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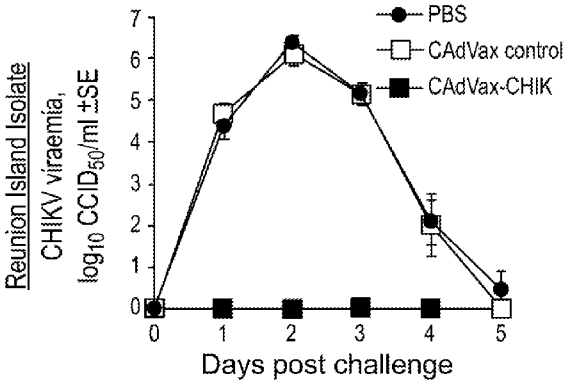
(54) Title: FLAVIVIRUS VACCINE WHICH MITIGATES CROSS-REACTIVE INFECTION BY OTHER FLAVIVIRUSES

FIG. 8



(57) Abstract: Disclosed are methods for mitigating vaccine-enhanced infection, such as vaccinations against a flavivirus such as Zika which can lead to enhanced infection of other viruses such as Chikungunya virus. Also disclosed are chimeric adenoviruses deleted for the E1A/B region wherein flavivirus proteins are included for vaccination. Also disclosed are chimeric adenoviruses comprising a Zika sequence, such as a full length prM-E ectodomain fusion region or an E gene deleted for a conserved fusion-loop epitope. Deletion of this epitope mitigates cross-infectivity.

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Flavivirus Vaccine Which Mitigates Cross-Reactive Infection by Other Flaviviruses

Reference to Prior Application

This application claims benefit of and priority to US Provisional Application 62/490,829 filed April 27, 2017, US Provisional Application 62/490,829 is hereby incorporated by reference in its entirety.

Technical Field

The present teachings disclose methods and compositions for the mitigation of cross-infectivity of Zika virus with other viruses.

Introduction

Zika virus (ZIKV) is a member of the genus Flavivirus, which also includes Dengue (DENV), yellow fever (YFV), and West Nile (WNV) viruses (Lazear, H.M., et al., J. Virol., 2016, 90, 4864-4875). Although the majority of human ZIKV infections result in asymptomatic or mild illness, there is strong epidemiological evidence associating ZIKV infection during pregnancy with severe congenital defects including microcephaly and spontaneous miscarriage (Zika Virus Microcephaly and Guillain-Barre Syndrome Situation Report, 2016, apps.who.int; Cauchemez, S., et al., Lancet, 2016, 387, 2125-2132). The unprecedented geographic expansion of the virus, with up to 1.3 million estimated human infections and 35 countries in the Americas reporting active ZIKV transmission (Cauchemez, S., et al., Lancet, 2016, 387, 2125-2132) has prompted the World Health Organization to declare that the ZIKV outbreak constituted a "Public Health Emergency of International Concern" (Gulland, A., BMJ, 2016, 352, i657). Besides the main mode of ZIKV transmission through mosquitoes of the Aedes genus (Fernandez-Salas, I., et al., Antiviral Res., 2015, 124, 30-42), ZIKV also can be spread by mother-to-child transmission (Martines, R.B., et al., MMWR, 2016, 65, 159-160; Mlakar, J., et al., N. Engl. J. Med., 2016, 374, 951-958; Quicke, K.M., et al., Cell Host Microbe, 2016, 20, 83-90), sexual contact (Venturi, G., Euro Surveill., 2016, 21, 30148; Moreira, J., et al., Clin. Infect. Dis., 2016, 63, 141-142; D'Ortenzio, E., et al., N. Engl. J. Med., 2016, 374, 2195-2198; Deckard, D.T., et al., M.M.W.R., 2016, 65, 372-374; Musso, D., et al., Emerging Infectious Diseases, 2015, 21, 359-361), and blood transfusion (Marano, G., et al., Blood transfusion, 2016, 14, 95-100; Musso, D., et al., Euro Surveill. 2014, 19, pii: 20761). There is an urgent need to expedite preclinical development and clinical translation of vaccines that can control current and future outbreaks of ZIKV infection in Latin America and prevent its further geographic spread.

ZIKV is an enveloped positive-sense RNA virus with a 10.7 kb genome. The genome encodes a polyprotein that is cleaved post-translationally into three structural proteins--the capsid (C), membrane precursor (prM), and envelope (E)--and seven non-structural (NS) proteins (Kuno, G., et al., Arch. Virol., 2007, 152, 687-696). The C protein binds to the viral RNA to form a nucleocapsid, prM prevents premature fusion with host membranes and forms heterodimers with the E protein that is essential for virion assembly. The pr region of the prM protein is subsequently cleaved by cellular furin-like proteases leaving the M-E proteins on mature virions (Zhu, Z., et al., Emerging Microbes & Infections, 2016, 5, e22). The E protein mediates cellular attachment, entry, and fusion (Mukhopadhyay, S., et al., Nat. Rev. Microbiol. 2005, 3, 13-22). Based on the biology of other flaviviruses, the E protein represents an attractive target for vaccine development because neutralizing antibodies against the E protein mediate protection from disease (Pierson, T.C., et al., Cell Host Microbe, 2008, 4, 229-238). The Zika virus E protein includes Domains I, II and III; Domain II mediates dimerization of E protein, and includes a "fusion loop" at amino acids 98-109 (Dai, L., et al., Cell Host & Microbe 2016, 19, 696-704) This fusion loop is highly conserved in flaviviruses (Dai, L., et al., Cell Host & Microbe 2016, 19, 696-704). Many of the most potently inhibitory anti-E antibodies against other flaviviruses (e.g., WNV and DENV) recognize quaternary epitopes that are present on E only when it is arranged on the icosahedral virion (Barba-Spaeth, G., et al., Nature, 2016, 536, 48-53; Rouvinski, A., et al., Nature, 2015, 520, 109-113; Dejnirattisai, W., et al., Nat. Immunol., 2015, 16, 170-177); Vogt, M.R., et al., J. Virology, 2009, 83, 6494-6507; Kaufmann, B., et al., Proc. Natl. Acad. Sci. U.S.A., 2010, 107, 18950-18955; Fibriansah, G., et al., Science, 2015, 349, 88-91; Fibriansah, G., et al., Nat. Commun., 2015, 6, 6341; Fibriansah, G., et al., EMBO Mol. Med., 2014, 6, 358-371). The prM protein also is a target of the anti-flavivirus response, however anti-prM antibodies generally are non-neutralizing (de Alwis, R., et al., PLoS Negl. Trop. Dis., 2011, 5, e1188; Dejnirattisai, W., et al., Science, 2010, 328, 745-748). Cryo-electron microscopy studies indicate that the ZIKV virion is highly mature, which would result in most of the pr antigen being cleaved in the late Golgi by furin-like proteases (Kostyuchenko, V.A., et al., Nature, 2016, 533, 425-428; Sirohi, D., et al., Science, 2016, 352, 467-470). Along with neutralizing antibodies, T cell responses contribute to protection against flaviviruses. In DENV infections, CD8+ T cells are associated with protection in patients living in hyperendemic areas (Weiskopf, D., et al., Proc. Natl. Acad. Sci. U.S.A., 2013, 110, E2046-2053), a role also observed in murine models (Yauch, L.E., et al., J. Immunol., 2009, 182, 4865-4873). Analogously, in the context of an inactivated WNV virion-based vaccine,

CD8+ T cell responses augmented vaccine immunity (Shrestha, B., et al., Vaccine, 2008, 26, 2020-2033). DENV-specific CD4+ T cells can produce IFN-γ, TNF-α, and IL-2, and this polyfunctionality correlated with an asymptomatic presentation (Hatch, S., et al., J. Infect. Dis., 2011, 203, 1282-1291; Mangada, M.M., et al., J. Infect. Dis., 2002, 185, 1697-1703). Thus, the possible protective roles of T cell responses need to be considered and optimized in the design of ZIKV vaccines.

