

DESCRIPTION

METHOD FOR PRODUCING INFLUENZA HA SPLIT VACCINE

5 TECHNICAL FIELD

[0001]

The present invention relates to a method for producing an influenza HA split vaccine.

BACKGROUND ART

[0002]

10 Current influenza haemagglutinin (hereinafter also abbreviated as “HA”) vaccines induce an anti-HA antibody, thereby exerting a protective effect against infection. The anti-HA antibody binds to a portion of a virus called a “head region” externally exposed from a virus membrane. This region most frequently undergoes structural change in a viral strain. Therefore, in some cases, the anti-HA antibody may fail to bind to a virus which causes antigenic variation
15 and is different from the vaccine strain, and the vaccine cannot exert the protective effect against the infection.

[0003]

Recently, it has been revealed that antibodies that bind to a stem region which is less likely to cause antigenic variation include protective antibodies against infection (Patent
20 Document 1). In order to efficiently induce the antibody that binds to the stem region, a HA stem protein having a stabilized stem portion has been developed, and its clinical trial in humans has been carried out: the stem portion, which is originally unstable, has been stabilized through artificial variation or binding of linkers.

[0004]

25 However, problems about the production for practical use still remain to be solved, and development of a HA vaccine antigen that can induce an anti-stem antibody more easily has

been expected.

CITATION LIST

PATENT DOCUMENT

[0005]

- 5 Patent Document 1: Japanese Unexamined Patent Publication (Translation of PCT International Application) No. 2016-516090

SUMMARY OF THE INVENTION

TECHNICAL PROBLEM

[0006]

- 10 In view of the foregoing, it is an object of the present invention to provide a method for producing an influenza HA split vaccine which produces an antibody that binds to a HA stem region, which is less likely to cause antigenic variation, of influenza.

SOLUTION TO THE PROBLEM

[0007]

- 15 The method for producing a HA split vaccine according to the present invention includes subjecting an influenza HA split vaccine to an acidic treatment, thereby producing an influenza HA split vaccine which produces an antibody that binds to a long alpha helix (LAH) of a HA stem region, and is effective against an influenza virus that causes antigenic variation.

[0008]

- 20 Specifically, the present invention relates to the following.

[Item 1]

A method for producing an influenza HA split vaccine which produces an antibody that binds to a LAH of a HA stem region, the method including: subjecting an influenza HA split vaccine to an acidic treatment.

- 25 [Item 2]

The production method according to Item 1, wherein the influenza HA split vaccine is also effective against an influenza virus that causes antigenic variation.

[Item 3]

5 A method for producing an influenza HA split vaccine which produces an antibody that binds to a LAH of a HA stem region and which is effective against an influenza virus that causes antigenic variation, the method including: subjecting an influenza HA split vaccine to an acidic treatment.

[Item 4]

10 The method of any one of Items 1 to 3, wherein the acidic treatment is performed at a pH of 4.4 to 5.8.

[Item 5]

The method of any one of Items 1 to 4, wherein the influenza HA split vaccine is of type H3N2 or type H1N1.

[Item 6]

15 An influenza HA split vaccine which produces an antibody that binds to a LAH of a HA stem region.

[Item 7]

The influenza HA split vaccine of Item 6, which is also effective against an influenza virus that causes antigenic variation.

20 [Item 8]

The influenza HA split vaccine of Item 6 or 7, which has a HA stem region exposed outside.

[Item 9]

25 The influenza HA split vaccine of any one of Items 6 to 8, wherein the HA stem region of the influenza HA split vaccine antigen, which is exposed outside, enhances the antigenicity

of the LAH of the HA stem region, and the influenza HA split vaccine is capable of producing an antibody that binds to the LAH of the HA stem region.

[Item 10]

An influenza HA split vaccine which is effective against an influenza virus that causes
5 antigenic variation, and which produces an antibody that binds to a LAH of a HA stem region, the vaccine being produced by subjecting an influenza HA split vaccine to an acidic treatment.

[Item 11]

An influenza HA split vaccine which is produced by subjecting an influenza HA split vaccine to an acidic treatment, produces an antibody that binds to a LAH of a HA stem region,
10 and is also effective against an influenza virus that causes antigenic variation.

