

DESCRIPTION

**Title of Invention: VACCINE COMPOSITION INCLUDING ZIKA VIRUS
INACTIVATED BY EPIGALLOCATECHIN-3-GALLATE AS ACTIVE
INGREDIENT**

TECHNICAL FIELD

[0001] The present invention relates to a vaccine composition including Zika virus inactivated by epigallocatechin-3-gallate (EGCG) as an active ingredient.

BACKGROUND ART

[0002] A method for inactivating viruses with formaldehyde, one of the various methods for preparing an inactivated vaccine, has been used for the past several decades in the manufacture of commercial vaccines against infection from a variety of viruses, including hepatitis A virus, respiratory syncytial virus (hereinafter referred to as RSV), influenza virus, Japanese encephalitis virus, polio virus, measles virus, rabies virus, etc. However, in the cases of RSV and polio virus vaccines inactivated with formaldehyde, adverse effects of reducing vaccine effectiveness have been reported due to antigen denaturation and partial destruction by formaldehyde treatment. In particular, in the case of RSV, virus infection was found to increase after vaccination, and the low level of virus neutralizing antibodies in the formaldehyde-inactivated RSV vaccine is considered one of the causes of this phenomenon. This weak immune response can increase the risk of reinfection and worsen the disease in vaccinated individuals.

[0003] The cause for the low level neutralizing antibodies against the virus after formaldehyde-inactivated vaccination is due to the extensive modification of the vaccine antigen protein by formaldehyde. Among the 20 amino acids that make up proteins, more than 15 amino acids, including lysine, undergo modification, which contributes to reduced vaccine efficacy. In particular, research results suggest that modifying lysine increasing the carbonyl group content can affect immunogenicity. Similar cases to those of the RSV vaccine have been observed in research on Zika virus vaccines currently under development; in the tests on primates and humans, the live attenuated vaccine elicited high neutralizing antibody titers ($>1:1,000$), whereas the vaccine inactivated by formaldehyde elicited very low neutralizing antibody titers ($>1:100$). As for the antibody immune response that contributes to various vaccine effects, humoral immunity, which includes neutralizing antibodies capable of directly neutralizing infectious agents, is primarily important. In addition thereto, antibodies can also induce protective function by attracting macrophages, etc., to virus-infected cells and causing antibody-dependent cytotoxicity (ADCC). This protective function is determined by the isotype type of an antibody. In the case of mice, among various major antibody isotypes including IgG1 (Th2 type function) and IgG2a (Th1 function), IgG2a plays a particularly prominent Th1 function and an important role in ADCC. Most of the existing formaldehyde-inactivated vaccines are adjuvanted with aluminum hydroxide gel to enhance immunity, but their weak Th1 function is considered a limitation in vaccine efficiency. Therefore, it remains a

challenge to overcome this disadvantage and develop a vaccine that has the effect of inducing enhancement of Th1 cellular immunity that causes ADCC in addition to the neutralizing function through humoral immunity. In particular, in the case of inactivated vaccines, it is common to use various adjuvants distinct from aluminum hydroxide gels to strengthen immune function. However, this poses a challenge for commercialization due to the need for expensive adjuvants. Therefore, the development of an effective inactivated Zika vaccine without using additional inactivating agents or adjuvant still remains a challenge.

[0004] [Prior Art Document]

[0005] [Patent Document]

[0006] (Patent Document 0001) 1. Korea Patent Application Publication No. 10-2019-0127605 (published on November 13, 2019)

DISCLOSURE OF THE INVENTION

TECHNICAL PROBLEM

[0007] An object of the present invention is to provide a vaccine composition including Zika virus inactivated by epigallocatechin-3-gallate (EGCG) as an active ingredient.

TECHNICAL SOLUTION

[0008] In order to achieve the above object, the present invention provides a vaccine composition including Zika virus inactivated by epigallocatechin-3-gallate (EGCG) as an active ingredient.