Chikungunya virus (CHIKV) is an alphavirus that has caused periodic but sporadic outbreaks in tropical Africa and Asia and has recently (2005–2007) caused the largest outbreak of this virus in recorded history. Over 260,000 cases (≈1/3 of the population) were reported in Réunion Island (France) (Pialoux, G., et al., Lancet Infect. Dis., 2007, 7, 319-327) with 1.39 million cases reported in India (Mavalankar, D., et al., Lancet Infect. Dis., 2007, 7, 306-307). Numerous imported cases have also been reported in Europe (Fernandez-Salas, I., et al., Antiviral Res 2015, 124, 30-42), Asia (Lim, P.L., J. Travel Medicine 2009, 16, 289-291) and the United States (Centers for Disease C, M.M.W.R., 2007, 56, 276-277). No vaccine or effective drug for CHIKV disease is currently commercially available. The disease usually involves weeks to months of debilitating arthralgia/arthritis, and can involve myalgia, fever, headache, nausea, vomiting and/or a rash (Brighton, S.W., et al., South African Medical Journal, 1983, 63, 313-315) with arthritis/arthralgia affecting 73–80% of patients (Pialoux, G., et al., Lancet Infect. Dis., 2007, 7, 319-327). Disease can persist for two years or more in some patients (de Andrade, D.C., et al., BMC Infectious Diseases, 2010, 10, 31; Larrieu, S., et al., J. Clinical Virology, 2010, 47, 85-88; Borgherini, G., et al., Clin. Infect. Dis., 2008, 47, 469-475). The recent epidemic involved the emergence of a new clade of CHIKV within the large East-, Central-, and South-African phylogroup (Schuffenecker, I., et al., PLoS Med., 2006, 3, e263). Viruses within the new clade of CHIKV are efficiently transmitted by Aedes albopictus mosquito, whereas the usual vector for CHIKV is Aedes aegypti (Tsetsarkin, K.A., et al., PloS One, 2009, 4, e6835; Tsetsarkin, K.A., et al., PLoS Pathog., 2007, 3, e201). The new viruses also appear to be associated with more severe disease in humans (Charrel, R.N., et al., The New England Journal of Medicine, 2007, 356, 769-771; Ng, L.F., et al., PloS One, 2009, 4, e4261). Importantly, viruses within this new clade have been associated for the first time with human mortality (Suryawanshi, S.D., et al., The Indian Journal of Medical Research, 2009, 129, 438-441; Economopoulou, A., et al., Epidemiology and Infection, 2009, 137, 534-541; Mavalankar, D., et al., Emerg. Infect. Dis. 2008, 14, 412-415).

Dendritic cells prime adaptive immune responses. The recognition that dendritic cells

(DCs) orchestrate and coordinate effective adaptive immune responses provides a strong rationale for targeting of antigen directly to these antigen presenting cells (APC) following vaccination (Grodeland, G., et al., PLoS One, 2013, 8, e80008; Macri, C., et al., Clin. Transl. Immunology, 2016, 5, e66; Palucka, K., et al., Immunity, 2010, 33, 464-478). Conventional myeloid DCs (cDCs) link innate and adaptive immunity and are responsible for the initiation and regulation of immune responses via the activation of T cells, natural killer cells and B cells. While much of cDC research has been carried out in mice, progress has been made in defining cDC subsets across species, including humans and monkeys (Dutertre, C.A., et al., Cell Immunol., 2014, 291, 3-10). Targeting of antigen to different molecules on APCs can polarize the immune response (Dutertre, C.A., et al., Cell Immunol., 2014, 291, 3-10; Grodeland, G., et al., Frontiers in Immunology, 2015, 6, 367). While targeting of vaccine antigens to MHC II molecules increases TH2 and IgG1 antibody responses, targeting to chemokine receptor XCR1 enhances TH1 and IgG2 responses, in addition to CD8+ T cell responses (Grodeland, G., et al., PLoS One, 2013, 8, e80008; Grodeland, G. et al., J. Immunol., 2013, 191, 3221-3231; Fossum, E., et al., European Journal of Immunology, 2015, 45, 624-635). Therefore, antigen targeting to specific APC types may tailor immune responses for optimal protection against individual pathogens. In this regard, the targeting to Clec9A+ DCs has been shown to enhance presentation of both MHC class I and II-restricted antigens (Caminschi, I., et al., Blood, 2008, 112, 3264-3273). Targeting of DCs via Clec9A also induced robust, long-lasting humoral responses even without adjuvants (Park, H.Y., et al., J. Immunology, 2013, 191, 4919-4925) in mice and non-human primates (Li, J. et al., European Journal of Immunology, 2015, 45, 854-864). Clec9A targeting induces follicular T helper (TFH) cells that are essential for germinal center formation and crucial for generating long-lived plasma cells (LLPCs) and memory B cells (MBCs), which are required for durable and anamnestic antibody responses (Kato, Y., et al., J. Immunol., 2015, 195, 1006-1014).

Adenoviral vector have utility for flavivirus vaccine development. Adenovirus (Ad)-based vectors are promising vaccine platforms to stimulate innate and adaptive immune responses (Hartman, Z.C., et al., Virology, 2007, 358, 357-372; Huang, X., et al., Human Gene Therapy, 2009, 20, 293-301; Lore, K., et al., J. Immunology, 2007, 179, 1721-1729). Of the 51 known human Ad serotypes, the most commonly employed vectors are based on serotypes 2 and 5 (Ad2 and Ad5) (Abbink, P., et al., J. Virology, 2007, 81, 4654-4663; Barouch, D.H., et al., Vaccine, 2011, 29, 5203-5209; Bassett, J.D., et al., Expert Rev. Vaccines, 2011, 10, 1307-1319). Ad5 vectors are highly utilized with the greatest number of clinical trials ongoing in the cancer vaccine and infectious disease fields (Gene Therapy

Clinical Trials Worldwide, www.wiley.com). Ad vectors have been used for the development of DENV vaccines (Holman, D.H., et al., Clin. Vaccine Immunol., 2007, 14, 182-189) including a tetravalent vaccine expressing domain III of the E protein (E-DIII) from the four different DENV serotypes (Khanam, S., et al., Vaccine, 2009, 27, 6011-6021). This vaccine candidate was tested using Ad5 vector as a priming immunization and DNA immunization as a boosting and induced neutralizing antibodies and T cell responses against all DENV serotypes. In rhesus macaques, tetravalent vaccination using a mixture of two bivalent Ad vectors encoding both the prM and the E proteins of DENV induced neutralizing antibodies and T cells responses against the 4 vaccination serotypes and protected monkeys against a live DENV challenge occurring at 4 or 24 weeks after two immunizations (Raviprakash, K., et al., J. Virology, 2008, 82, 6927-6934).

Murine models of ZIKV. Recently, the Diamond laboratory and other groups have developed mouse models of ZIKV pathogenesis that recapitulate many features of human disease (Aliota, M.T., et al., PLoS Negl. Trop. Dis., 2016, 10, e0004750; Aliota, M.T., et al., PLoS Negl. Trop. Dis., 2016, 10, e0004682; Lazear, H.M., et al., Cell Host Microbe, 2016, 19, 720-730; Rossi, S.L. et al., Am. J. Trop. Med. Hyg., 2016, 94,1362-1369). Whereas 4- to 6-week-old wild-type (WT) mice did not develop overt clinical illness after infection with contemporary clinical strains of ZIKV, mice lacking the ability to produce or respond to type I interferon (IFN) (e.g., Ifnar1-/- mice) developed severe neurological disease that was associated with high viral loads in the brain and spinal cord, chronic infection in the testes, and substantial lethality. In a complementary approach using WT mice treated with a blocking anti-ifnar antibody (MAR1-5A3), a less severe model of ZIKV pathogenesis that also resulted in replication of ZIKV in several organs was developed (Lazear, H.M., et al., Cell Host Microbe, 2016, 19, 720-730). These animals, however, survived infection and did not develop neurological disease. In more recent studies, the Diamond laboratory has generated an adapted ZIKV strain that causes significant morbidity and mortality in adult WT mice treated with a blocking anti-Ifnar antibody; this model allows for induction of vaccine-derived immune responses in WT immunocompetent mice, and then after administration of the anti-Ifnar antibody, a stringent challenge model of protection against ZIKV infection. In addition to these models, the Diamond laboratory also has generated an in utero transmission model of ZIKV infection and pathogenesis. To evaluate ZIKV infection during pregnancy, Miner, J.J., et al., (Cell, 2016, 165, 1081-1091) used Ifnar1-/- females crossed to WT males and used pregnant WT females treated with an anti-ifnar-blocking antibody. These studies revealed that ZIKV infects pregnant dams including the placenta. This ZIKV infection results

in damage to the placental barrier and infection of the developing fetus, as well as placental insufficiency and intrauterine growth restriction. In severe cases, ZIKV infection led to fetal demise. These findings establish models for studying mechanisms of in utero transmission and testing of vaccines that could prevent or mitigate intrauterine infection with ZIKV.

