real time stability studies was at 2-8°C. These temperature ranges were chosen based on the fact that HPV VLP inactivation rate is very sensitive to temperature. Previous conformational integrity data via biophysical measurements have shown that increasing the temperature to above 40-45°C induces significant conformational changes in the HPV VLP in solution, a condition which needs to be avoided in accelerated stability studies.

EXAMPLE 2

[0036] Effects of salt and excipients on stability The effects of NaCl concentration and added excipients on the stability of HPV16 VLP-aluminum formulations at 2-8°C was investigated. Formulations of 160 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant in different NaCl concentration solution with/without the addition of 0.01% Polysorbate 80, 10 mM histidine were incubated at 2-8°C. The in vitro antigenicity of the formulations was then assayed after different incubation times by Biacore analysis. Results are shown in FIGURE 1A. The experiment was then repeated for incubation at 15°C. FIGURE 1B shows the results when incubated at 15°C. In both graphs, closed squares are the points for 0.15M NaCl; open squares are the points for 0.30M NaCl; closed circles are for 0.30 M NaCl, 0.01% Polysorbate 80, and 10 mM histidine. The data indicate that increasing salt concentration enhances the vaccine stability and the addition of buffer and polysorbate further enhances the stability.

EXAMPLE 3

[0037] Effects of stabilizers The effects of stabilizing excipients on the accelerated stability of HPV 16 VLP-aluminum formulations was investigated. Formulations of 200 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant with/without the addition of different stabilizing excipients were incubated at pH 6.2 and 25°C. The in vitro antigenicity of the formulations was then assayed after different incubation times by Biacore analysis. The formulation compositions are shown in FIGURE 2: closed circles are for 0.3M NaCl + 0.1% polyglutamic acid (poly-Glu); crossed squares are for 0.3M NaCl + 0.1% polyadenylic acid (poly-A); open squares are for 0.3M NaCl + 10 mM histidine + 0.01% polysorbate 80; open triangles are for 0.3M NaCl; and open circles are for 0.15M NaCl. The data indicate that at 25°C, increasing salt concentration from 0.15 to 0.3 M and adding buffer and polysorbate enhances the stability of the formulation. The addition of a polyanion, however, more dramatically enhances the stability of aluminum adsorbed HPV VLPs against heat induced loss of in vitro antigenicity.

EXAMPLE 4

[0038] Effects of polyanions on stability The effects of polyanion excipients on the accelerated stability of HPV 16 VLP-aluminum formulations was investigated in more detail. Formulations of 160 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant in 0.3 M NaCl, 0.01% Polysorbate 80, 10 mM Histidine, pH 6.2 with/without the addition of a polyanion excipient were incubated at 37°C for 1 week. The in vitro antigenicity of the formulations was then assayed by Biacore analysis. The kinds and concentrations of polyanion excipients added are as shown in FIGURE 3, from left to right: control; 0.1 % poly Adenylic acid (Poly A); 0.1% poly Cytidylic acid (poly C); 0.1% poly Aspartic acid (poly Asp) (5-15K); 0.1% poly Glutamic acid (poly-Glu) (5-15K); 0.1% poly Glutamic acid (poly-Glu) (50-100K); 0.1% bovine serum albumin
EXAMPLE 5

[0040] The effect of carboxymethyl cellulose. The effect of carboxymethyl cellulose (CMC) concentration on the accelerated stability of HPV16 VLP-aluminum formulations was examined. Formulations of 160 mcg/ml HPV16 on 450 mcg/ml aluminum adjuvant in 0.3 M NaCl, 0.01 % polysorbate 80, 10 mM Histidine, pH 6.2 with/without the addition of different amount of CMC (200 cps) were incubated at 37°C for 1 week. The in vitro antigenicity of the formulations was then assayed by Biacore analysis. Results are shown in FIGURE 4 for 0, 0.005%, 0.01%, 0.05%, 0.1% and 0.2% CMC. The data indicate that the stability enhancement is a function of added CMC concentration. In vitro antigenicity is retained in the presence of at least 0.05% CMC.