ADVANTAGEOUS EFFECTS

[0009] According to the present invention, the Zika virus

vaccine inactivated with epigallocatechin-3-gallate (EGCG) has a superior vaccine effectiveness due to a neutralizing antibody compared to conventional formaldehyde-inactivated Zika virus vaccines. In addition, Zika virus vaccine inactivated with EGCG significantly improves a cytotoxic ADCC immune responses caused by an antibody, and thus, EGCG alone can be effectively utilized as a Zika virus vaccine composition without additional inactivating material or combination with an adjuvant.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1 shows the results of analyzing the antiviral effect of formaldehyde and EGCG against Zika virus. FIGS. 1a and 1b show the antiviral effect against ZIKV when treated with 0.1% FA, and FIGS. 1c, 1d, and 1e show the antiviral effects against ZIKV when treated with 0.1% EGCG, 0.2% EGCG, and 0.5% EGCG, respectively.

[0011] FIG. 2 shows the results of analyzing the toxicity of an inactivated Zika virus vaccine; and a mixture of the vaccine and an adjuvant (aluminum hydroxide gel).

[0012] FIG. 3 shows the results of analyzing the IgG antibody titer in serum obtained after inoculating the vaccine into mice in order to confirm the immunogenicity of the inactivated Zika virus vaccine.

[0013] FIG. 4 shows the results of analyzing the antibody titers of IgG and subtypes (IgG1 and IgG2a) in serum obtained after the second vaccination of the vaccine in mice in order to confirm the immunogenicity of the inactivated Zika virus vaccine. FIG. 4a shows the analysis results of antibody titers of an IgG antibody, FIG. 4b shows the analysis results

of antibody titers of an IgG1 antibody, and FIG. 4c shows the analysis results of antibody titers of an IgG2a antibody.

[0014] FIG. 5 shows the results of analyzing the antibody effect of the Zika virus vaccine against the Zika virus E protein using enzyme-linked immunosorbent assay. FIG. 5a shows the analysis results of the effect of an IgG antibody, FIG. 5b shows the analysis results of the effect of an IgG1 antibody, and FIG. 5c shows the analysis results of the effect of an IgG2a antibody.

[0015] FIG. 6 shows the results of analyzing immunogenicity through enzyme-linked immunosorbent assay after inducing structural changes in the Zika virus E protein at acidic condition of pH 5, similar to the environment of endosomes in cells. FIG. 6a is the analysis results of an IgG antibody, FIG. 6b shows the analysis results of an IgG1 antibody, and FIG. 6c shows the analysis results of an IgG2a antibody.

[0016] FIG. 7 shows the results of analyzing the effect of the inactivated Zika virus vaccine on neutralizing antibodies.

MODE FOR CARRYING OUT THE INVENTION

[0017] Hereinafter, the present invention will be described in more detail.

[0018] The present invention provides a vaccine composition including Zika virus inactivated by epigallocatechin-3-gallate (EGCG) as an active ingredient.

[0019] The EGCG can bind to the Zika virus protein.

[0020] Additionally, the EGCG can inactivate the Zika virus and improve cytotoxic immune efficacy.

[0021] The Zika virus may originate from Brazil or Boracay, but is not limited thereto.

[0022] The composition may include 0.1 to 0.5% (w/v) of EGCG.

[0023] Additionally, the composition may be one in which EGCG is dissolved in a solution capable of adjusting the pH to neutral, preferably at a concentration of 0.2%(w/v) in phosphate-buffered saline.

[0024] Additionally, the composition may further include an adjuvant, in which the adjuvant may be at least one selected from the group consisting of aluminum hydroxide gel, monophosphoryl lipid A, MF59, AS03, AF03, AS01, AS04, CpG ODN, and virosome, but is not limited thereto.

[0025] The MF59, AS03, AF03 as Freund's adjuvants, and the AS01, AS04, CpG ODN, and virosome as Toll-like receptor (TLR)-based adjuvants.

