COMPOSITIONS AND METHODS FOR ADMINISTRATION OF VACCINES AGAINST DENGUE VIRUS

PRIORITY

[001] This application claims the benefit under 35 USC § 119(e) of provisional U.S. patent application Serial No. 61/183,020 filed on June 01, 2009, which is incorporated herein by reference in its entirety.

FIELD

[002] Embodiments of the present invention report compositions and methods for administering a vaccine to a subject against dengue viruses. In some embodiments, vaccine compositions may be administered by intradermal injection. In certain embodiments, intradermal injection in a subject of a vaccine against dengue virus may include one or more intradermal boosts after initial vaccination. Other embodiments include intradermal injection of a vaccine composition against dengue virus wherein the composition provides protection against more than one serotype of dengue virus, such as DEN-1, DEN-2, DEN-3 and DEN-4.

BACKGROUND

[003] Vaccines for protection against viral infections have been effectively used to reduce the incidence of human disease. One of the most successful technologies for viral vaccines is to immunize animals or humans with a weakened or attenuated strain of the virus (a "live, attenuated virus"). Due to limited replication after immunization, the attenuated strain does not cause disease. However, the limited viral replication is sufficient to express the full repertoire of viral antigens and can generate potent and long-lasting immune responses to the virus. Thus, upon subsequent exposure to a pathogenic strain of the virus, the immunized individual is protected from disease. These live, attenuated viral vaccines are among the most successful vaccines used in public health.

SUMMARY

[003a] In a first aspect, there is provided a single vaccine composition against dengue virus for use in a method for inducing protection in a subject against three or four dengue virus serotypes, wherein the single vaccine composition against dengue virus is a mixture of live attenuated dengue viruses against three or four dengue virus serotypes to be administered to a subject by intradermal introduction to thereby induce protection in the subject against three or four dengue virus serotypes.

[003b] In a second aspect, there is provided a composition for protecting a subject against a dengue virus serotype comprising, an intradermally (ID) administered single vaccine composition of predetermined ratio of three or four dengue virus serotypes made up of live attenuated dengue viruses.

[003c] In a third aspect, there is provided a system for administering a dengue vaccine composition to a subject comprising, a single vaccine composition against three or four dengue virus serotypes wherein the single vaccine composition comprises three or four dengue virus serotypes and an apparatus capable of introducing the vaccine composition intradermally by needle-free administration to a subject.

[003d] In a fourth aspect, there is provided a vaccine kit comprising;

a vaccine composition against dengue virus comprising the composition as defined above; and

an apparatus capable of administering the composition intradermally to a subject.

[003e] In a fifth aspect, there is provided the use of a single vaccine composition against dengue virus in the manufacture of a medicament for inducing protection in a subject against three or four dengue virus serotypes, wherein the single vaccine composition against dengue virus is to be administered to the subject by intradermal introduction to thereby induce protection in the subject against multiple dengue virus serotypes.

[004] Embodiments of the present disclosure generally relate to methods and compositions for inducing protection in a subject against dengue virus by, for example, administering a vaccine to a subject against dengue viruses. Some embodiments can include introducing a vaccine composition to a subject via intradermal (ID) injection. In accordance with these embodiments,

the vaccine composition can be introduced to a subject intradermally to, for example, protect against one or more than one dengue serotype (*e.g.* cross protection). In certain embodiments, a vaccine composition can include, but is not limited to, a single dose of one serotype of dengue virus (*e.g.* DENVax 4) administered to a subject. In other embodiments, a vaccine composition may include, but is not limited to; an initial dose of one serotype of dengue virus (*e.g.* DENVax 4 or other serotype) and then one or more boosts of the same, a combination or a different serotype can be administered to a subject.

[005] Other embodiments herein can concern inducing a cellular immune response in a subject by, for example, introducing a vaccine composition to a subject via intradermal introduction wherein the vaccine composition includes, but is not limited to, a dengue virus vaccine. In accordance with these embodiments, compositions disclosed can be administered intradermally to a subject for modulating neutralizing antibody production in the subject against dengue virus serotypes. Some embodiments concern predetermined composition ratios (*e.g.* 1:1, 1:2, 1:4, any ratio of two or more serotypes is contemplated) of the various serotypes of dengue virus or fragments thereof or attenuated compositions thereof in a single vaccine composition in order to increase cross protection in a subject against some or all dengue virus serotypes when the subject is administered the single vaccine composition intradermally.