ADVANTAGES OF THE INVENTION

[0009]

According to the present invention, an influenza HA split vaccine which produces an antibody that binds to a HA stem region of influenza is obtained by a simple technique, the HA
15 stem region being less likely to cause antigenic variation. Therefore, an influenza HA split vaccine which is also effective against an influenza virus which causes antigenic variation is obtained.

BRIEF DESCRIPTION OF THE DRAWINGS

20 [0010]

[FIG. 1] FIG. 1 is a schematic diagram illustrating an influenza virus.

[FIG. 2] FIG. 2 is a graph showing an increase in the titer of an anti-LAH antibody in sera of mice inoculated with a membrane fusion-type H3N2 HA split vaccine.

[FIG. 3] FIG. 3 is a graph showing an improvement in the cross-protective capacity of
25 mice inoculated with the membrane fusion-type H3N2 HA split vaccine against an antigenic

variant.

[FIG. 4] FIG. 4 is a graph showing an increase in the titer of an anti-LAH antibody in sera of mice inoculated with a membrane fusion-type H1N1 HA split vaccine.

[FIG. 5] FIG. 5 is a graph showing an improvement in the cross-protective capacity of mice inoculated with the membrane fusion-type H1N1 HA split vaccine against an antigenic variant.

[FIG. 6] FIG. 6 shows graphs each indicating that a LAH binding monoclonal antibody binds more strongly to a membrane fusion-type HA split vaccine than to a current HA split vaccine.

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DESCRIPTION OF EMBODIMENTS

[0011]

Embodiments of the present invention will be described in detail below with reference to the accompanying drawings. However, the embodiments are intended to facilitate understanding of the principle of the present invention, and the scope of the invention is not limited to the following embodiments. Other embodiments, in which the configuration of the following embodiments has been appropriately replaced by those skilled in the art, are also encompassed in the scope of the present invention.

[0012]

20 The method for producing an influenza HA split vaccine according to this embodiment includes a step of subjecting an influenza HA split vaccine to an acidic treatment.

[0013]

An influenza HA split vaccine is prepared through a treatment of a whole-virus vaccine with ether to remove lipid components which become pyrogens. The influenza HA split vaccine has HA protein as the main ingredient because the influenza HA split vaccine is produced by

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collecting the HA protein, which is required for immunization, from the surfaces of the virus particles by density gradient centrifugation.

[0014]

Glycoprotein called “spike protein” protrudes from the surface of an influenza virus (FIG. 1). An influenza A virus has two types of spike proteins, namely, HA and NA (neuraminidase), which help the virus cause the infection. HA binds to a cell to be infected and helps the entry of the virus into the cell. HA frequently causes antigenic variation. NA unbinds the infected cell from HA, and serves to release the replicated viruses from the cell.

[0015]

HA of the influenza A virus is divided into two regions, namely, a head region and a stem region (FIG. 1). The head region contains a receptor binding site at which the virus binds to a target cell. The stem region contains a fusion peptide sequence necessary for membrane fusion between the viral membrane and the cell membrane of the target cell.

[0016]

An acidic treatment on the influenza HA split vaccine changes the structure of the HA protein to a structure called membrane fusion-type. In the membrane fusion-type HA protein, the stem region is exposed outside from the viral membrane instead of the head region, with a large structural change in the conformation of an antigen stem. The present inventors have found in vivo that when the membrane fusion-type HA protein is used as a vaccine, an antibody that binds to a LAH of the stem region is induced, and that this antibody has a protective effect against a virus strain that causes antigenic variation. The present invention has been made based on this fact.

[0017]

The acidic treatment is not particularly limited, and may be performed at a pH of, for example, 3.0 to 6.5, preferably 4.0 to 6.0, and more preferably 4.4 to 5.8. The acid for use in

the acidic treatment is not particularly limited, and may be, for example, phosphoric acid, citric acid, maleic acid, or any other suitable acid.