Thus far, a number of CHIKV vaccines have been developed, but none have been licensed. Formalin-killed CHIKV vaccines have been shown to be immunogenic in humans (Edelman, R., et al., J. Infectious Diseases, 1979, 140, 708-715), nonhuman primates (Nakao, E., et al., Bulletin of the World Health Organization, 1973, 48, 559-562) and mice (Tiwari, M., et al., Vaccine, 2009, 27, 2513-2522). However, growth of large quantities of CHIKV for vaccine manufacture is complicated by the requirement for appropriate BSL3 containment. A live-attenuated CHIKV vaccine strain, known as TSI-GSD-218, induced neutralizing responses and protected mice and monkeys against challenge (Levitt, N.H., et al., Vaccine, 1986, 4, 157-162). However, in a phase II human trial, this vaccine caused side effects in several recipients that included arthralgia (Edelman, R., et al., Am. J. Trop. Med. Hyg., 2000, 62, 681-685). DNA-based CHIK vaccines encoding E1, E2 and capsid on three separate plasmids have been shown to be immunogenic in mice (Muthumani, K., et al., Vaccine, 2008, 26, 5128-5134). Unfortunately, DNA vaccines have so far not been particularly effective at generating antibody responses in humans (Kutzler, M.A., Nature Reviews Genetics, 2008, 9, 776-788), which is a concern as antibodies are believed to be required for protection against CHIKV infections (Couderc, T., et al., J. Infectious Diseases, 2009, 200, 516-523). Chimeric live-attenuated CHIK vaccines encoding CHIKV E1, E2 and capsid with the non-structural genes from Venezuelan Equine Encephalitis virus (VEEV), Eastern Equine Encephalitis virus (EEEV) or Sindbis virus (SINV) have been shown to be immunogenic and protective against nasal CHIKV challenge in mice (Wang, E., et al., Vaccine, 2008, 26, 5030-5039). However, manufacturing and safety remain significant hurdles given the known ability of alphaviruses to recombine and generate replication competent viruses (Strauss, J.H., et al., Seminars in Virology, 1997, 8, 85-94). Recently, the first alphavirus Virus-like particle (VLP) CHIKV vaccine was produced and tested in non-human primates (Akahata, W., et al., Nature Medicine, 2010, 16, 334-338). Although this vaccine, based on the West African CHIKV genotype, provided protection, three doses were required.

Vaccination by current methods against one flavivirus or alphavirus, such as, without limitation, Zika or CHIK, can induce antibodies to other flaviviruses or alphaviruses but can, paradoxically, enhance infection with these infectious agents.

Summary

The inventors have developed a potency-enhanced adenovirus vaccine based upon dendritic cell targeting. In addition to enhanced potency, a vaccine of the present teachings can encode flavivirus or alphavirus antigens modified to mitigate the induction of immunization against alternative flavivirus or alphavirus. The modifications can mitigate paradoxical infections that can occur with other vaccines. Zika vaccination is demonstrated, and this approach is generalized to other flaviviruses and alphaviruses.

In various embodiments, a vaccine of the present teachings can comprise a dendritic cell-targeted adenovirus that is deleted for an E1A/B region and can also comprise at least one structural gene or a portion thereof of a heterologous virus. In some configurations, a flavivirus antigen such as a Zika virus-like particle (VPL) antigen can be modified so that it does not induce activating antibodies against other arbovirus. A vaccine of the present teachings can mitigate induction of vaccine-enhanced infection by other viruses. In various configurations, a heterologous virus of the present teachings can be a flavivirus or an alphavirus. In some configurations, a structural gene of a flavivirus such as, without limitation, Zika, can be a capsid (C), membrane precursor (prM) or envelope (E) gene. In various configurations, at least one structural gene can be prM-E. In various configurations, the prM-E gene can be deleted for a conserved fusion-loop epitope in the E protein. In various configurations, the prM-E gene can contain one or more point mutations that destroy a conserved three-dimensional structure of a conserved fusion-loop epitope in the E protein. In various configurations, the prM-E gene can contain one or more mutations that destroy a conserved fusion-loop epitope in the E protein. In various configurations, the structural gene can be E. In various configurations, the at least one structural gene of a flavivirus can be at least two structural genes of a flavivirus. In various configurations, the at least one structural gene of a flavivirus can be at least three structural genes of a flavivirus. In some configurations, the structural genes of a flavivirus can be E1, E2, capsid, or a combination thereof. In some configurations, the structural genes of a flavivirus can be E1, E2 and capsid. In various configurations, the vaccine can mitigate induction of vaccine-enhanced infection by other flaviviruses. In various configurations, the vaccine can mitigate induction of vaccine-enhanced infection by chikungunya virus. In various configurations, the at least one flavivirus or alphavirus can be Zika virus (ZIKV), Chikungunya virus (CHIK), Dengue virus (DENV), yellow fever virus (YFV), or West Nile (WNV) virus. In various configurations, the at least one heterologous virus can be Zika virus or Chikungunya virus (CHIK). In various

configurations, the at least one flavivirus can be Zika virus (ZIKV). In various configurations, the at least one alphavirus can be CHIK. In various configurations, a vaccine of the present teachings can be a vaccine that confers immunity against a flavivirus such as Zika virus (ZIKV). In various configurations, a vaccine of the present teachings can be a vaccine that confers immunity against an alphavirus such as Chikungunya virus (CHIK).

In various embodiments, the present teachings include a vaccine comprising a dendritic cell-targeted adenovirus that is deleted for an E1A/B region, and at least one structural gene of a heterologous virus, wherein the vaccine mitigates induction of vaccine-enhanced infection by other viruses. In some configurations, the heterologous virus can be a flavivirus or an alphavirus. In various configurations, the heterologous virus can be, without limitation, a Zika virus or a CHIK virus. In some configurations, the at least one structural gene can be C, prM or E, or a combination thereof. In some aspects, the prM-E gene can be a prM-E gene deleted for a conserved fusion-loop epitope in the E protein. In some aspects, the at least one structural gene can be E.

In some configurations, a vaccine of the present teachings can mitigate induction of vaccine-enhanced infection by other flaviviruses. In some configurations, a vaccine of the present teachings can mitigate induction of vaccine-enhanced infection by chikungunya virus.

In various configurations, a vaccine of the present teachings can confer immunity against at least one flavivirus such as Zika virus (ZIKV), Dengue virus (DENV), yellow fever virus (YFV), or West Nile (WNV) virus.

In some embodiments, the present teachings include vaccination methods. In various configurations, a method of vaccinating a subject against a flavivirus or an alphavirus can comprise administering to a subject a vaccine in accordance with the present teachings. In various configurations, vaccination methods of the present teachings can be used to induce immunity against a flavivirus or an alphavirus without inducing immunization to another flavivirid or alphavirus.

Brief Description of the Drawings

FIG. 1 illustrates a dendritic cell targeted adenoviral vaccine for Zika which circumvents flavivirus cross-reactivity.

FIG. 2 illustrates ZIKV protein E expression using Ad5ZprM-E-ecto vector.

FIG. 3 illustrates a structural comparison between wild type Ad5 fiber and a fiber-fibritin-ligand chimera.

FIG. 4 illustrates Ad5FF1.8 vector targeting to DC via the fiber knob replacement

with sdAb Nb1.8.

FIG. 5 illustrates a lethal challenge model of WT mice with ZIKV and protection with antibodies.

FIG. 6 illustrates daily weights measured for a lethal challenge model of WT mice with ZIKV and protection with antibodies.

FIG. 7 illustrates survival curves for a lethal challenge model of WT mice with ZIKV and protection with antibodies.

FIG. 8 illustrates mouse viremia after challenge with the Réunion Island isolate of CHIK.

FIG. 9 illustrates mouse foot swelling after challenge with the Réunion Island isolate of CHIK.

FIG. 10 illustrates mouse viremia after challenge with the Asian isolate of CHIK.

FIG. 11 illustrates mouse foot swelling after challenge with the Asian isolate of CHIK.