[006] In certain embodiments, some advantages of using intradermal introduction of a vaccine against dengue virus can include, but are not limited to, multiple protection against some or all dengue virus serotypes in a subject, reduced cost by using small doses compared to subcutaneous injection, modulation of antibodies produced against some or all dengue virus serotypes in a subject and reduced pain at a site of administration in a subject administered a composition of vaccine against dengue virus.

[007] In some embodiments, a single dose vaccine against dengue virus can include one or more dengue virus serotype(s). In accordance with these embodiments, a subject may be treated with at least one additional intradermal injection(s) administered at a separate site from the first injection, for example, next to or in a separate anatomical site on the subject. In addition, at least one additional intradermal injection(s) may be performed less than 30 days after the first administration to the subject. Vaccine compositions of these and other embodiments disclosed herein may include two or more dengue virus serotypes at a predetermined ratio.

Brief Description of the Drawings

[008] The following drawings form part of the present specification and are included to further demonstrate certain embodiments. Some embodiments may be better understood by reference to one or more of these drawings alone or in combination with the detailed description of specific embodiments presented.

[009] Fig. 1 represents an example of an intradermal injection device currently available.

[0010] Fig. 2 represents examples of injection sites in a subject having intradermal introduction of a vaccine against dengue virus.

[0011] Fig. 3 represents a bar graph comparison of subcutaneous versus intradermal injection of a vaccine against dengue virus and neutralizing antibody titer produced against different dengue virus serotypes after a primary administration.

[0012] Fig. 4 represents a bar graph comparison of subcutaneous versus intradermal injection of a vaccine against dengue virus and neutralizing antibody titer produced against different dengue virus serotypes after a second, boosting administration.

[0013] Fig. 5 represents a histogram plot of subcutaneous and intradermal immunizations with a vaccine against a dengue virus serotype in mice.

[0014] Figs. 6A and 6B represent graphic depictions of a challenge experiment using two different dengue virus serotypes on a dengue serotype-immune mouse population following vaccination of the mice with another dengue virus serotype.

Definitions

[0015] As used herein, "a" or "an" may mean one or more than one of an item.

[0016] As used herein, vessel can include, but is not limited to, test tube, mini- or micro-fuge tube, channel, vial, microtiter plate or container.

[0017] As used herein the specification, "subject" or "subjects" may include but are not limited mammals such as humans or mammals, domesticated or wild, for example dogs, cats, other household pets (*e.g.*, hamster, guinea pig, mouse, rat), ferrets, rabbits, pigs, horses, cattle, prairie dogs, or zoo animals.

[0018] As used herein, "about" can mean plus or minus ten percent.

[0019] As used herein, "attenuated virus" can mean a virus that demonstrates reduced or no clinical signs of disease when administered to a subject such as a mammal (*e.g.*, human or an animal).

DESCRIPTION

[0020] In the following sections, various exemplary compositions and methods are described in order to detail various embodiments. It will be obvious to one skilled in the art that practicing the various embodiments does not require the employment of all or even some of the specific details outlined herein, but rather that concentrations, times and other specific details may be modified through routine experimentation. In some cases, well-known methods or components have not been included in the description.

[0021] Certain aspects of the present invention include, but are not limited to, administration of vaccine compositions against dengue virus.

[0022] Embodiments of the present invention generally relate to methods and compositions for inducing protection in a subject against dengue virus serotypes. Other embodiments can include introducing a vaccine composition to a subject via intradermal (ID) injection wherein the vaccine composition introduced intradermally induces cross protection against some or all dengue serotypes. In certain embodiments, the vaccine composition comprises a single dose of a vaccine against dengue virus serotype 4 (DENVax 4) administered to a subject. In other embodiments, the vaccine composition comprises an initial dose of DENVax 4 then, one or more boosts of the vaccine administered to a subject.