[0026] Additionally, the composition may include 5 to 20 parts by weight of an adjuvant based on 100 parts by weight of the inactivated Zika virus.

[0027] As used herein, the term "vaccine" is used in the broadest sense to mean a composition that positively affects the immune response of a subject. The vaccine composition provides a subject with an enhanced systemic or local immune response triggered by a cellular immune response, which is exemplified by cytotoxic T lymphocytes (CTLs), or a humoral immune response, which is exemplified by an antibody.

[0028] The vaccine composition of the present invention may additionally include a pharmaceutically acceptable carrier. The term "pharmaceutically effective amount" herein refers to an amount sufficient to achieve prevention, alleviation, or therapeutic efficacy against diseases or pathological symptoms caused by viruses. Pharmaceutically acceptable carriers that can be included in the composition of the

present invention are those commonly used in the course of formulation, such as lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, and mineral oil, but are not limited thereto. In addition to the above-mentioned components, the composition of the present invention may further include lubricants, wetting agents, sweeteners, flavoring agents, emulsifiers, suspending agents, preservatives, etc. The vaccine composition of the present invention may include other components such as stabilizers, excipients, other pharmaceutically acceptable compounds, or any other antigen or portion thereof. Vaccines may be in the form of freeze-dried preparations or suspensions, both of which are common in the field of vaccine production.

[0029] The dosage form of the vaccine composition of the present invention may be in the form of an enteric-coated unit, inoculations for intraperitoneal, intramuscular, or subcutaneous administration, aerosol spray, or oral or intranasal use. It is also possible to administer the vaccine composition in drinking water or edible pellets. The vaccine composition of the present invention may be delivered as a single vaccine by expressing immunomodulatory molecules, such as heterologous antigens and cytokines, in the same recombinant, or may be used as a "cocktail" including two or more viral vectors carrying different foreign genes or adjuvants.

[0030] As used herein, the term "adjuvant" generally refers

to any material that increases the humoral or cellular immune response to an antigen (e.g., alum, Freund's complete adjuvant, Freund's incomplete adjuvant, LPS, poly IC, poly AU, etc.).

[0031] Hereinafter, the present invention will be described in detail through examples to aid understanding of the present invention. However, the following examples only illustrate the content of the present invention and the scope of the present invention is not limited to the following examples. Examples of the present invention are provided to more completely explain the present invention to those skilled in the art.

[0032] [Experimental Example 1] Preparation of viruses and animal cells

[0033] Zika virus (hereinafter referred to as ZIKV) (Brazil, 16321, NCCP43245) was provided by the National Culture Collection for Pathogens of the National Institute of Health of the Korea Disease Control and Prevention Agency (KDCA). The virus was inoculated into monkey kidney cells and allowed to propagate for 3 days. The culture of the propagated virus was then harvested and stored in an ultra-low temperature freezer (-80°C). Monkey kidney cells were cultured in a nutrient medium consisting of minimum essential medium (MEM) supplemented with 10% fetal bovine serum (hereinafter referred to as FBS) and cultured at 37°C in a 5% CO_2 atmosphere.

[0034] [Experimental Example 2] Preparation of inactivating materials

[0035] Epigallocatechin-3-gallate (hereinafter referred to as EGCG) and formaldehyde (hereinafter referred to as FA)

were used as inactivating materials for the ZIKV vaccine. EGCG (98% purity) was purchased from Changsha Sunfull Biotech (Changsha, China). FA was purchased from Sigma-Aldrich (St. Louis, USA). The two types of inactivation materials were dissolved in distilled water (hereinafter referred to as DW) or phosphate-buffered saline (hereinafter referred to as PBS) and purified using a 0.2 μ m syringe filter.