Detailed Description

The inventors have developed an adenovirus vaccine targeted to dendritic cells. An adenovirus-based vaccine of the present teachings has enhanced potency, and can encode one or more flavivirus or alphavirus antigens modified to mitigate the induction of immunization against alternative flavivirus or alphavirus. The modifications can mitigate paradoxical infection that can occur with other vaccines. In some configurations, the adenoviral vector can be replication incompetent. In some configurations, the adenoviral vector can be deleted for the viral E1A/B genes. In some configurations, an adenoviral vector of the present teachings can comprise a full length prM-E ectodomain fusion region of a flavivirus such as a Zika virus, or a flavivirus E gene deleted or mutated for a conserved fusion-loop epitope. In some configurations, a flavivirus prM-E can be modified with one or more mutations that destroy a conserved fusion-loop epitope in the E protein, so that an adenovirus vaccine can provide protection against Zika without paradoxical augmentation of infection by other arboviruses.

Methods

The methods and compositions described herein utilize laboratory techniques well known to skilled artisans, and can be found in laboratory manuals such as Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001; Spector, D. L. et al., Cells: A Laboratory Manual, Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998; Nagy, A., Manipulating the Mouse Embryo: A Laboratory Manual (Third Edition), Cold Spring Harbor, NY, 2003 and Harlow, E., Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999. Methods of administration of pharmaceuticals and dosage regimes, can be determined according to standard principles of pharmacology well known skilled artisans, using methods provided by standard reference texts such as Remington: the Science and Practice of Pharmacy (Alfonso R. Gennaro ed. 19th ed. 1995); Hardman, J.G., et al., Goodman & Gilman's The Pharmacological Basis of Therapeutics, Ninth Edition, McGraw-Hill, 1996; and Rowe, R.C., et al., Handbook of Pharmaceutical Excipients, Fourth Edition, Pharmaceutical Press, 2003. As used in the present description and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context indicates otherwise.

Examples

The present teachings include descriptions provided in the examples that are not intended to limit the scope of any aspect or claim. Unless specifically presented in the past tense, an example can be a prophetic or an actual example. The following non-limiting examples are provided to further illustrate the present teachings. Those of skill in the art, in light of the present disclosure, will appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the present teachings.

Example 1

This example illustrates expression of ZIKV soluble E protein by recombinant Ad vector. To validate the expression of ZIKV proteins by Ad5 vector we incorporated the DNA sequence encoding the full prM gene and the ectodomain of E of ZIKV (strain H/PF/2013 from French Polynesia) containing a heterologous N-terminal IL-2 signal peptide and C-terminal hexahistidine tag (6-His) under transcriptional control of cytomegalovirus (CMV) immediate early promoter in place of the early E1A/B genes deleted in Ad5 genome. This construct produces high levels of soluble E protein only, as prM/M is cleaved completely by the host signalase, and in the absence of the transmembrane domain of E, prM and E do not stably associate as heterodimers (Cockburn, J.J., et al., EMBO J., 2012, 31, 767-779). The generated Ad5ZprM-E-ecto vector was used to infect A549 cells to validate the expression of secreted E protein. As

can be seen in FIG. 3-4, the 6-His-tagged E protein band with molecular mass of approximately 48 kDa was detected 48 and 72 hours post-infection in both cell lysates and culture medium by Western blotting. A549 cells were infected with Ad5ZprM-E-ecto vector at a multiplicity of infection (MOI) of 900. The cells and culture medium samples mixed with Laemmli loading buffer, boiled, and run on 4-20% gradient SDS-PAGE as follows. (1) Prestained protein standards, molecular masses are indicated in kDa on the left.; (2) Culture medium 48-h post-infection (pi); 3) Culture medium 72-h pi; 4) Uninfected cells culture medium; 5) Cells 48-h pi; 6) Cells 72-h pi; 7) Mock-infected cells; 8) Purified ZIKV protein E (25 ng/lane). Electrophoretically separated proteins were transferred to PVDF membrane and probed with anti-His tag and secondary anti-mouse AP-conjugated antibodies. The ZIKV protein E purified from culture medium of HEK293 cells transiently expressing the same CMV-driven prM-E-ecto plasmid was used as a positive control. These data demonstrate soluble ZIKV E expression using recombinant Ad5ZprM-E-ecto vector.

Example 2

This example illustrates Ad targeting to DCs via functional replacement of fiber knob with sdAb ligand. Ad5 cellular entry is mediated by distinct binding and internalization events; the knob domain of Ad5 fiber initiates attachment through interactions with coxsackie virus and adenovirus receptor (CAR) expressed on epithelial cells (Bergelson, J.M., et al., Science, 1997, 275, 1320-1323), whereas internalization is mediated by distinct interactions between integrins and the RGD motif within the Ad5 penton (Tsetsarkin, K.A., et al., PloS One, 2009, 4, e6835) as illustrated in FIG. 3 (see also WO/2015/161314 by Curiel, D.T., et al.). FIG. 3 illustrates a structural comparison between wild type Ad5 fiber and a fiber-fibritin-ligand chimera, which comprises a phage T4 fibritin trimerization foldon to replace the Ad5 fiber knob. The sdAb Nb1.8 targeting ligand is fused to the foldon C-terminus.

To generate a DC-targeted Ad, we employed the nanobody Nb1.8, a camelid sdAb raised against murine bone marrow-derived DCs (BMDC). This nanobody recognizes immature BMDC (iBMDC) in vitro and in vivo (De Groeve, K., et al., J. Nuclear Medicine, 2010, 51, 782-789). The sdAb Nb1.8-coding sequence was incorporated in-frame following fiber-fibritin fusion within Ad5 genome by homologous recombination in E. coli and the resultant viral genome was rescued in 293F28 cells (Belousova, N., et al., J. Virology, 2003, 77, 11367-11377) to generate replication incompetent Ad5FF1.8 vector essentially as described (Kaliberov, S.A., et al., Laboratory investigation, 2014, 94,893-905) (FIG. 1). Both

fiber and Nb1.8 incorporation in the context of assembled virions were verified by Western blotting on purified Ad5FF1.8 particles (data not shown).

Example 3

This example illustrates that Ad5FF1.8 vector mediates DCs transduction. To establish the efficacy of the Ad5FF1.8 vector gene transfer in a relevant animal DC substrate, we used murine iBMDC to assess their transduction using GFP reporter gene expression. Ad5FF1.8 showed superior gene transfer into DCs compared to the control Ad5 vector, as demonstrated by the markedly increased number of GFP-positive DCs (FIG. 4). FIG. 4 illustrates Ad5FF1.8 vector targeting to DC via the fiber knob replacement with sdAb Nb1.8. In FIG. 4, the upper panel illustrates iBMDC monolayers infected with the indicated Ad vectors imaged 40 h post-infection using epifluorescence microscopy. Representative images are shown at a magnification of 40×. The lower panel in FIG. 4 illustrates a flow cytometry analysis of gene transfer levels in iBMDC transduced with Ad5FF1.8 or control Ad5 vector, measured as the percentage GFP+CD11c+ cells.

To confirm that the specificity of Ad5FF1.8 transduction is mediated by sdAb Nb1.8, we transduced DCs with Ad5FF1.8 in the presence or absence of soluble Nb1.8. Increasing the concentration of soluble Nb1.8 resulted in a dose-dependent decrease of gene transfer by Ad5FF1.8 (data not shown). These data demonstrate Ad vector targeting to DCs via genetic sdAb incorporation into viral capsid.

Example 4

This example illustrates generation of sdAb ligands binding Clec9A. To target Ad vector to specific DC subsets we have identified several surface markers that are conserved across species and present on CD8α+/CD141+ DCs (flavivirus E gene deleted or mutated for a conserved fusion-loop, C.A., et al., Cell Immunol., 2014, 291, 3-10; Poulin, L.F., et al., Blood, 2012, 119, 6052-6062; Schreibelt, G., et al., Blood, 2012, 119, 2284-2292) including the C-type lectin receptor Clec9A (or DNGR-1), a damage-associated molecular pattern (DAMP) receptor (Poulin, L.F., et al., Blood, 2012, 119, 6052-6062; Poulin, L.F., et al., J. Exp. Med., 2010, 207, 1261-1271). The generation of sdAb binding Clec9A was carried out using the recombinant murine Clec9A protein (MyBioSource, Inc., San Diego, CA) to immunize alpacas. Seven unique sdAb clones were selected using the constructed phage-display sdAb library and validated by ELISA for Clec9A recognition. Most of the selected sdAbs showed high binding efficiency in the range of 1 nM or less (data not shown) thus

providing several sdAb-coding sequences to be exploited for genetic capsid incorporation to achieve GAd vector targeting to CD8α+/Clec9A+ DC subset.