[0023] Other aspects of the present invention include modulating an immune response to a vaccine against dengue virus administered intradermally compared to subcutaneously to a subject. Vaccines against dengue virus may include a composition comprising ratios of serotypes of dengue virus, live attenuated dengue virus, or fragments thereof such as proteins or nucleic acids derived or obtained from dengue virus serotypes. Ratios of various serotypes may be equal or certain serotypes may be represented more than others depending on need or exposure or potential exposure to the virus. In accordance with these embodiments, a ratio may be a 1:2, 1:3, 1:4, 1:10, 1:20; 1:1:1, 1:2:2, 1:2:1, 1:1:1:1, 1:2:1:2; 1:3:1:3, 2:3:3:3, 5:4:5:5, 1:2:2 or any ratio for any of serotypes 1, 2, 3 and/or 4, depending on for example, number of serotypes represented in the formulation, predetermined response and effect desired. It is contemplated that any dengue virus serotype formulation may be used to generate a vaccine (*e.g.* attenuated virus etc.) of use in intradermal administration to a subject in need thereof. It is contemplated that some formulations may be more effective than others when introduced intradermally than other formulations.

[0024] In other embodiments, compositions of dengue virus vaccine formulations may be introduced intradermally to a subject prior to, during or after exposure to dengue virus by the subject. In accordance with these embodiments, a subject may receive a single intradermal injection or more than one injection comprising a dengue virus formulation, optionally, followed by one or more additional injections. Intradermal applications of formulations described herein may be combined with any other anti-viral treatment or administration mode of vaccine (*e.g.* subcutaneous injection) to a subject. In some embodiments, it is contemplated that intradermal introduction of a formulation contemplated herein may be administered to any appropriate region of a subject's body (*e.g.* arm, hip, etc). In addition, intradermal administration of vaccine formulations herein as primary or boost administrations may occur in the same day, consecutive days, weekly, monthly, bi-monthly or other appropriate treatment regimen.

Methods

Nucleic Acid Amplification

[0025] Nucleic acids may be used in any formulation or used to generate any formulation contemplated herein. Nucleic acid sequences used as a template for amplification can be isolated viruses (*e.g.* dengue viruses), according to standard methodologies. A nucleic acid sequence may be genomic DNA or fractionated or whole cell RNA. Where RNA is used, it may be desired to convert the RNA to a complementary cDNA. In some embodiments, the RNA is whole cell RNA and is used directly as the template for amplification. Any method known in the art for amplifying nucleic acid molecules is contemplated (*e.g.*, PCR, LCR, Qbeta Replicase, etc).

Expressed Proteins or Peptides

[0026] Genes can be expressed in any number of different recombinant DNA expression systems to generate large amounts of the polypeptide product, which can then be purified and used in methods and compositions reported herein. Any method known in the art for generating and using constructs is contemplated. In certain embodiments, genes or gene fragments encoding one or more polypeptide may be inserted into an expression vector by standard cloning or subcloning techniques known in the art.

[0027] Proteins, peptides and/or antibodies or fragments thereof may be detected or analyzed by any means known in the art. In certain embodiments, methods for separating and analyzing molecules may be used such as gel electrophoresis or column chromatography methods.

Electrophoresis

[0028] Electrophoresis may be used to separate molecules (*e.g.*, large molecules such as proteins or nucleic acids) based on their size and electrical charge. There are many variations of electrophoresis known in the art. A solution through which the molecules move may be free, usually in capillary tubes, or it may be embedded in a matrix or other material known in the art. Common matrices can include, but are not limited to, polyacrylamide gels, agarose gels, mass spec, blotting and filter paper.

[0029] Some embodiments, using a gene or gene fragment encoding a polypeptide may be inserted into an expression vector by standard subcloning techniques. An expression vector may be used which produces the recombinant polypeptide as a fusion protein, allowing rapid affinity purification of a peptide or protein. Examples of such fusion protein expression systems are the glutathione S-transferase system (Pharmacia, Piscataway, NJ), the maltose binding protein system (NEB, Beverley, MA), the FLAG system (IBI, New Haven, CT), and the 6xHis system (Qiagen, Chatsworth, CA).