[0018]

Based on differences in antigenicity, HA of the influenza A virus is classified into 18
5 subtypes (H1 to H18), and NA into 9 subtypes (N1 to N9). The influenza HA split vaccine of
the present invention is applicable to all of these subtypes. In addition, the method for producing
the influenza HA split vaccine according to the present invention can produce a vaccine which
is effective against not only the influenza A virus, but also an influenza B virus having HA.

[0019]

10 The influenza split HA vaccine obtained by the production method according to the
present invention produces an antibody that binds to a LAH which is less likely to cause
variation. Therefore, the vaccine can be cross-protective against an influenza virus, which is
known as an antigenic variant, as long as the virus has the same HA subtype. Furthermore, the
influenza split HA vaccine obtained by the production method according to the present
15 invention may be cross-reactive between HA subtypes of similar amino acid sequences of LAH
(e.g., H3 and H7).

[0020]

In a preferred embodiment, the influenza HA split vaccine obtained by the production
method of the present invention binds to a LAH binding monoclonal antibody more strongly
20 than a current HA split vaccine. For example, the influenza HA split vaccine binds to the LAH
binding monoclonal antibody at least 1.05 times, preferably at least 1.1 times, more preferably
at least 1.5 times, and even more preferably at least two times more strongly than the current
HA split vaccine. In this context, "the influenza HA split vaccine binds at least 1.05 times, at
least 1.1 times, at least 1.5 times, or at least two times more strongly than the current HA split
25 vaccine" means, for example, that the reciprocal of the antibody concentration at the time when

an absorbance determined by regression is 0.7 is at least 1.05 times, at least 1.1 times, at least 1.5 times, or at least two times the reciprocal of the antibody concentration of the current HA split vaccine. In a preferred embodiment, the binding capacity of the influenza HA split vaccine of the present invention to the LAH binding monoclonal antibody is higher than that of the current HA split vaccine. Although the upper limit is not particularly limited, the binding capacity may be in a range of, for example, 1.05 to 200 times, 1.1 to 150 times, 1.5 to 100 times, or 2 to 50 times. Alternatively, the range of the binding capacity of the influenza HA split vaccine of the present invention to the LAH binding monoclonal antibody compared to that of the current HA split vaccine may be indicated by a combination of the lower limit value selected from 1.05, 1.1, 1.5, 2, 3, 4, and 5 and the upper limit value selected from 200, 150, 100, 50, 30, and 20. For the measurement of the binding capacity of the influenza HA split vaccine to the LAH binding monoclonal antibody, any method can be used without particular limitations, and a common method known to those skilled in the art can be employed. For example, the binding capacity can be measured by a method described in examples of the present application.

[0021]

In the present application, the “LAH binding monoclonal antibody” means a monoclonal antibody which binds to the LAH. For the production of the monoclonal antibody, any method may be used without particular limitations, and a common method known to those skilled in the art may be employed. In the measurement of the binding capacity of the influenza HA split vaccine to the LAH binding monoclonal antibody, it is assumed that the LAH binding monoclonal antibody is capable of binding to a peptide corresponding to at least a portion of the LAH of an influenza virus from which the influenza HA split vaccine is derived.

[0022]

In this application, the “current HA split vaccine” means a vaccine from which lipid components that become pyrogens are removed through a treatment of the whole-virus vaccine

with ether, and can be produced by a method described in Example 1 of the present application, for example. The current HA split vaccine may also be an influenza HA split vaccine produced without being subjected to an acidic treatment, in contrast with the influenza HA split vaccine of the present invention prepared by a method including the following acidic treatment.

5 [0023]

The method for producing the influenza HA split vaccine of the present invention may include a step of adding an adjuvant. Examples of the adjuvant include, but are not limited to, aluminum salts such as aluminum hydroxide and aluminum phosphate, chitosan, oligodeoxynucleotides, and oil-in-water emulsions. Among them, aluminum hydroxide is preferred, and use of aluminum hydroxide as the adjuvant can enhance the immunogenicity.