Example 5

This example illustrates mouse models of ZIKV pathogenesis. Within the last six months, the Diamond laboratory has developed several mouse models of ZIKV pathogenesis in mice deficient in type I interferon (IFN) signaling. A loss of Ifnar expression or blockade of Ifnar function is necessary because ZIKV does not replicate efficiently in wild-type (WT) mice due in part to a species-specific lack of antagonism of mouse Stat2 (Grant, A., Cell Host Microbe, 2016, 19, 882-890) a key signaling intermediate downstream of type I IFN signaling. The different adult mouse models have possible utility for vaccine testing, each with its own limitations. Lethal infection of contemporary ZIKV strains in adult Ifnar-/- mice (Lazear, H.M., et al., Cell Host Microbe, 2016, 19, 720-730) has the advantage of stringent challenge of protection, however, it is limited because immunization occurs in an immunocompromised Ifnar-/- mouse, which could skew or dampen responses. The inoculation of WT adult C57BL/6 mice treated with an anti-Ifnar blocking antibody and contemporary American/Asian ZIKV strains (Lazear, H.M., et al., Cell Host Microbe, 2016, 19, 720-730) allows immunization in WT mice, however, it provides only a virological read-out because infection causes mild morbidity and no mortality. The present teachings allow for lethal infection of an adapted African ZIKV strain in adult WT mice (FIG. 5-7). In these experiments, adult WT C57BL/6 mice were passively transferred 2 mg of anti-Ifnar1 mAb and 250 μgs of the indicated mAbs (CHK-166, ZV-54, or ZV-57) via an intraperitoneal injection one day before subcutaneous inoculation with 10^5 FFU of adapted ZIKV Dakar 41519. On day 3 after infection, serum was collected for analysis of viremia by qRT-PCR for a lethal challenge model of WT mice with ZIKV and protection with antibodies. For FIG. 5-6, statistical significance was analyzed by a one-way ANOVA (**, $P < 0.01$; ***, $P < 0.001$). FIG. 7 illustrates survival curves for a lethal challenge model of WT mice with ZIKV and protection with antibodies. Anti-ZIKV mAbs provided statistically significant protection in the percentage of surviving animals compared to the control CHK-166 mAb (***, $P < 0.001$, log rank test for ZV-54 and ZV-67). The results are pooled from independent experiments; n = 8-9 mice for each treatment condition. The present methods thus allow for immunization in WT mice with a stringent requirement for protection against challenge. However, an African ZIKV strain is used, although concern is mitigated by immunization with an Ad encoding structural genes from a contemporary Asian (or American) isolate. This model can be high-

throughput (WT mice can be purchased in large cohorts from commercial vendors), uses immune-competent mice for induction of vaccine responses, and challenges with a heterologous ZIKV strain (which can account for breadth/diversity of response). This model can be used for vaccination and lethal challenge.

In addition to these infection models in adult mice, the Diamond laboratory has developed parallel models (Ifnar-/- pregnant females infected with contemporary ZIKV strains or WT pregnant females treated with an Ifnar blocking mAb and infected with contemporary or adapted ZIKV strains) of in utero transmission of ZIKV (Miner, J.J., et al., Cell, 2016, 165, 1081-1091). Depending on the specific model, we observed placental infection, injury and insufficiency, fetal resorption, and fetal brain injury and neuronal cell death. Moreover, we identified ZIKV within trophoblasts and fetal endothelial cells in the placenta, consistent with a tropism for cells lining the maternal-fetal interface and a trans-placental route of infection. The different in utero models have possible utility for vaccine testing, again each with limitations. Based on the rationale described above, we will immunize WT females with GAd encoding ZIKV genes, allow for immune responses to develop, breed them with WT males, administer a blocking anti-Ifnar antibody, challenge with the adapted African ZIKV strain, and monitor clinical and virological parameters in the mother and developing fetus according to published methods (Miner, J.J., et al., Cell, 2016, 165, 1081-1091). These considerations would be fully operative for the context of CHIK as well.

Example 6

This example illustrates CHIK challenge of CAdVax-CHIK immunized C57BL/6 mice.

Recombinant adenovirus vectors have been widely tested in humans and have been shown to be safe in an extensive series of human trials. They are also stable, immunogenic and relatively easy to manufacture (Seregin, S.S., et al., Expert Opinion on Biological Therapy, 2009, 9, 1521-1531). The non-replicating Complex Adenovirus vaccine (CAdVax) vectors (Wang, D., et al., J. Virol. 2006, 80, 2738-2746) contain deletions in E1, E3 and most of the E4 regions (except orf6) of the adenovirus 5 (Ad5) genome. These deletions, coupled with multiple engineered transgene-expression sites, allow the insertions of multiple antigens at different locations, or a large antigen insert at the location of choice within the Ad5 genome. The technology has been used to generate recombinant dengue, influenza, Marburg, Ebola, West Nile and Rift Valley fever virus vaccines, and has been shown to be efficacious

in murine, guinea pig, ferret and non-human primate models (Raviprakash, K., et al., J. Virology, 2008, 82, 6927-6934; Pratt, W.D., et al., Clinical and vaccine immunology: CVI, 2010, 17, 572-581; Holman, D.H., et al., Clinical and vaccine immunology: CVI, 2009, 16, 1624-1632; Holman, D.H., et al., Vaccine, 2008, 26, 2627-2639; Swenson, D.L., et al., Clinical and Vaccine Immunology: CVI, 2008, 15, 460-467; Schepp-Berglind, J., et al., Clinical and vaccine immunology: CVI, 2007, 14, 1117-1126; Wang, D., et al., Virology, 2006, 353, 324-332).

Herein we describe the application of the CAdVax technology to the design of a CHIKV vaccine. In this CAdVax-CHIK vaccine, a single insert encoding the structural polyprotein (comprising the envelope glycoproteins E1, E2 and capsid) of CHIKV was inserted in the right hand of the genome. The major advantage of this particular configuration of the CAdVax is that it prevents the generation of replication-competent adenovirus through homologous recombination in the packaging cell line, HEK293, a common problem of first generation Ad5 vectors. The antigen sequences are from a CHIKV isolate from the recent epidemic on Réunion Island, and the complete structural polyprotein gene was expressed in order to retain the native processing sequences. A single dose of the vaccine completely protected mice against viremia and disease in recently developed adult wild-type mouse models of CHIKV-induced arthritis (Gardner, J., et al., J. Virology, 2010, 84, 8021-8032). We would anticipate augmentations in these positive results with our DC targeting.

Mice (n=3–6 per group) were vaccinated with CAdVax-CHIK, a control CAdVax vaccine or PBS. At 6.5 weeks post-immunization, mice were challenged with CHIKV. FIG. 8 illustrates viremia after challenge with the Réunion Island isolate. Viremia was significantly different between CAdVax-CHIK and CAdVax-control vaccinated groups on days 1–3 (all $p<0.037$, Mann Whitney U test). FIG. 9 illustrates foot swelling after challenge with the Réunion Island isolate. Swelling (represented as a cross sectional area in mm^2) was significantly different between CAdVaxCHIK and CAdVax-control vaccinated groups on days 3–8 (all $p<0.02$, Mann Whitney U test). FIG. 10 illustrates viremia after challenge with the Asian isolate. Viremia was significantly different between CAdVax-CHIK and CAdVax-control vaccinated groups on days 1–4 (all $p<0.014$, Mann Whitney U test). FIG. 11 illustrates foot swelling after challenge with the Asian isolate. Swelling was significantly different between CAdVax-CHIK and CAdVax-control vaccinated groups on days 3-10 (all $p<0.04$, Mann Whitney U test).

All publications referenced herein are incorporated by reference, each in its entirety.

Claims

What is claimed is:

1. A vaccine comprising:

dendritic cell-targeted adenovirus that is deleted for an E1A/B region; and

at least one structural gene of a heterologous virus, wherein the vaccine mitigates induction of vaccine-enhanced infection by other viruses.

2. A vaccine in accordance with claim 1, wherein the heterologous virus is selected from the group consisting of a flavivirus and an alphavirus.

3. A vaccine in accordance with claim 1, wherein the heterologous virus is a flavivirus.

4. A vaccine in accordance with claim 3, wherein the at least one structural gene is selected from the group consisting of C, prM and E.

5. A vaccine in accordance with claim 3, wherein the at least one structural gene is prM-E.

6. A vaccine in accordance with claim 5, wherein the prM-E gene has been deleted for a conserved fusion-loop epitope in the E protein.