Pharmaceutical Formulations

[0030] Any pharmaceutical formulation known in the art for a vaccine is contemplated herein. In certain embodiments, a formulation can contain one or more DEN serotype in various ratios, depending on predetermined exposure to or existence of dengue virus subtypes. It is contemplated that formulations can contain other agents of use in vaccination of a subject including, but not limited to other active or inactive ingredients or compositions known to one skilled in the art.

Kits

[0031] Other embodiments concern kits of use with the methods (*e.g.* methods of application or administration of a vaccine) and compositions described herein. Some embodiments concern kits having vaccine compositions of use to prevent or treat subjects having, exposed or suspected of being exposed to one or more dengue viruses. In certain embodiments, a kit may contain one or more than one formulation of dengue virus serotype(s) (*e.g.* attenuated vaccines) at predetermined ratios. Kits can be portable, for example, able to be transported and used in

remote areas such as military installations or remote villages. Other kits may be of use in a health facility to treat a subject having been exposed to one or more dengue viruses or suspected of being at risk of exposure to dengue virus.

[0032] Kits can also include a suitable container, for example, vials, tubes, mini- or microfuge tubes, test tube, flask, bottle, syringe or other container. Where an additional component or agent is provided, the kit can contain one or more additional containers into which this agent or component may be placed. Kits herein will also typically include a means for containing the agent, composition and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained. Optionally, one or more additional agents such as immunogenic agents or other anti-viral agents, anti-fungal or anti-bacterial agents may be needed for compositions described, for example, for compositions of use as a vaccine against one or more additional microorganisms.

[0033] The following examples are included to demonstrate certain embodiments presented herein. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered to function well in the practices disclosed herein. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the certain embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope herein.

EXAMPLES

Example 1

[0034] Previous studies revealed that natural infection with each DEN (dengue virus) serotype leads to long-lived protection against dengue fever caused by the homologous serotype. In certain embodiments, administration of an effective dengue vaccine closely mimics natural infection and can serve as a mode for administering vaccines against dengue virus. Embodiments reported herein can concern a natural infection route of dengue virus (DEN) infection, similar to intradermal delivery by the transporting host, a mosquito bite. In certain embodiments, intradermal injection to deposit the vaccine viruses into the same tissue can be used. Skin is a highly accessible organ and represents an effective immune barrier, mainly attributed to the presence of Langerhans cells (LCs) residing in the epidermis. Skin

immunization elicits a broad range of immune responses, including humoral, cellular, and mucosal and has the potential to bypass the effect of pre-existing immunity on the immunogenicity of administered vaccines.

[0035] Some embodiments for intradermal (ID) administration of the tetravalent dengue vaccines in a subject in need of such a treatment are reported. One exemplary method of intradermal administration was performed on four Cynomologous macaques administered a 5:5:5:5 DENVax (dengue virus vaccine) by intradermal administration. To achieve an equivalent dose of virus, 0.15 ml of vaccine was deposited ID in three closely spaced sites using a needle-free jet injector (see Figs. 1 and 2, below). Fig. 1 represents an intradermal inject (*e.g.*, PharmaJet®) device used for intradermal inoculations.

[0036] Fig. 2 illustrates inoculation sites on Cynomolgus macaques post vaccination with PharmaJet device. Animals were boosted 60 days later with the same formulation by the same route. Serum samples were collected at predetermined intervals, days 15, 30, 58, 74, and 91 and were tested for the presence of neutralizing antibodies directed against the four dengue serotypes. PRNT (plaque reduction neutralization test, known in the art for quantifying levels of anti-DEN neutralizing antibodies) were performed on the sera samples.

[0037] It was demonstrated that the neutralizing antibody titers are significantly higher after ID administration as compared to SC administration after a primary administration (see Fig.3) or after a secondary administration (See Fig. 4). Since the sites were closely spaced in the same area, and each innoculum consists of all four viruses, this mode of vaccine delivery closely resembles a single administration of DENVax. Fig 3 illustrates 50%PRNT Geometric Mean Titers at Day 58 (58 days after the primary administration). Fig. 4 illustrates 50%PRNT Geometric Mean Titers at Day 74 (14 days after the secondary administration on Day 60). As can be seen in the figures, the neutralizing antibody titers to all four dengue viruses were higher after intradermal versus subcutaneous administration. In addition, the number of animals that demonstrated neutralizing antibody responses ("seroconversion" defined as PRNT > 10) was greater after the first dose of vaccine (see Table 1, the percentage of animals that seroconverted to each of the four dengue serotypes is shown after primary and secondary immunization).