[0024]

The influenza HA split vaccine obtained by the production method of the present invention can be used, for example, for additional inoculation after a predetermined period after the initial inoculation. The period after the initial inoculation and before the additional inoculation is not particularly limited, but may be, for example, twenty days to three years, preferably three months to two years, more preferably six months to one year. The amount of the influenza HA split vaccine for the initial and additional inoculations is not particularly limited, but may be, for example, 1 μ g to 200 μ g, preferably 10 μ g to 30 μ g, more preferably 15 μ g, per dose. A single dose is, for example, 0.5 mL. Any administration method may be used for the initial and additional inoculations without particular limitations, and for example, nasal, subcutaneous, intradermal, transdermal, intraocular, mucosal, or oral administration may be employed. Intramuscular administration is preferred.

[0025]

The influenza HA split vaccine obtained by the production method of the present invention has a protective effect against a virus strain that causes antigenic variation. For

example, if a current HA split vaccine is prepared from particles of H3N2 influenza virus (A/Fujian/411/02 (H3N2)) and subjected to an acidic treatment, the vaccine can have a protective effect against infection of not only A/Fujian/411/02 (H3N2), but also A/Guizhou/54/89 (H3N2), A/OMS/5389/88 (H3N2), A/Beijing/32/92 (H3N2),
5 A/England/427/88 (H3N2), A/Johannesburg/33/94 (H3N2), A/Leningrad/360/86 (H3N2), A/Mississippi/1/85 (H3N2), A/Philippines/2/82 (H3N2), A/Shangdong/9/93 (H3N2), A/Shanghai/16/89 (H3N2), A/Shanghai/24/90 (H3N2), A/Sichuan/2/87 (H3N2), A/Kitakyushyu/159/93 (H3N2), A/Akita/1/94 (H3N2), A/Panama/2007/99 (H3N2), A/Wyoming/03/03 (H3N2), A/New York/55/2004 (H3N2), or A/Hiroshima/52/2005 (H3N2) ,
10 for example. Also, for example, if a current HA split vaccine is prepared from particles of H1N1 influenza virus (A/Puerto Rico/8/34 (H1N1)) and subjected to an acid treatment, the vaccine can also have a protective effect against infection of not only A/Puerto Rico/8/34 (H1N1), but also A/Narita/1/09 (H1N1), A/Beijing/262/95 (H1N1), A/Brazil/11/78 (H1N1), A/Chile/1/83 (H1N1), A/New Jersey/8/76 (H1N1), A/Taiwan/1/86 (H1N1), A/Yamagata/32/89 (H1N1),
15 A/New Caledonia/20/99 (H1N1), A/Solomon Islands/3/2006 (H1N1), A/Brisbane/59/2007 (H1N1), or A/Mexico/4108/2009 (H1N1), for example.

[Examples]

[0026]

1. Preparation of HA Split Vaccine

20 Tween 80 was added to particles of H3N2 influenza virus (strain X31) or particles of H1N1 influenza virus (A/Puerto Rico/8/34 strain) suspended in phosphate buffered saline to a final concentration of 0.2%, and suspended therein. Diethyl ether was added and suspended, and the suspension was left stand until an aqueous layer and a diethyl ether layer were completely separated, and then the diethyl ether layer was removed. After repeating this ether
25 extraction, diethyl ether remaining in the recovered aqueous layer was distilled off at normal

pressure to obtain an HA split vaccine.

[0027]

2. Acidic Treatment

The HA split vaccine was suspended in phosphate buffered saline, and an acidic
5 treatment was then performed by adding 0.15 M citrate buffer (pH 3.5) to bring the pH to 5.0.
After standing at room temperature for 30 minutes, 1 M Tris buffer (pH 8.0) was added so that
the pH was returned to 7.3. Thereafter, centrifugation was performed to obtain a membrane
fusion-type HA split vaccine. Formalin was added to the membrane fusion-type HA split
vaccine thus prepared to a final concentration of 0.05 %, and left stand for several days.

10 [0028]

A current HA split vaccine was prepared in the same manner as described in 1 above
except that no acidic treatment was provided.