7. A vaccine in accordance with claim 3, wherein the at least one structural gene is E.

8. A DC targeted adenoviral vector in accordance with claim 3, wherein the vaccine mitigates induction of vaccine-enhanced infection by other flaviviruses.

9. A DC targeted adenoviral vector in accordance with claim 1, wherein the vaccine mitigates induction of vaccine-enhanced infection by chikungunya virus.

10. A DC targeted adenoviral vector in accordance with claim 1, wherein the at least one flavivirus is selected from the group consisting of Zika virus (ZIKV), Dengue virus (DENV), yellow fever virus (YFV), and West Nile (WNV) virus.

11. A DC targeted adenoviral vector in accordance with claim 1, wherein the at least one flavivirus is Zika virus (ZIKV).

12. A DC targeted adenoviral vector in accordance with claim 2, wherein the at least one alphavirus is Chikungunya virus (CHIK).

13. A method of vaccinating a subject against a flavivirus or alphavirus without inducing immunization of other flavivirid or alphavirid, comprising administering to a subject a vaccine in accordance with any one of claims 1-10.

FIG. 1

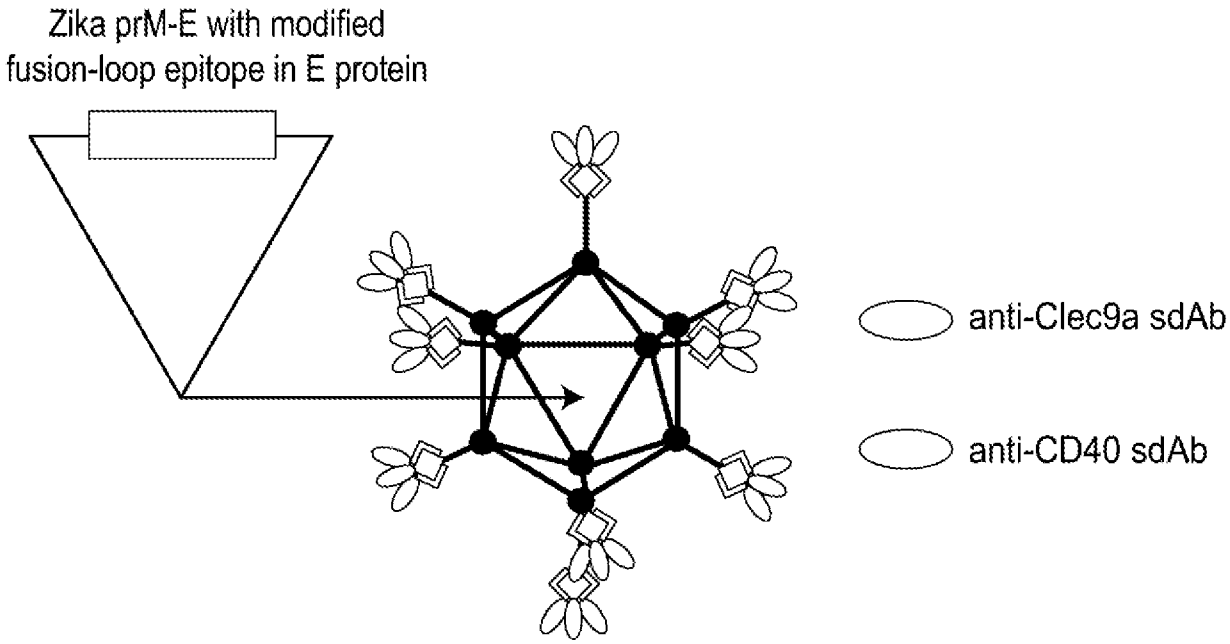
Zika prM-E with modified
fusion-loop epitope in E protein
anti-Clec9a sdAb
anti-CD40 sdAb

FIG. 2

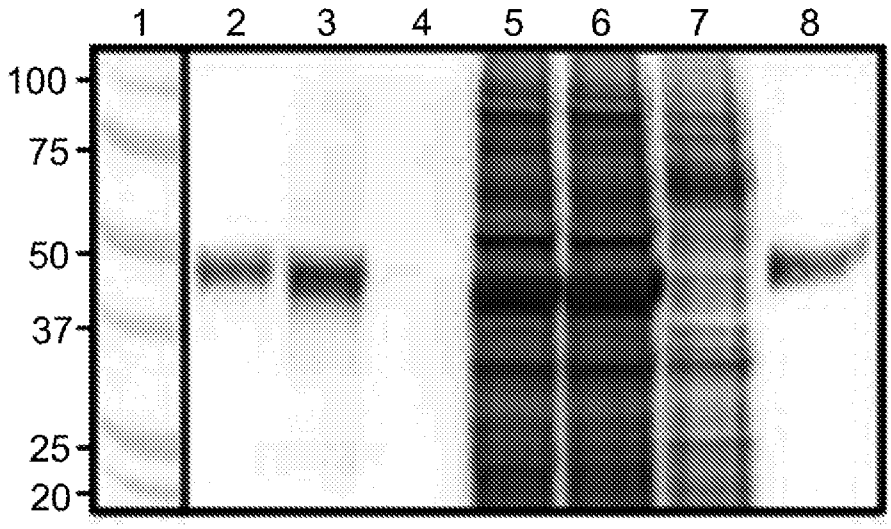
1
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FIG. 3

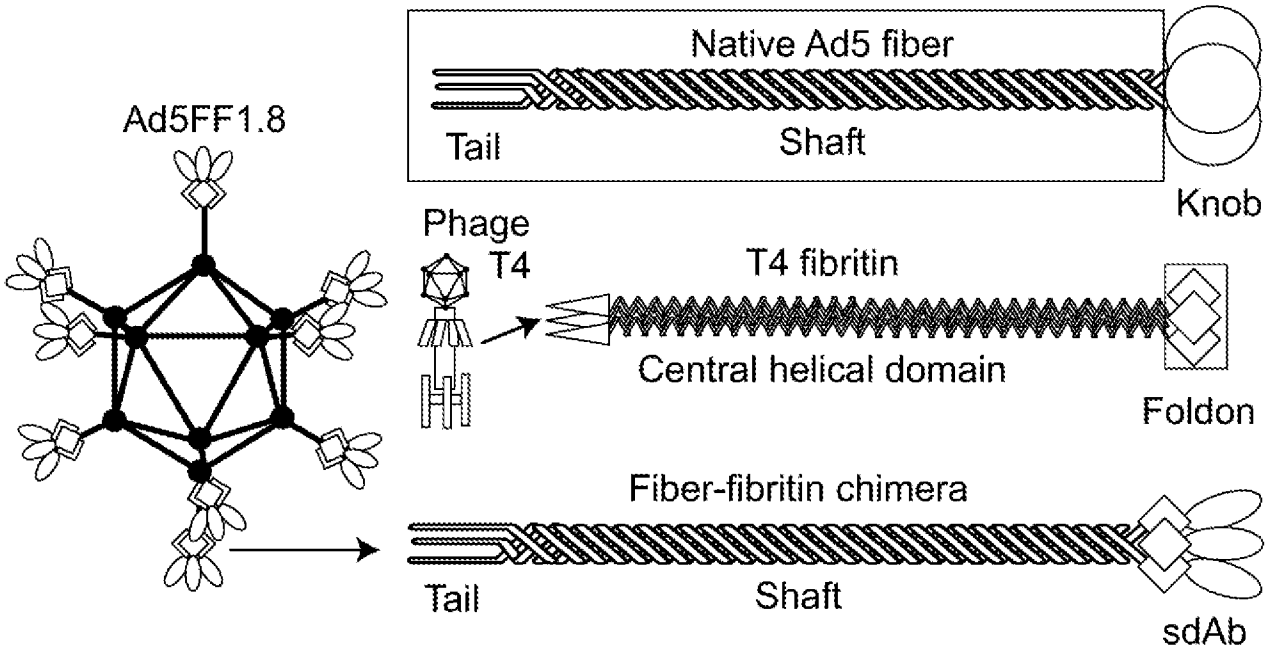

Ad5FF1.8
Native Ad5 fiber
Tail
Shaft
Knob
Phage
T4
T4 fibritin
Central helical domain
Foldon
Fiber-fibritin chimera
Tail
Shaft
sdAb