[0036] [Experimental Example 3] Preparation of ZIKV vaccine

[0037] In order to prepare a ZIKV vaccine, ZIKV was inactivated with inactivating materials (EGCG and FA). Specifically, in the case of EGCG, EGCG was dissolved in DW or PBS at 0.1%(w/v), 0.2%(w/v), 0.5%(w/v), and 1%(w/v), and then the EGCG solution was mixed with ZIKV and stored at 35°C for 24 hours. Titters of 10^5 PFU/mL, 10^6 PFU/mL, and 10^7 PFU/mL of ZIKV were used in the experiment. In the case of FA, 0.1% (v/v) FA was mixed with an equal amount of ZIKV and the mixture was stored at 35°C for 24 hours.

[0038] [Experimental Example 4] Measurement of plaque titer

[0039] In order to confirm the ZIKV vaccine prepared in Experimental Example 3, the measurement method of plaque titer was performed. Monkey kidney cells were cultured in 6-well cell culture plates using nutrient medium consisting of minimum essential medium (MEM) supplemented with FBS. The plates on which the cells were cultured were washed with PBS, and 500 μ L of 10-fold diluted ZIKV vaccine was infected in each well of the culture plates. Plates infected with the ZIKV vaccine were incubated at 37°C for 2 hours. Then, the cells were washed with PBS to remove the inoculum, and the cells were covered with a mixture of 2x Dulbecco's modified eagle medium (DMEM) and 2% low melting point agarose. The 6-

well plates were cultured for 3 days at 37°C in a 5% CO₂ atmosphere. After viral plaques were formed, the cells were fixed by adding 4% FA thereto. Then, the mixture covering the cells was removed and stained with crystal violet for visualization.

[0040] [Experimental Example 5] Toxicity analysis of ZIKV vaccine

[0041] In order to confirm the toxicity of the ZIKV vaccine, animal experiments were performed. Five-week-old C57BL/6 female mice were purchased from Orient Bio Co., Ltd. Animal experiments were performed at the International Vaccine Institute under specific pathogen-free conditions. All animal experiments were performed in accordance with the guidelines of the Animal Experiment Ethics Committee of the International Vaccine Institute. After an acclimatization period of one week, for toxicity testing, mice (5 per group) were inoculated intramuscularly with 0.1% FA or 0.2% EGCG dissolved in DW or PBS, and the body weight of the mice was measured for 7 days.

[0042] Then, mice (5 per group) were inoculated intramuscularly with a titer of 4.5×10^5 PFU of a ZIKV vaccine inactivated with 0.1% FA or 0.2% EGCG. The ZIKV vaccine was mixed with 2% aluminum hydroxide gel (adjuvant) in a 9:1 ratio. The inactivated material was diluted with DW or PBS. Two weeks after the first vaccination, mice were inoculated with the same amount of vaccine as the first vaccination. PBS mixed with 2% aluminum hydroxide gel (adjuvant) was set as a negative control. Blood samples were collected twice at 2-week intervals. The collected blood

samples were centrifuged at 4°C and 10,000 RPM for 20 minutes. After removing blood clots, the supernatant serum was stored at -20°C.

[0043] [Experimental Example 6] Pretreatment of ZIKV E protein at low pH

[0044] Before performing enzyme-linked immunosorbent assay, the ZIKV SPH2015 envelope protein (Sino Biological) was pretreated under a low pH condition. E protein and sodium acetate (Sigma-Aldrich) at pH 5.0 were mixed in a 1:1 ratio, and the mixture was stored at room temperature for 30 minutes. For the enzyme-linked immunosorbent assay, E protein pretreated at pH 5.0 was coated on a 96-well Nunc immunoplate, which was then stored at 4°C.