EXAMPLE 6

[0041] Effects of size of CMC. The effects of carboxymethyl cellulose (CMC) molecular weight and NaCl concentration on the accelerated stability of HPV16 VLP-aluminum formulations was examined. Formulations of 160 mcg/ml HPV16 VLP on 450 mcg/ml aluminum adjuvant in 0.3 M NaCl, 0.01 % polysorbate 80, 10 mM Histidine, pH 6.2 with/without the addition of different amount of CMC (200 cps) were incubated at 37°C for 1 week. The in vitro antigenicity of the formulations was then assayed by Biacore analysis. Results are given in FIGURE 5: open circle is for 0.32 M NaCl; and closed circle is for 0.15M NaCl, CMC 200 cps; open square with dotted line is for 0.32 M NaCl; and closed circle is for 0.15M NaCl. The data indicate the CMC with smaller molecular weight is either 10-20 cps or 200 cps level. Results are given in FIGURE 5: open circle is for 0.32 M NaCl, CMC 200 cps; open square with solid line is for 0.32 M NaCl, CMC 10-20 cps; closed diamond is for 0.15M NaCl, CMC 10-20 cps; open square with dotted line is for 0.32 M NaCl; and closed circle is for 0.15M NaCl. The data indicate the CMC with smaller molecular weight (about 10-20 cps) basically provides similar stability enhancement to HPV VLP-aluminum formulations with CMC 200 cps and that the presence of CMC allows the vaccine preparation to be formulated at a physiological salt concentration (0.15 M NaCl).

Claims

1. A human papillomavirus (HPV) vaccine formulation comprising:
   a) HPV virus-like particles (VLPs) which are adsorbed on an aluminum adjuvant each HPV VLP type being present in an amount of 10-200 μg/ml, the VLPs being independently selected from HPV6a, HPV6b, HPV11, HPV16 and HPV18;
   b) a salt selected from NaCl, KCl, Na2SO4, (NH4)2SO4, sodium phosphate and sodium citrate;
   c) a buffer which is histidine or imidazole which provides for a pH range of the vaccine solution of from pH 6.0 to 6.5; and
   d) a non-ionic surfactant chosen from polyoxyethylene sorbitol fatty acid esters, polyoxyethylene alkyl ethers, Triton X-100®, Triton X-114®, NP-40®, Span 85 and the Pluronic series of non-ionic surfactants.

2. A vaccine according to Claim 1 wherein the salt is present in a concentration of from 0.10M to 0.5M.

3. A vaccine according to Claim 2 wherein the salt is 0.32M NaCl.

4. A vaccine according to Claim 1, 2 or 3 wherein the buffer is present in a concentration of 2 mM to 100 mM.

5. A vaccine according to Claim 4 wherein the buffer is 10 mM histidine, pH 6.2.

6. A vaccine according to anyone of Claims 1 to 5 wherein the non-ionic surfactant is selected from: Polysorbate 20, Polysorbate 80, NP-40®, Triton X-100®, Triton X-114®, Span 85, Brij 35®, Brij 58®.

7. A vaccine according to any preceding Claim wherein the surfactant is present in a concentration of from 0.0005% to 0.5% (wt/vol).

8. A vaccine formulation according to Claim 1 comprising:
   b) 0.32 M NaCl;
   c) 10 mM histidine buffer, pH 6.2; and
   d) 0.01 % Polysorbate 80.

9. A vaccine formulation according to any preceding Claim which is in an aqueous solution.

10. A vaccine according to any preceding Claim further comprising a polymeric polyanionic stabilizer.

11. A vaccine according to Claim 10 wherein the stabilizer is selected from: carboxymethyl cellulose (CMC), heparin, Poly(Glu), Poly(Asp), Poly (Glu, Phe), oxidized glutathione, polycytidylic acid, polya- denylic acid, RNA, DNA, and serum albumins.
12. A vaccine according to Claim 10 or 11 wherein the concentration of stabilizer is from 0.01% to 0.5% (by weight/volume).