FIG. 4

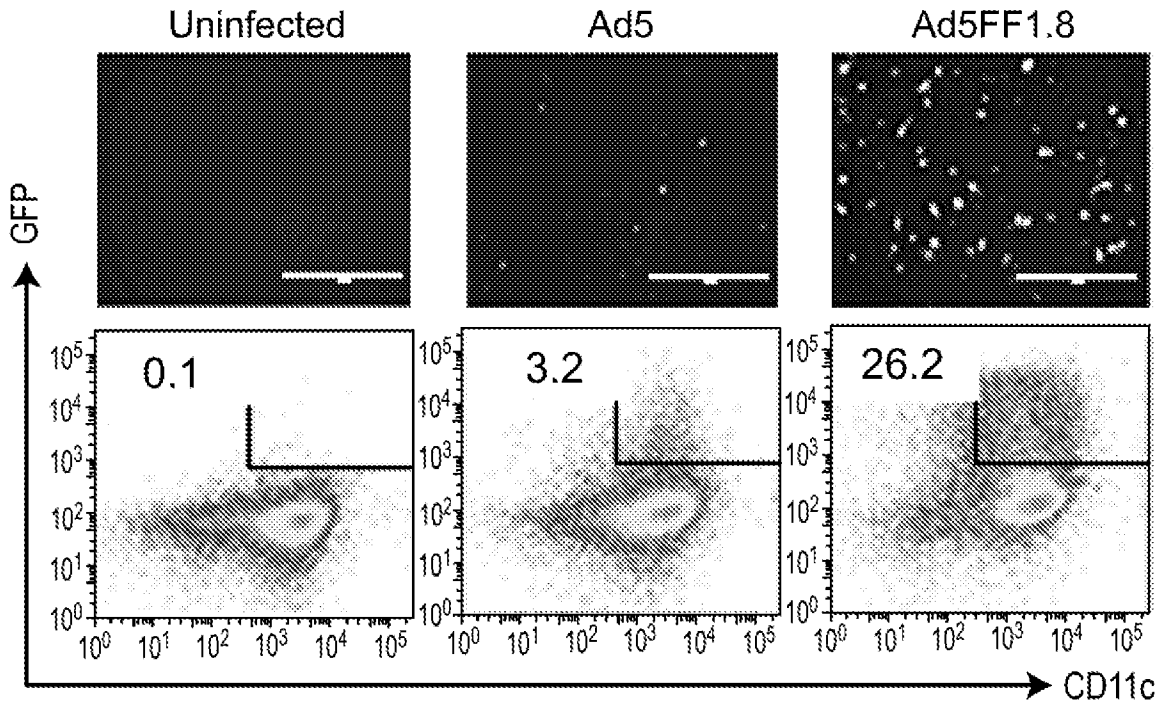

Uninfected
Ad5
Ad5FF1.8
GFP
0.1
3.2
26.2
CD11c

FIG. 5

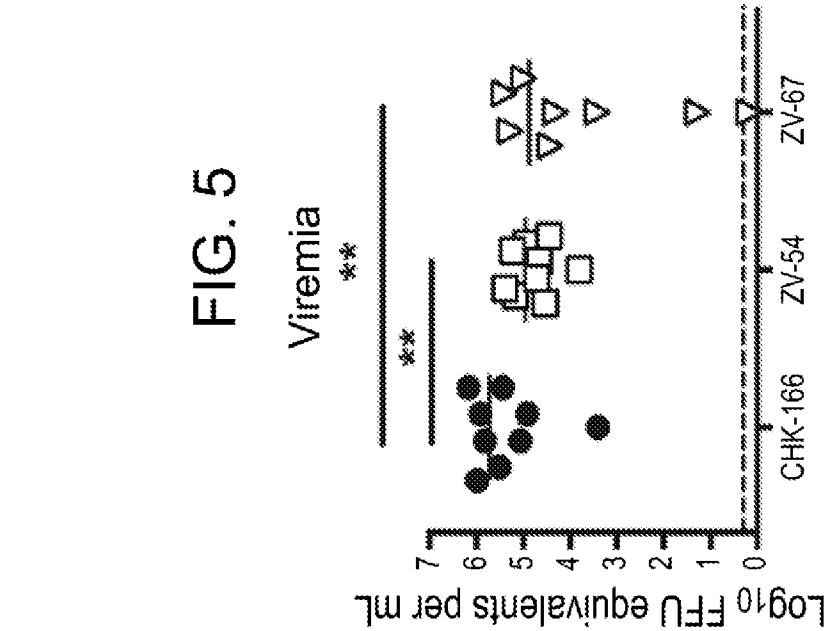


FIG. 6

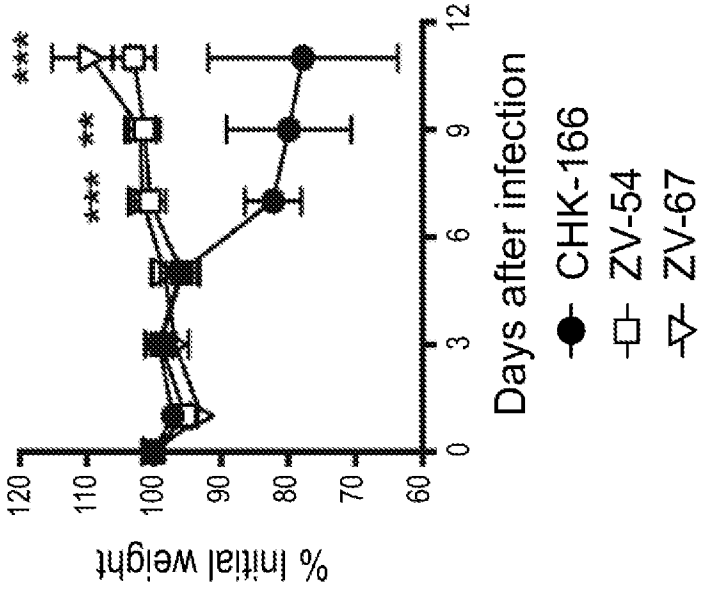


FIG. 7

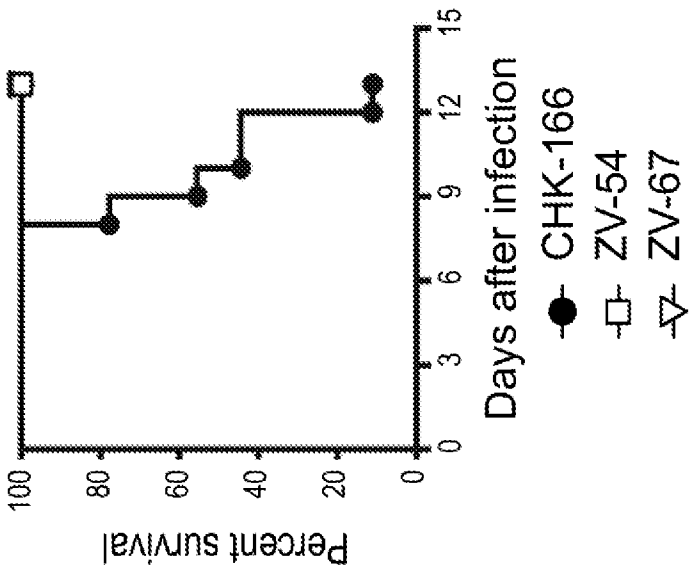


FIG. 8

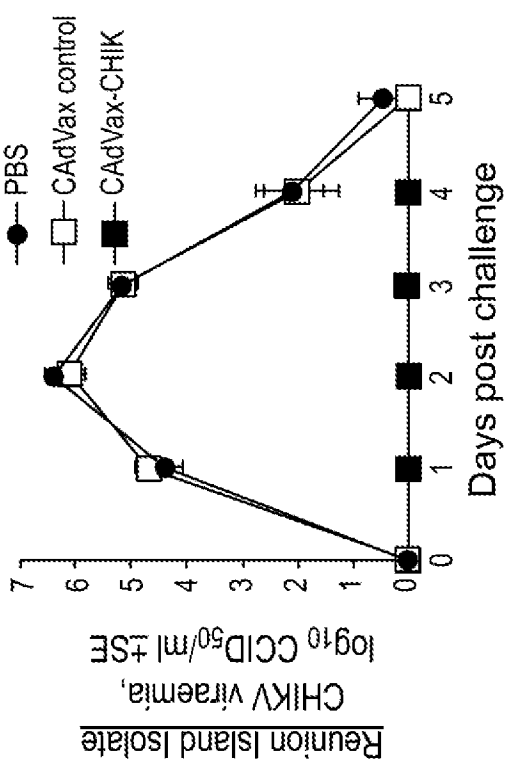


FIG. 9

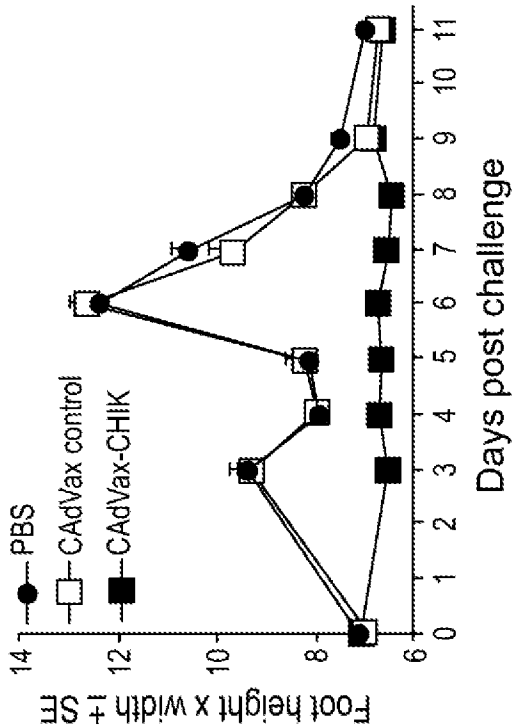


FIG. 10

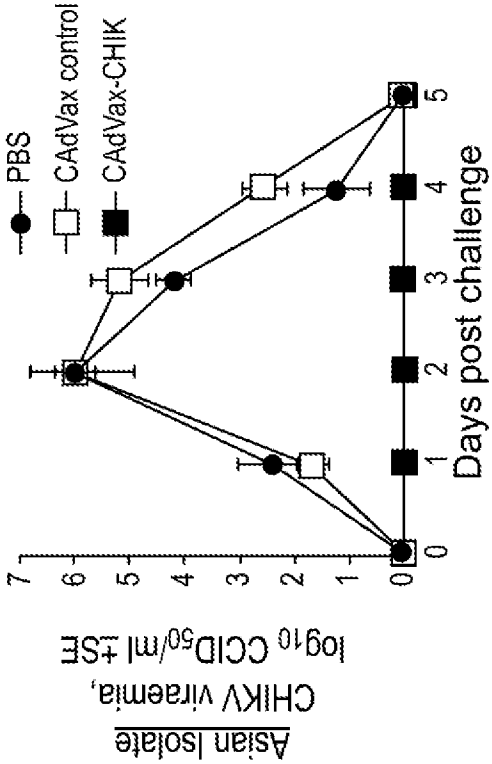
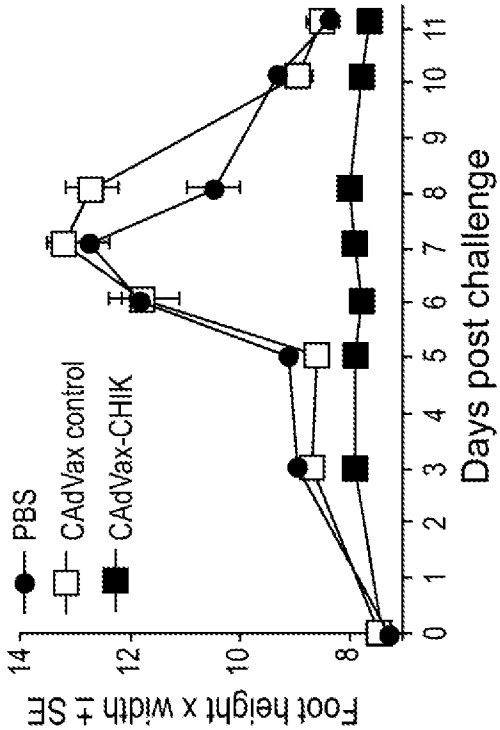


FIG. 11



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/029909

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2018.01) A61K 39/12, C12N 7/00, A61P 31/14, C12N 15/861

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (2018.01) A61K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See extra sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2006078279 A2 UNIV PENNSYLVANIA [US]; WILSON JAMES M [US] 27 Jul 2006 (2006/07/27) Abstract,page 2 lines 1-5, page 2 line 24-page 3 line 5, page 7 lines 22-24, page 18 line 16-page 19 line 2, page 26, linees 3-6 and 19-21, page 27 line 18-page 19 line 5, page 41, example 3	1-13
Y	WO 2015161314 A1 UNIV WASHINGTON [US]; CURIEL DAVID [US]; KALIBEROV SERGEY [US] 22 Oct 2015 (2015/10/22) page 4 second paragraph, paragraph 6, example 14	1-13
Y	MONATH, Thomas P. Recombinant, chimeric, live, attenuated vaccines against Flaviviruses and Alphaviruses. In: Replicating Vaccines. Springer, Basel, 2011. p. 349-438. [Online] [retrieved on 2018-07-23]. Retrieved from the Internet <URL: https://link.springer.com/chapter/10.1007/978-3-0346-0277-8_16> <DOI: 10.1007/978-3-0346-0277-8_16> 29 Sep 2010 (2010/09/29) the whole document, especially section 3.1 on page 354, page 355 second paragraph, page 388, second paragraph, page 396 third paragraph, sections 3.4.1, 3.4.2.	4-7,10,13

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 Jul 2018	29 Jul 2018
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer RON-COHEN Yael Telephone No. 972-2-5651737

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/029909

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WANG, Danher, et al. A complex adenovirus vaccine against chikungunya virus provides complete protection against viraemia and arthritis. Vaccine, 2011, 29.15: 2803-2809. [Online] [retrieved on 2018-07-22]. Retrieved from the Internet <URL: https://www.sciencedirect.com/science/article/pii/S0264410X11001800>< <doi:10.1016/j.vaccine.2011.01.108> 24 Mar 2011 (2011/03/24) abstract, the whole document	9,12