[0038] Table 1: Seroconversion of non-human primates after dengue immunization

DENVax Formulation	% Seroconversion							
	DEN-1		DEN-2		DEN-3		DEN-4	
	Prime	Boost	Prime	Boost	Prime	Boost	Prime	Boost
5:5:5:5 SC	87.5%	100.0%	100.0%	100.0%	75.0%	100.0%	50.0%	100.0%
5:5:5:5 ID	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

[0039] The immunized animals were tested for protection against challenge with wild type dengue viruses. In cynomolgus macaques, wild type dengue virus infection leads to virus replication and viremia, but no clinical signs. At day 91, two monkeys were challenged with DENV-1 (dengue virus serotype 1) and two monkeys challenged with DEN-2 (dengue virus serotype 2). Serum samples were collected daily for 11 days after challenge. Levels of dengue virus RNA were measured in the samples by quantitative real-time polymerase chain reaction technology (q-rtPCR) and titers of viable virus were measured by virus isolation and plaque formation on Vero cells. The results are shown in Tables 2 and 3. Neutralizing antibodies against DEN-1 at Day 91, just prior to challenge ("Pre-Challenge") and Day 105, 14 days after challenge ("Post"). Viremia is given as the number of days that live DEN-1 virus could be isolated from blood samples ("Duration") and the log10 of the peak titer isolated from each animal. Viral RNA is given as the number of days viral RNA could be detected in the serum samples ("Duration") and peak viral RNA levels in each monkey, expressed as the log10 of the number of viral RNA genomes detected.

Table 2: Responses after challenge with DEN-1

Monkey	Formulation	DEN-1 PRNT		Viremia		Viral RNA	
		Pre-Challenge	Post	Duration	Peak	Duration	Peak
CY0174	5:5:5:5 SC	240	240	0	0	0	0
CY0181	5:5:5:5 SC	640	61440	0	0	5	5.6
CY0192	5:5:5:5 ID	1920	1280	0	0	0	0
CY0194	5:5:5:5 ID	7680	1920	0	0	0	0
CY0061	Controls	1	2560	6	2.0	9	5.7
CY0193	Controls	25	2560	3	2.7	7	6.4
CY0058	Controls	1	640	5	2.9	7	5.5
CY0073	Controls	1	1280	5	3.6	10	6.2

Table 3: Responses after challenge with DEN-2

Monkey	Formulation	DEN-2 PRNT		Viremia		Viral RNA	
		Pre-Challenge	Post	Duration	Peak	Duration	Peak
CY0172	5:5:5:5 SC	3413	3413	0	0	1	3.9
CY0177	5:5:5:5 SC	853	533	0	0	0	0
CY0198	5:5:5:5 ID	240	320	0	0	0	0
CY0201	5:5:5:5 ID	1920	1600	0	0	0	0
CY0088	Controls	6	10240	6	2.3	8	5.1
CY0199	Controls	1	3840	5	1.8	9	4.7
CY0065	Controls	1	10240	5	2.9	8	5.8
CY0104	Controls	1	10240	4	2.4	8	5.7

[0040] After challenge, the SC and ID immunized animals were completely protected from DEN-1 or DEN-2 induced viremia (compared to the control animals that demonstrated significant viremia of long duration). In all of the ID immunized animals, but not all of the SC immunized animals, there was also an absence of viral RNA replication and a lack of an increase in antibody titer after challenge (compare the ID animals to SC injected CY0181, CY0172 or the control animals). These data suggest that protection is "sterilizing" and prevents any virus replication after challenge.