13. A vaccine according to Claim 11 or 12 wherein the stabilizer is CMC.

14. An HPV vaccine formulation according to Claim 13 comprising:
   
   b) 0.32 M NaCl;
   c) 10 mM histidine buffer, pH 6.2;
   d) 0.01% Polysorbate 80; and
   e) 0.05% carboxymethyl cellulose.

Patentansprüche

1. Humane Papillomavirus (HPV)-Impfstoffformulierung umfassend:
   
   a) HPV-virusähnliche Partikel (VLP), die an ein Aluminium-Adjuvans adsorbiert sind, wobei jeder HPV-VLP-Typ in einer Menge von 10 - 200 μg/ml vorliegt, wobei die VLP unabhängig aus HPV6a, HPV6b, HPV11, HPV16 und HPV 18 ausgewählt sind;
   b) ein Salz, das aus NaCl, KCl, Na₂SO₄, (NH₄)₂SO₄, Natriumphosphat und Natriumcitrat ausgewählt ist;
   c) einen Puffer, der Histidin oder Imidazol darstellt, der einen pH-Bereich der Impfstofflösung von pH 6.0 bis 6.5 abdeckt; und
   d) ein nicht ionisches Tensid, das aus Polyoxyethylensorbitol-Fettsäureestern, Polyoxyethylenalkylethern, Triton-100®, Triton X-114®, NP-40®, Span 85 und der Pluronic-Reihe nicht ionischer Tenside ausgewählt ist.

2. Impfstoff nach Anspruch 1, worin das Salz in einer Konzentration von 0,10 M bis 0,5 M vorliegt.

3. Impfstoff nach Anspruch 2, worin das Salz 0,32 M NaCl darstellt.

4. Impfstoff nach Anspruch 1, 2 oder 3, worin der Puffer in einer Konzentration von 2 mM bis 100 mM vorliegt.

5. Impfstoff nach Anspruch 4, worin der Puffer 10 mM Histidin, pH 6,2, darstellt.

6. Impfstoff nach einem der Ansprüche 1 bis 5, worin das nicht ionische Tensid ausgewählt ist aus: Polysorbat 20, Polysorbat 80, NP-40®, Triton X-100®, Triton X-114®, Span 85, Brij 35® und Brij 58®.

7. Impfstoff nach einem der vorangehenden Ansprüche, worin das Tensid in einer Konzentration von 0,0005 % bis 0,5 % (G/V) vorliegt.

8. Impfstoffformulierung nach Anspruch 1, umfassend:
   
   b) 0,32 M NaCl;
   c) 10 mM Histidinpuffer, pH 6,2; und
   d) 0,01 % Polysorbat 80.

9. Impfstoffformulierung nach einem der vorangehenden Ansprüche, die in einer wässrigen Lösung vorliegt.

10. Impfstoff nach einem der vorangehenden Ansprüche, weiter umfassend einen polymeren polyanionischen Stabilisator.

11. Impfstoff nach Anspruch 10, worin der Stabilisator ausgewählt ist aus: Carboxymethylcellulose (CMC), Heparin, Poly(Glu), Poly(Asp), Poly(Glu, Phe), oxidiertem Glutathion, Polycytidylsäure, Polyadenylsäure, RNA, DNA und Serumalbuminen.

12. Impfstoff nach Anspruch 10 oder 11, worin die Konzentration des Stabilisators von 0,01 % bis 0,5 % (G/V) beträgt.

13. Impfstoff nach Anspruch 11 oder 12, worin der Stabilisator für CMC steht.

14. HPV-Impfstoffformulierung nach Anspruch 13, umfassend:
   
   b) 0,32 M NaCl;
   c) 10 mM Histidinpuffer, pH 6,2;
   d) 0,01 % Polysorbat 80; und
   e) 0,05 % Carboxymethylcellulose.