Y	ABBINK, Peter, et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. Science, 2016, aah6157. [Online] [retrieved on 2018-07-25]. Retrieved from the Internet <URL: http://science.sciencemag.org/content/early/2016/08/03/science.aah6157><DOI: 10.1126/science.aah6157> 04 Aug 2016 (2016/08/04) the whole document, especially page 1130-1132, FIGs 5 and 6)	10,11
Y	SUMATHY, K., et al. Protective efficacy of Zika vaccine in AG129 mouse model. Scientific reports, 2017, 7: 46375. [Online] [retrieved on 2018-07-25]. Retrieved from the Internet <URL: https://www.nature.com/articles/srep46375/><DOI: 10.1038/srep46375> 12 Apr 2017 (2017/04/12) the whole document, especially abstract, "Immunogenicity" and "Discussion" sections, FIG 2f	10,11
P,X	SHARMA, Piyush K., et al. Development of an adenovirus vector vaccine platform for targeting dendritic cells. Cancer gene therapy, 2017, 1. [Online] [retrieved on 2018-07-22]. Retrieved from the Internet <URL: https://www.nature.com/articles/s41417-017-0002-1><doi:10.1038/s41417-017-0002-1.> 15 Dec 2017 (2017/12/15) the whole document	1-13

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2018/029909

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
WO 2006078279 A2	27 Jul 2006	WO 2006078279 A2	27 Jul 2006
		WO 2006078279 A8	14 Sep 2006
		WO 2006078279 A3	19 Apr 2007
		AT 497785 T	15 Feb 2011
		CA 2563538 A1	27 Jul 2006
		CA 2563538 C	17 Dec 2013
		DE 602005026269 D1	24 Mar 2011
		EP 1742668 A2	17 Jan 2007
		EP 1742668 B1	09 Feb 2011
		ES 2361000 T3	13 Jun 2011
		JP 2007535550 A	06 Dec 2007
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		US 8394386 B2	12 Mar 2013
WO 2015161314 A1	22 Oct 2015	WO 2015161314 A1	22 Oct 2015
		EP 3132038 A1	22 Feb 2017
		EP 3132038 A4	29 Nov 2017
		US 2017044269 A1	16 Feb 2017

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/029909

B. FIELDS SEARCHED:

* Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: NCBI, Esp@cenet, Google Patents, CAPLUS, BIOSIS, MARPAT, PubMed, Google Scholar, DWPI
Search terms used: Vaccine, Adenovirus vector, dendritic cell- targeted, deletion E1 A/B region, Heterologous virus, alphavirus , flavivirus, structural genes, C gene, prM-E gene, E gene, Dengue virus, Zika virus, yellow fever virus, West Nil virus, Chikungunya virus, Ad5FF1.8, applicant