Example 2

[0041] In another example, an optimized DENVax formulation delivered in different locations and with different timings will be tested in non-human primates. Groups of eight Cynomolgus macaques will be immunized with a DENVax formulation containing 1×10^5 plaque forming units (pfu), 1×10^4 pfu, 1×10^5 pfu and 1×10^5 pfu of DENVAx-1, DENVax-2, DENVax-3 and DENVax-4, respectively (abbreviated 5:4:5:5). Two doses will be administered in 0.1 ml ID. Groups will be immunized with either one dose in each arm at Day 0, one dose in one arm at Day 0 and one dose in the other arm at Day 7, or one dose in one arm at Day 0 and one dose in the other arm at Day 60. These groups will be compared to a group that receives the same dose (5:4:5:5) in three sites in the same are on Day 0 and three sites in the other arm on Day 60 as well as a group that receives the same dose in a single 0.5 ml SC immunization in one arm at Day 0 and in the other arm at Day 60. A control group will be immunized with vaccine excipients only (no vaccine viruses). Following immunization, blood samples will be collected on days 0, 7 (for peak viremia), 15, 30, 60, and 90 to test the neutralizing antibodies against the four dengue virus serotypes by PRNT50. PBMCs collected on days 30, 60, 90 will

be also monitored for IFN-γ secretion by an ELISPOT assay. On day 90, two animals from each group will be challenged with wild type of DEN-1, DEN-2, DEN-3, or DEN-4 viruses. Challenged animals will be monitored for clinical signs and temperature (twice daily), changes in food consumption (once daily) and body weight (weekly). In addition, all animals will be bled daily for 11 days post-challenge to monitor viremia and hematological parameters. Again, the speed and duration of PRNT responses to all four DEN viruses and protection after day 90 challenge will be assessed. It is believed that intradermal administration in multiple sites and in distinct anatomical locations may be more effective than subcutaneous administration as a single bolus. Multiple sites can provide exposure of the vaccine to more antigen presenting cells. Distinct anatomical locations can permit vaccine access to multiple lymph nodes. In addition, booster immunizations of dengue vaccines have only been administered after the development of antibody responses in mice, primates and human clinical trials, thirty days or longer. At this time, neutralizing antibodies inhibit the response to the live viral vaccines. It was previously shown that boosting primates one month after primary immunization was less effective than dosing four months after primary immunization. It was speculated that high levels of homologous and heterologous antibodies that circulate after the initial immunization can inhibit viral replication in a second dose. While prolonged (two months or longer) immunization may circumvent this inhibition, it has not been tested whether accelerated immunization regimen with shorter immunization intervals, before the development of potent neutralizing antibody responses may be advantageous. Such a shortened regimen may be an advantage in endemic countries or for travelers, where exposure to dengue viruses in between the immunizations may put them at risk of disease.

Example 3

[0042] In another example, a human clinical trial has been initiated, studying the safety and immunogenicity of two DENVax formulations, administered in 0.1 ml either by ID or SC injection. Groups of 12 individuals will be immunized with for example, a low dose DENVax formulation (8×10^3 pfu, 5×10^3 pfu, 1×10^4 pfu and 2×10^5 pfu of DENVax-1, -2, -3 and -4, respectively) or a high dose (2×10^4 pfu, 5×10^4 pfu, 1×10^5 pfu and 3×10^5 pfu of DENVax-1, -2, -3 and -4, respectively) of DENVax ID or SC on Days 0 and 90. Two control groups will be injected SC or ID with phosphate-buffered saline. Patients will be monitored for any adverse events, and for any significant changes in hematological or blood chemistry parameters. Serum

samples will be collected to measure vaccine virus replication and neutralizing antibody responses at periodic intervals.

Example 4

[0043] Immunogenicity and efficacy of DENVax administered intradermally in AG129 mice. In another example, two studies were performed to compare the effect of route of *administration on immunogenicity and efficacy of DENVax in AG129 mice.* In one example, the immunogenicity of monovalent DENV[a]x-4 (*e.g.* vaccine against one Dengue virus serotype) was compared in AG129 mice by measuring the neutralizing antibody responses following SC injection under the skin on the back or ID injection into the foot pad using a needle and syringe. Groups of 8 AG129 mice were injected ID or SC with 10^5 PFU/dose of chimeric DENVax-4 vaccine in 50µl and 100µl final volume, respectively. Six weeks after priming, animals from each treatment group were boosted via the corresponding ID or SC route with 10^5 PFU of DENVax-4 or TFA. Mice were bled on Day 31 and 58 and collected sera were pooled to measure neutralizing antibody responses.