[0045] [Experimental Example 7] Enzyme-linked immunosorbent assay

[0046] In order to identify antibodies specific for ZIKV and ZIKV E protein, enzyme-linked immunosorbent assay was performed. Nunc-immune 96-well plates were coated with ZIKV (10^5 PFU/well) and ZIKVSPH2015 envelope protein (0.1 µg/well) and stored at 4°C overnight. The coated plates were blocked with bovine serum albumin [1%(v/v)] for 1 hour. Mouse sera were diluted at a 1:50 ratio in serum dilution buffer (0.25% bovine serum albumin mixed in PBS with Tween 20 surfactant). After washing the coated plates with PBS containing Tween 20 surfactant, serum serially diluted two-fold was added thereto and stored at room temperature for 1 hour. Horseradish peroxidase (HRP)-conjugated goat antimouse IgG-h+1, IgG1 or IgG2a antibody was diluted at a 1:10000 ratio, placed on serum-adhered plates, and stored at room temperature for 1 hour. The plates were washed with PBS containing Tween 20

surfactant, placed in TMB (substrate), and stored at room temperature for 30 minutes. After 30 minutes, in order to stop the color reaction, a 2 N sulfuric acid solution was added thereto. The absorbance of the plates was analyzed at 450 nm using an enzyme-linked immunosorbent assay plate meter.

[0047] [Experimental Example 8] Plaque reduction neutralization test method

[0048] In order to measure neutralizing antibody titers against ZIKV, a plaque reduction neutralization test was performed. Monkey kidney cells were cultured in 6-well plates at 37°C overnight. Mouse sera were diluted at a 1:25 ratio in minimal essential medium (MEM) and heat inactivated at 56°C for 30 minutes. The inactivated mouse serum was serially diluted two-fold, mixed with an equal amount of ZIKV (5.0×10^3 PFU/ml), and stored at 37°C for 1 hour. The mixture was placed in plates cultured with monkey kidney cells, and were then subjected to a plaque assay. The plates were incubated for 2 days at 37°C and in a 5% CO₂ atmosphere. Plaque Reduction Neutralization Test 50 Titer was determined as the serum dilution factor that reduces the number of plaques by 50% compared to the negative control group. An anti-ZKA185 human IgG1 antibody and immunized C57BL/6 mouse serum were obtained from the International Vaccine Institute and used as a control for the plaque reduction neutralization test.

[0049] [Example 1] pH Analysis of inactivating materials

[0050] The pH values of the two types of inactivating materials (FA and EGCG) prepared in Experimental Example 2 were compared. As a result, as shown in Table 1, it was confirmed that the pH values of the inactivating materials

dissolved in DW were lower than that of PBS at all concentrations, and specifically, that the pH value of EGCG decreased as its concentration increased.

[0051] 【Table 1】

Inactivating Materials	Solvent	3%	2%	1%	0.2%	0.1%
FA	DW	—	—	—	—	4.87
	PBS	—	—	—	—	7.46
EGCG	DW	—	—	4.90	5.22	5.17
	PBS	—	—	6.93	7.27	7.39

[0052] [Example 2] Analysis of antiviral effect of inactivating materials

[0053] The antiviral effects of the inactivating materials prepared in Experimental Example 2 against ZIKV were analyzed. As a result, as shown in FIG. 1, when treated with 0.1% FA, 0.2% EGCG, and 0.5% EGCG, ZIKV at 1×10^6 PFU/mL and ZIKV at 1×10^7 PFU/mL were completely inactivated, and when treated with 0.1% EGCG, ZIKV at 1×10^5 PFU/mL and ZIKV at 1×10^6 PFU/mL were completely inactivated, whereas ZIKV at 1×10^7 PFU/mL was confirmed to be 56-fold inactivated compared to the control group.

[0054] [Example 3] Analysis of toxicity of ZIKV vaccine

[0055] In order to confirm the toxicity of the ZIKV vaccine inactivated with the inactivating material, the ZIKV vaccine inactivated with 0.2% EGCG or 0.1% FA was inoculated into mice, and then the change in body weight for 7 days was measured. As a result, as shown in FIG. 2A, it was confirmed that there was no significant change in body weight between the experimental groups. Additionally, after inoculating mice with a mixture of the ZIKV vaccine and aluminum hydroxide gel (adjuvant), the change in body weight was measured for 7 days. As a result, as shown in FIG. 2B, it

was confirmed that there was no significant change in body weight between the experimental groups.