[0029]

3. Measurement of Titer of anti-LAH Antibody by ELISA

15 3-1. Inoculation of H3N2 Influenza Vaccine

BALB/c mice (female, 6 to 12 weeks old) were intraperitoneally inoculated with the
current H3N2 HA split vaccine or the membrane fusion-type HA split vaccine (10 µg of vaccine
+ 20 µg of AddaVax adjuvant (InvivoGen) dissolved in phosphate buffered saline to a liquid
volume of 200 µl). Twenty eight days after the initial inoculation, the mice were
20 intraperitoneally inoculated with the membrane fusion-type HA vaccine (10 µg of the vaccine
alone was dissolved in phosphate buffered saline to a liquid volume of 200 µl). At least 14 days
after the additional inoculation, blood was collected from the mice inoculated with the vaccine,
from which sera were collected.

[0030]

25 3-2. Measurement by ELISA

The concentration of the anti-LAH antibody in the sera of BALB/c mice intraperitoneally inoculated with the current H3N2 HA split vaccine or the membrane fusion-type HA split vaccine was measured by ELISA (Enzyme-Linked Immuno Sorbent Assay) in the following manner.

5 [0031]

Specifically, a synthetic peptide (H3; Ac-RIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDLTDSMNKLFKTRRQLRENADYKDDDDKC) (SEQ ID NO: 1) corresponding to a portion (long alpha helix) of the stem portion was dissolved in phosphate buffered saline (pH 7.3) at 10 µg/ml, and added to 96-well plates
10 by 100 µl each. After standing overnight at 4°C, each well was washed three times with phosphate buffered saline, and 150 µl of phosphate buffered saline containing 1 % bovine serum albumin was added. After standing at room temperature for two hours, each well was washed three times with phosphate buffered saline. Then, 100 µl of a mouse serum serially diluted with phosphate buffer containing 0.05 % of Tween 20 and 1 % bovine serum albumin, and 100 µl of
15 a standard monoclonal antibody of known concentration (H3; clone name V15-5) were added to each well. After standing at room temperature for two hours, each well was washed three times with phosphate buffered saline (containing 0.05 % of Tween 20), and 100 µl of a peroxidase-labeled anti-mouse IgG antibody (Southern Biotech) diluted with phosphate buffered saline containing 0.05 % Tween 20 and 1 % bovine serum albumin was added to each
20 well. After standing at room temperature for two hours, each well was washed three times with phosphate buffered saline (containing 0.05 % of Tween 20). Then, 30 mg of o-phenyldiamine tablet (Sigma) and 24 µl of 30% hydrogen peroxide solution (30% w/w; Sigma) were added to 60 ml of citrate buffer (pH 5.0) as a substrate, and 100 µl of the resultant was added to each well. After the color development, 50 µl of 2N sulfuric acid (Wako Pure Chemical Industries,
25 Ltd.) was added to stop the reaction, and an absorbance value at 490 nm was measured using a

Microplate Reader 450 (Biorad).

[0032]

As shown in FIG. 2, the titer of the anti-LAH antibody in the serum of the BALB/c mice intraperitoneally inoculated with the membrane fusion-type HA split vaccine was significantly higher than the titer of the anti-LAH antibody in the serum of BALB/c mice intraperitoneally inoculated with the current HA split vaccine.

[0033]

4. Cross-Protection against Antigenic Variant

In an experiment on protection against infection with the H3N2 virus, 200 µl of a serum collected from uninoculated mice, 200 µl of a serum collected from mice inoculated with the current H3N2 HA split vaccine, or 200 µl of a serum collected from mice inoculated with the membrane fusion-type HA split vaccine was intraperitoneally administered to BALB/c mice (female, 6 to 12 weeks old).

[0034]

Three hours after the serum administration, another H3N2 influenza virus (A/Guizhou/54/89) having different antigenicity from the vaccine strain was intranasally administered at 5 mouse lethal dose 50 (five times the amount of virus lethal to 50% of mice) under anesthesia.

[0035]

Mice were weighed and observed daily for 21 days from the viral infection to study the change in body weight and the survival rate. Mice that lost 25% of their weight were sacrificed.

[0036]

As shown in FIG. 3, regarding the BALB/c mice inoculated with the membrane fusion-type HA split vaccine, the decrease in the survival rate was significantly curbed on and after the

ninth day after the infection with the other H3N2 influenza virus of different antigenicity.