[0044] Immunization of DENVax-4 via the ID route elicited a 5-fold higher neutralizing antibody response to DEN-4 after the boost compared to the response induced via the SC route (see for example, Fig. 4). The anti-DEN-4 response elicited by either route of immunization had a marked cross-neutralizing activity against DEN-3 but not against DEN-1 or DEN-2 serotypes. Fig. 4 represents neutralizing antibody responses following primary and secondary immunization of AG129 mice with chimeric DENVax-4. Mice were bled on Day 31 and 58 and collected sera were pooled to measure neutralizing antibody responses using the plaque reduction assay (PRNT50).

[0045] Two weeks after the boost animals from each group were split in to two groups and challenged with 10^6PFU of DEN-1 (Mochizuki virus strain) or DEN-2 (New Guinea C strain) viruses. Challenged animals were monitored for clinical signs of disease and survival rates were recorded over a period of 5 weeks. Mice immunized via the ID route showed no signs of disease after DEN-1 challenge (Fig. 5A). In the SC immunized group only one mouse succumbed to infection while the rest of animals had no any apparent signs of infection (Fig. 5B). In contrast, all control animals succumbed to infection by day 13 after DEN-1 challenge (Fig. 5A). Following DEN-2 challenge, all animals immunized with only DENVax-4 via the SC route succumbed to infection by day 25 with mean survival time (MST) of 19.5 days as

compared to the control (TFA) mice that all succumbed by day 17 (MST=12.5 days) post-challenge (Fig. 5B). In contrast, fifty percent of ID DENVax-4 immunized mice survived the infection until the end of the 5 week monitoring period (Fig. 5B). Figs. 5A and 5 B represent survivals of DENVax-4 immune AG129 mice following challenge with DEN-1 (a) or DEN-2 (b) viruses. Challenged animals were monitored for clinical signs of disease and survival rates were recorded over a period of 5 weeks.

[0046] In a second study, immunogenicity of tetravalent DENVax vaccine administered SC or ID in mice (e.g. AG129) was tested. Groups of AG129 mice, six per group were injected SC or ID with the DENVax in 100µl or 50µl (final volume), respectively. Mice were immunized with DENVax at a 5:4:5:5 (10^5 PFU of DENVax-1,-3 and -4 and 10^4 PFU of DENVax-2) dose level of composite chimeric vaccines. All immunized animals received a booster injection of 5:4:5:5 DENVax 42 days' post-primary inoculation. Blood samples were collected on days 42 and 56 to measure neutralizing antibody responses to each DEN virus serotype.

[0047] As represented in Table 4, both primary and secondary neutralizing antibody responses to all four DEN serotypes were induced. Following the boost, the neutralizing anti-DEN-1, DEN-3 and DEN-4 antibody titers were increased by 2, 5 and 2 fold, respectively in the group of mice injected ID as compared to the SC immunized animals. Neutralizing responses to DEN-2 virus were comparable in both groups. Immunization via the SC route resulted in a profile of dominant neutralizing antibody responses against DEN-1>DEN-2>DEN-3>DEN-4, with neutralizing titers 5120, 1280, 640 and 80, respectively. The hierarchy of neutralizing antibody responses after ID administration had shifted as follows; DEN-1>DEN-3>DEN-2>DEN-4 with neutralizing antibody titers 10240, 3840, 1280 and 160, respectively.

[0048] Table 4 is a comparison of the immunogenicity of tetravalent DENVax bearing the ratio 5:4:5:5 PFU of each composite chimeric virus (10^5 PFU of DENVax-1,-3 and -4 and 10^4 PFU of DENVax-2) after SC or ID immunization of mice. Blood samples were collected on days 42 and 56 to measure neutralizing antibody responses to each DEN virus serotype.

Table 4

DENVax Formulation	Neutralizing Antibody Titers (GMT)							
	DEN-1		DEN-2		DEN-3		DEN-4	
	Prime	Boost	Prime	Boost	Prime	Boost	Prime	Boost
5:4:5:5/SC	1920	5120	3200	1280	1280	640	80	80
5:4:5:5/ID	2560	10240	1280	1280	1600	3840	120	160

Materials and Methods

[0049] Mice: AG129 mice have an "intact" immune system; deficient for the interferon (IFN)–α/β and –γ receptors. Dengue infection has been described. Other studies: pathogenesis, cell tropism, and ADE have been examined. This model permits challenge with DEN-1 and DEN-2.