[0056] [Example 4] Analysis of immunogenicity of ZIKV vaccine

[0057] In order to confirm the immunogenicity of the ZIKV vaccine, ZIKV vaccine inactivated with 0.2% EGCG or 0.1% FA (4.5×10^5 PFU/100 μ L); or a mixture of the ZIKV vaccine and aluminum hydroxide gel (adjuvant) in a 9:1 ratio was inoculated (intramuscular injection; IM) twice at 2-week intervals in C57BL/6 mice, and serum was obtained from the mice after 2 weeks. Then, immunogenicity was analyzed using enzyme-linked immunosorbent assay. The number of mice per experimental group was set to 5. As a result, as shown in FIG. 3, it was confirmed that the vaccine inactivated with 0.2% EGCG showed a higher IgG antibody titer than the vaccine inactivated with 0.1% FA.

[0058] In addition, antibody titers of IgG1 and IgG2a belonging to the IgG subtypes against ZIKV were confirmed using serum obtained after the second vaccination in C57BL/6 mice. As a result, as shown in FIG. 4, it was confirmed that the vaccine inactivated with EGCG showed higher antibody titers than FA for both IgG1 and IgG2a, and the vaccine inactivated with 0.2% EGCG dissolved in PBS showed the highest antibody titer for the IgG2a subtype.

[0059] [Example 5] Analysis of antibody effect on ZIKV E protein

[0060] In order to confirm the effect of antibodies against ZIKV E protein, antibody titers of IgG and subtypes thereof were measured by enzyme-linked immunosorbent assay using the sera obtained after the second vaccination in C57BL/6 mice.

As a result, as shown in FIG. 5, it was confirmed that in the case of IgG, the highest antibody titer was seen in the vaccine inactivated with 0.2% EGCG, and the vaccine inactivated with 0.2% EGCG or 0.1% FA showed a higher antibody titer when dissolved in PBS than in DW. Additionally, in the case of IgG1, the vaccine inactivated with 0.2% EGCG showed a higher IgG1 antibody titer when dissolved in DW than in PBS, whereas the vaccine inactivated with 0.1% FA showed a higher antibody titer when dissolved in PBS than in DW. Additionally, it was confirmed that in the case of IgG2a, the vaccine inactivated with 0.2% EGCG dissolved in PBS showed the highest antibody titer, whereas the vaccine inactivated with 0.1% FA dissolved in DW showed no antibody titer, and the vaccine inactivated with 0.1% FA dissolved in PBS showed a lower antibody titer compared to the vaccine inactivated with 0.2% EGCG.

[0061] In addition, the ZIKV E protein was treated with sodium acetate to adjust pH 5 state similar to the environment of endosomes in cells to induce structural changes in the E protein, and the immunogenicity was analyzed using enzyme-linked immunosorbent assay. As a result, as shown in FIG. 6, it was confirmed that the overall results were similar to those where the ZIKV E protein was not treated with sodium acetate. Specifically, the vaccine inactivated with 0.2% EGCG dissolved in PBS showed the highest IgG and IgG2a antibody titers, and the vaccine inactivated with 0.1% FA showed higher IgG, IgG1, and IgG2a antibody titers when dissolved in PBS than in DW.