Revendications

1. Formulation vaccinale contre le virus du papillome humain (VPH) comprenant:
   
   a) des particules pseudo-virales (PPV) VPH qui sont adsorbées sur un adjuvant aluminium, chaque type de PPV VPH étant présent en une quantité de 10-200 μg/ml, les PPV étant indépendamment sélectionnées parmi VPH6a, VPH6b, VPH11, VPH16 et VPH18;
   b) un sel sélectionné parmi NaCl, KCl, Na₂SO₄, (NH₄)₂SO₄, le phosphate de sodium et le citrate de sodium;
   c) un tampon qui est de l’histidine ou de l’imidazole, lequel assure à la solution vaccinale une plage de pH de pH 6,0 à 6,5; et
   d) un tensioactif non ionique choisi parmi des esters d’acides gras dérivés de polyoxyéthylène sorbitol, des éthers de polyoxyéthylène-alkyle,
Triton X-100®, Triton X-114®, NP-40®, Span 85 et la série pluronique de tensioactifs non ioniques.

2. Vaccin selon la revendication 1, dans lequel le sel est présent en une concentration de 0,10 M à 0,5 M.

3. Vaccin selon la revendication 2, dans lequel le sel est du NaCl 0,32 M.

4. Vaccin selon la revendication 1, 2 ou 3, dans lequel le tampon est présent en une concentration de 2 mM à 100 mM.

5. Vaccin selon la revendication 4, dans lequel le tampon est de l'histidine 10 mM, pH 6,2.

6. Vaccin selon l’une quelconque des revendications 1 à 5, dans lequel le tensioactif non ionique est sélectionné parmi: le Polysorbate 20, Polysorbate 80, NP-40®, Triton X-100®, Triton X-114®, Span 85, Brij 35®, Brij 58®.

7. Vaccin selon l’une quelconque des revendications précédentes, dans lequel le tensioactif est présent en une concentration de 0,0005% à 0,5% (poids/volume).

8. Formulation vaccinale selon la revendication 1, comprenant:
   b) du NaCl 0,32 M;
   c) un tampon histidine 10 mM, pH 6,2; et
   d) du Polysorbate 80 0,01%.

9. Formulation vaccinale selon l’une quelconque des revendications précédentes qui est dans une solution aqueuse.

10. Vaccin selon l’une quelconque des revendications précédentes comprenant un stabilisant polymère polyanionique.

11. Vaccin selon la revendication 10, dans lequel le stabilisant est sélectionné parmi: la carboxyméthylcellulose (CMC), l’héparine, Poly(Glu), Poly(Asp), Poly(Glu, Phe), le glutathion oxydé, l’acide polycytidylique, l’acide polyadénylique, de l’ARN, de l’ADN et des sérum-albumines.

12. Vaccin selon la revendication 10 ou 11, dans lequel la concentration de stabilisant est de 0,01% à 0,5% (en poids/volume).

13. Vaccin selon la revendication 11 ou 12, dans lequel le stabilisant est de la CMC.

14. Formulation vaccinale contre le VPH selon la revendication 13, comprenant:
   b) du NaCl 0,32 M;
   c) un tampon histidine 10 mM, pH 6,2; et
   d) du Polysorbate 80 0,01%; et
   e) de la carboxyméthylcellulose 0,05%.
Figure 2: Graph showing the relative bioreactor response (% of ref. at target conc.) over time (months) at 25°C for different conditions:

- 0.32 M NaCl + 0.1% POLY (Glu)
- 0.32 M NaCl + 0.1% POLY (A)
- 0.3 M NaCl + 10 mM HISTIDINE + 0.01% POLYSORBATE80
- 0.3 M NaCl
- 0.15 M NaCl
FIG. 5

Relative biacore response (% of ref. at target conc.)

Days at 37°C

37°C

- 0.32 M NaCl, CMC200
- 0.32 M NaCl, CMC20
- 0.15 M NaCl, CMC20
- 0.32 M NaCl
- 0.15 M NaCl
REFERENCES CITED IN THE DESCRIPTION

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