[0050] Non-human primates: Cynomolgus, rhesus macaques carry virus (viremia), but no disease manifests.

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What is claimed is:

1. A single vaccine composition against dengue virus for use in a method for inducing protection in a subject against three or four dengue virus serotypes, wherein the single vaccine composition against dengue virus is a mixture of live attenuated dengue viruses against three or four dengue virus serotypes to be administered to a subject by intradermal introduction to thereby induce protection in the subject against three or four dengue virus serotypes.

2. The single vaccine composition against dengue virus of claim 1, wherein at least one additional intradermal injection(s) of the vaccine against dengue virus is to be administered.

3. The single vaccine composition against dengue virus of claim 1, wherein at least one additional intradermal injection(s) is to be performed at a separate site on the subject.

4. The single vaccine composition against dengue virus of claim 1, wherein at least one additional intradermal injection(s) is to be performed less than 30 days after the first administration to the subject.

5. The single vaccine composition against dengue virus of claim 1, wherein the vaccine composition comprises three or four dengue virus serotypes at a predetermined ratio.

6. The single vaccine composition against dengue virus of claim 1, wherein the vaccine composition comprises all four dengue virus serotypes.

7. The single vaccine composition against dengue virus of claim 1, wherein at least one immunostimulatory adjuvant is to be administered to the subject.

8. The single vaccine composition against dengue virus claim 7, wherein the immunostimulatory adjuvant comprises at least one Toll receptor (TLR) ligand(s).

9. A composition for protecting a subject against a dengue virus serotype comprising, an intradermally (ID) administered single vaccine composition of predetermined ratio of three or four dengue virus serotypes made up of live attenuated dengue viruses.

10. The composition of claim 9, wherein the composition comprises all four dengue virus serotypes.

11. The composition of claim 10, wherein the four serotypes are in a predetermined ratio.

12. The composition of claim 9, further comprising at least one immunostimulatory adjuvant.

13. The composition of claim 9, further comprising at least one Toll Receptor (TLR) ligand(s) or other adjuvant.

14. The composition of claim 13, wherein the at least one Toll Receptor ligand(s) comprises CpG-ODN, Poly I:C, imiquimod, inulin-derived adjuvants, MPL a Toll receptor 4 ligand, poly I:U a Toll receptor 3 ligand, heat labile enterotoxin, non toxic mutants of heat labile enterotoxin and a combination thereof.

15. A system for administering a dengue vaccine composition to a subject comprising, a single vaccine composition against three or four dengue virus serotypes wherein the single vaccine composition comprises three or four dengue virus serotypes and an apparatus capable of introducing the vaccine composition intradermally by needle-free administration to a subject.

16. A vaccine kit comprising;

a vaccine composition against dengue virus comprising the composition according to claim 9; and

an apparatus capable of administering the composition intradermally to a subject.

17. The kit of claim 16, further comprising an immunostimulatory adjuvant.

18. The kit of claim 16, further comprising a Toll Receptor ligand(s).

19. Use of a single vaccine composition against dengue virus in the manufacture of a medicament for inducing protection in a subject against three or four dengue virus serotypes, wherein the single vaccine composition against dengue virus is to be administered to the subject by intradermal introduction to thereby induce protection in the subject against multiple dengue virus serotypes.

20. The use of claim 19, wherein one additional intradermal injection(s) of the vaccine against dengue virus is to be administered.

21. The use of claim 19, wherein one additional intradermal injection(s) is to be performed at a separate site on the subject.

22. The use of claim 19, wherein one additional intradermal injections(s) is to be performed less than 30 days after the first administration to the subject.

23. The use of claim 19, wherein the vaccine composition comprises the three or four dengue virus serotypes at a predetermined ratio.

24. The use of claim 19, wherein the vaccine composition comprises all four dengue virus serotypes.

25. The use of claim 19, wherein at least one immunostimulatory adjuvant is to be administered to the subject.

26. The use of claim 25, wherein the immunostimulatory adjuvant comprises at least one Toll receptor (TLR) ligand(s).