[0037]

5. Measurement of Titer of anti-LAH Antibody by ELISA

5-1. Particles of H1N1 Influenza Virus

5 C57BL/6 mice (female, 6 to 12 weeks old) were intraperitoneally inoculated with a current H1N1 HA split vaccine or a membrane fusion-type HA split vaccine (10 µg of vaccine + 10 µg of CpG-ODN 1760 suspended in phosphate buffered saline and mixed with an equal volume of Freund's incomplete adjuvant (ROCKLAND) to a liquid volume of 200 µl). Twenty eight days after the initial inoculation, the mice were intraperitoneally inoculated with the
10 membrane fusion-type HA split vaccine (10 µg of vaccine + 10 µg of CpG-ODN suspended in phosphate buffered saline and mixed with an equal volume of Freund's incomplete adjuvant (ROCKLAND) to a liquid volume of 200 µl, in the same manner as the initial inoculation). At least 14 days after the additional inoculation, blood was collected from the mice inoculated with the vaccine, from which sera were collected.

15 [0038]

5-2. Measurement by ELISA

The concentration of the anti-LAH antibody in the sera of C57BL/6 mice intraperitoneally inoculated with the current H1N1 HA split vaccine or the membrane fusion-type HA split vaccine was measured by ELISA in the following manner.

20 [0039]

The measurement was performed in the same manner as described above except that a synthetic peptide (H1; Ac-RIENLNKKVDDGFLDIWTYNAELLVLENERTLDYHDSNVKNLYEKVRSQKNNAD YKDDDDKC) (SEQ ID NO: 2) corresponding to a portion (long alpha helix) of the stem
25 portion was used and a standard monoclonal antibody of known concentration (H1; clone name

F2) was used.

[0040]

As shown in FIG. 4, the titer of the anti-LAH antibody in the sera of the C57BL/6 mice intraperitoneally inoculated with the membrane fusion-type HA split vaccine was significantly
5 higher than the titer of the anti-LAH antibody in the sera of the C57BL/6 mice intraperitoneally inoculated with the current HA split vaccine.

[0041]

6. Cross-Protection against Antigenic Variant

In an experiment on protection against infection with the H1N1 virus, 200 µl of a serum
10 collected from uninoculated mice, 200 µl of a serum collected from mice inoculated with the current H1N1 HA split vaccine, or 200 µl of a serum collected from mice inoculated with the membrane fusion-type HA split vaccine was intraperitoneally administered to C57BL/6 mice (female, 6 to 12 weeks old).

[0042]

15 Three hours after the serum administration, another H1N1 influenza virus (A/Narita/1/09) having different antigenicity from the vaccine strain was intranasally administered at 5 mouse lethal dose 50 (five times the amount of virus lethal to 50% of mice) under anesthesia.

[0043]

20 Mice were observed daily for 20 days from the viral infection to study the survival rate. As shown in FIG. 5, regarding the C57BL/6 mice inoculated with the membrane fusion-type HA split vaccine, the decrease in the survival rate was significantly curbed on and after the ninth day after the infection with the other H1N1 influenza virus of different antigenicity.

[0044]