[0062] [Example 6] Analysis of immune inducing effect of ZIKV vaccine

[0063] In order to confirm the effect of the ZIKV vaccine on inducing Th1 and Th2 type immunity, antibodies obtained from C57BL/6 mice vaccinated with 0.1% FA or 0.2% EGCG were used. The effect of antibodies against ZIKV, ZIKV E protein, or ZIKV E protein pretreated at pH 5 was analyzed by enzyme-linked immunosorbent assay. As a result, as shown in Table 2, when the antibody titer was calculated as the IgG2a/IgG1 ratio, it was confirmed that the IgG2a ratio of the vaccine inactivated with 0.2% EGCG was higher than that inactivated with 0.1% FA. Specifically, it was shown that the antibody titer ratio of the EGCG-inactivated vaccine compared to the FA-inactivated vaccine was 15-fold higher for ZIKV and 500-fold or higher for ZIKV E protein and ZIKV E protein pretreated at pH5. The results above confirmed the effect shown without additional adjuvant. From the above results, it was confirmed that EGCG, as a single ingredient, has a reprogram function from Th2 immune response to Th1 immune response as well as an inactivation function, thereby significantly increasing the efficacy of the ZIKV vaccine.

[0064] **【Table 2】**

	Vaccine	IgG1	IgG2a	IgG2a/IgG1	Fold Change of Ratio of EGCGi/FAi
ZIKV	0.1% FA	25600	100	0.004	15
	0.2% EGCG	102400	6400	0.063	
ZIKV E Protein	0.1% FA	3200	200	0.062	>500
	0.2% EGCG	1600	51200	32	
ZIKV E Protein (pretreatment at pH 5)	0.1% FA	1600	100	0.062	>500
	0.2% EGCG	800	25600	32	
EGCGi = ZIKV vaccine inactivated with EGCG					
FAi = ZIKV vaccine inactivated with FA					

[0065] **【Example 7】** Analysis of neutralizing antibody effect

of ZIKV vaccine

[0066] In order to confirm the neutralizing antibody effect of the ZIKV vaccine, the neutralizing antibody titer of the vaccine was measured using the plaque reduction neutralization test using the sera obtained after the second vaccination of the ZIKV vaccine in mice. As a result, as shown in FIG. 7, the vaccine inactivated with 0.1% FA and 1% EGCG showed almost no neutralizing antibody titer against ZIKV (below the detection limit; indicated by a dotted line), whereas the vaccine inactivated with 0.2% EGCG dissolved in DW, the neutralizing antibody titer increased 4 to 6-fold, and the vaccine inactivated with 0.2% EGCG dissolved in PBS, the neutralizing antibody titer increased 30-fold or more. From the results above, it was confirmed that EGCG can increase neutralizing antibody titer about 6-fold when dissolved in PBS compared to when dissolved in DW under a neutral pH condition.

[0067] While the specific parts of the present invention have been described in detail above, it is obvious to those skilled in the art that these specific techniques are merely preferred embodiments and that the scope of the present invention is not limited thereby. That is, the actual scope of the present invention is defined by the appended claims and equivalents thereof.

CLAIMS

1. A vaccine composition comprising Zika virus inactivated by epigallocatechin-3-gallate (EGCG) as an active ingredient.

2. The vaccine composition of claim 1, wherein the Zika virus originates from Brazil or Boracay.

3. The vaccine composition of claim 1, wherein the EGCG inactivates the Zika virus and enhances cytotoxic immune efficacy.

4. The vaccine composition of claim 1, wherein the composition comprises EGCG in an amount of 0.1 to 0.5% (w/v).

5. The vaccine composition of claim 1, wherein in the composition, EGCG is dissolved in a solution capable of adjusting the pH to neutral.

6. The vaccine composition of claim 5, wherein in the composition, EGCG is dissolved in phosphate-buffered saline (PBS) at 0.2% (w/v).

7. The vaccine composition of claim 1, wherein the composition further comprises an adjuvant.

8. The vaccine composition of claim 7, wherein the adjuvant is at least one selected from the group consisting of aluminum hydroxide gel, monophosphoryl lipid A, MF59, AS03,

AF03, AS01, AS04, CpG ODN, and virosome.

9. The vaccine composition of claim 7, wherein the composition comprises 5 to 20 parts by weight of an adjuvant relative to 100 parts by weight of inactivated Zika virus.