25 7. Binding Capacity of Antibody to LAH Epitope

Binding of anti-LAH monoclonal antibodies prepared from murine or human peripheral blood infected with strain X31 to a current HA split vaccine or a membrane fusion-type HA split vaccine was measured by ELISA (Enzyme-Linked Immuno Sorbent Assay). The current HA split vaccine or membrane fusion-type HA split vaccine of an H3N2 influenza virus (strain X31) was dissolved in phosphate buffered saline (pH 7.3), and added to a 96-well plate by 50 µl each. After standing overnight at 4°C, each well was washed three times with phosphate buffered saline, and 150 µl of phosphate buffered saline containing 1 % bovine serum albumin was added. After standing at room temperature for two hours, each well was washed three times with phosphate buffered saline (containing 0.05 % of Tween 20), and 50 µl of the LAH binding monoclonal antibody serially diluted with phosphate buffer containing 1 % bovine serum albumin was added. After standing overnight at 4°C, each well was washed three times with phosphate buffered saline (containing 0.05 % of Tween 20), and 100 µl of a peroxidase-labeled anti-mouse IgG antibody (Southern Biotech) diluted with phosphate buffered saline containing 0.05 % Tween 20 and 1 % bovine serum albumin was added to each well. After standing at room temperature for two hours, each well was washed three times with phosphate buffered saline (containing 0.05 % of Tween 20). Then, 30 mg of o-phenylendiamine tablet (Sigma) and 24 µl of 30% hydrogen peroxide solution (30% w/w; Sigma) were added to 60 ml of citrate buffer (pH 5.0) as a substrate, and 50 µl of the resultant was added to each well. After the color development, 25 µL of 1 mol/L sulfuric acid (Wako Pure Chemical Industries, Ltd.) was added to stop the reaction, and an absorbance value at 490 nm was measured using Microplate Reader 450 (Biorad). The change in binding capacity was calculated from the absorbance values measured with respect to the current HA split vaccine or the membrane fusion-type HA split vaccine.

[0045]

As shown in FIG. 6, the binding capacity of the LAH binding monoclonal antibody to

the membrane fusion-type HA split vaccine was 1.05 to 21 times greater than the binding capacity to the current HA split vaccine. The results show that an acidic treatment of the HA split vaccine enhances the binding capacity of the antibody to the LAH epitope.

INDUSTRIAL APPLICABILITY

5 [0046]

The present invention is useful for the production of influenza vaccines.

[Sequence Listing Free Text]

[0047]

SEQ ID NO: 1, 2: Synthetic Peptide

10 [Sequence Listing]

CLAIMS

1. A method for producing an influenza HA split vaccine which induces *in vivo* production of an antibody that binds to a long alpha helix (LAH) of a HA stem region, the
5 method comprising subjecting an influenza HA split vaccine which has not undergone a formalin treatment to an acidic treatment,

wherein the acidic treatment is performed at a pH of 3.0 to 6.5.

2. The method according to Claim 1, wherein the influenza HA split vaccine also
10 has a protective effect against an antigen-mutated influenza virus.

3. A method for producing an influenza HA split vaccine which induces *in vivo* production of an antibody that binds to a LAH of a HA stem region and which has a protective effect against an antigen-mutated influenza virus, the method comprising subjecting an
15 influenza HA split vaccine which has not undergone a formalin treatment to an acidic treatment, wherein the acidic treatment is performed at a pH of 3.0 to 6.5.

4. The method of any one of Claims 1 to 3, wherein the acidic treatment is performed at a pH of 4.0 to 6.0.

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5. The method of any one of Claims 1 to 4, wherein the acidic treatment is performed at a pH of 4.4 to 5.8.

6. The method of any one of Claims 1 to 5, wherein the influenza HA split

vaccine is of type H3N2 or type H1N1.

7. An influenza HA split vaccine which induces *in vivo* production of an antibody that binds to a LAH of a HA stem region, the vaccine being produced by a method comprising subjecting an influenza HA split vaccine which has not undergone a formalin treatment to an acidic treatment,

wherein the acidic treatment is performed at a pH of 3.0 to 6.5.

8. An influenza HA split vaccine which is produced by a method comprising subjecting an influenza HA split vaccine which has not undergone a formalin treatment to an acidic treatment, induces *in vivo* production of an antibody that binds to a LAH of a HA stem region, and also has a protective effect against an antigen-mutated influenza virus,

wherein the acidic treatment is performed at a pH of 3.0 to 6.5.

9. The influenza HA split vaccine of Claim 7 or 8, wherein the acidic treatment is performed at a pH of 4.0 to 6.0.

10. The influenza HA split vaccine of any one of Claims 7 to 9, wherein the acidic treatment is performed at a pH of 4.4 to 5.8.

11. The influenza HA split vaccine of any one of Claims 7 to 10, wherein the influenza HA split vaccine is of type H3N2 or type H1N1.

12. An agent for protection from infection with influenza, comprising the influenza HA split vaccine of any one of Claims 7 to 11.