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Development and application of a bioluminescent imaging mouse model for Chikungunya virus based on pseudovirus system

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ABSTRACT

Chikungunya virus (CHIKV) is an arthropod-borne virus that is transmitted to humans primarily via the bite of an infected mosquito. Infection of humans by CHIKV can cause chikungunya fever which is an acute febrile illness associated with severe, often debilitating polyarthralgias. Since a re-emergence of CHIKV in 2004, the virus has spread into novel locations in nearly 40 countries including non-endemic regions and has led to millions of cases of disease throughout countries. Handling of CHIKV is restricted to the high-containment Biosafety Level 3 (BSL-3) facilities, which greatly impede the research progress of this virus. In this study, an envelope-pseudotyped virus expressing the firefly luciferase reporter protein (pHIV–CHIKV–Fluc) was generated. An *in vitro* sensitive neutralizing assay and an *in vivo* bioluminescent-imaging-based mouse infection model had been developed based on the CHIKV pseudovirus. Utilizing the platform, protection effect of DNA vaccine was evaluated. Therefore, this study provides a safe, sensitive and visualizing model for evaluating vaccines and antiviral therapies against CHIKV in low containment BSL-2 laboratories.

1. Introduction

Chikungunya virus (CHIKV) was first discovered in 1953 in Africa and circulated in Africa and Southeast Asia with periodic outbreaks for many years [1]. Currently, CHIKV is geographically distributed from Africa through Southeast Asia and South America. CHIKV is an alphavirus belonging to the *Togaviridae* family and is transmitted to human by mosquitoes. Chikungunya fever, the disease caused by CHIKV, is characterized by a polyarthralgia, high fever, headache, vomiting, myalgia and rash in most patients [2]. Although the acute febrile phase of the illness normally resolves within a few days, polyarthralgia typically persists for weeks or months causing serious economic and social impacts [3]. Since 2004, there has been an increased observed incidence rate of CHIKV infection in nearly 40 countries including non-endemic regions, such as Europe, Central America, South America, and North America [2,4–8]. Despite of its importance as a re-emerging virus and potential biological weapon, there are no licensed vaccines or specific antiviral drug and treatment for Chikungunya virus currently.

CHIKV is a positive-sense single-stranded RNA virus. The genome is approximately 11.8 kb in length and contains two open reading frames (ORFs), a 5′ cap structure and a 3′ poly A tail. One ORF encodes the non-structural proteins (NSs), and another ORF encodes the viral structural proteins: the capsid protein and envelope glycoproteins with E1, E2, E3 and 6k. E1 and E2 carry the main antigenic determinants and form an icosahedral shell at the surface of virion.

Recently, nonpathogenic, replication-defective pseudotyped viruses were widely used in research of serology, antibody standard development, vaccine immunogenicity testing and gene therapy of enveloped viruses such as Ebola virus, Middle Eastern Respiratory syndrome (MERS) virus, hepatitis C virus and rabies virus [9–13]. Pseudotyped virus provides a safe tool to study viral biology of highly pathogenic viruses in a low containment BSL-1/2 laboratory. It is known that the CHIKV glycoprotein is responsible for viral attachment, receptor binding, carrying important determinants of virulence, and entry of target cells, and it is therefore a major target of vaccine and drug design efforts [2,14]. In this study,

Abbreviations: pHIV-CHIKV-Fluc – p, pseudovirus; pHIV-CHIKV-Fluc – HIV, backbone; pHIV-CHIKV-Fluc – CHIKV, Chikungunya virus; pHIV-CHIKV-Fluc – Fluc, reporter gene; IV, intravenous; IP, intraperitoneal.

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we developed an envelope-pseudotyped virus expressing the Fluc reporter protein (pHIV–CHIKV–Fluc) using envelope-defective HIV-1 (strain SG3) that can be applied as a safe and sensitive neutralization assay to substitute wild CHIKV. It is known that bioluminescence live imaging system can provide an alternative approach in which every animal is served as its own control and the length of virus persistence in animals can be followed over the length of the experiment by detecting the signal of the firefly luciferase, which is expressed by pseudotyped viruses in mice. In this study, we established ideal animal model with these pseudovirus-infected mice and employed bioluminescence live imaging to evaluate protective effect of CHIKV DNA vaccine *in vivo*.

2. Materials and methods

2.1. Cell culture and animals

HEK293T(ATCC,CRL-3216), HEK293FT(Invitrogen, Carlsbad, CA, USA, R70007), HEK293(ATCC, CRL-1573), HepG2(ATCC, HB-8065), Vero (ATCC,CCL81), VeroE6(ATCC, CRL-1586), A549(ATCC,CCL-185), Hela(ATCC,CCL-2) and BHK21(ATCC,CCL-10) cells were grown in Dulbecco's modified Eagle's medium (HyClone, South Logan, UT) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), 1% Penicillin-Streptomycin Solution (Gibco, Carlsbad, CA, USA), 2% 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (Gibco, Carlsbad, CA, USA) at 37 °C under 5% CO_2. K-562 (ATCC, CCL-243) cells were grown in 1640 (HyClone, South Logan, UT) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin Solution.

2.2. DNA vaccine and other plasmids construction

The gene covering envelope glycoproteins and capsid protein C of CHIKV 37997 (Genebank Accession NO. AY726732, nt7569-11315) was used for synthesis, optimizing and subcloned into pcDNA3.1 vector to construct pcDNA3.1-CHIKV for packaging of pseudovirus. The same structural protein gene was also subcloned into pDRVISV1.0 vector to construct DNA vaccine (pSV1.0-CHIKV). The DNA sequences encoding the partial structural protein were amplified by a upstream primer containing a start codon and a Sal I site (5′-GCAGTCACCGTCGTCGACGCCACCATGGAGTTCATCCC TACCCAAACC-3′) and a downstream primer containing a stop codon and a EcoR V site (5′-CTAGAGCGGCCGCGATATCT CAGTGTCTGGAGAAGCTCACAC-3′). The PCR product was subcloned into pDRVISV1.0 vector to construct pSV1.0-CHIKV. The HIV framework plasmids pSG3Δenv.cmv.Fluc, pSG3Δenv.Fluc, pSG3.Fluc, pNL4-3.Luc.R-.E- were described previously [13].

2.3. Pseudotyped virus preparation

Production of CHIKV pseudovirouses in mammalian cells were similar to HIV pseudovirus described previously [15,16]. In brief, mammalian cells monolayer were cotransfected with CHIKV glycoprotein expression plasmid pcDNA3.1-CHIKV and the HIV framework plasmids pSG3.Δenv.cmv.Fluc at certain ratios using lipofectamine 3000 (Invitrogen, Carlsbad, CA) transfection reagents. Four hours post transfection, the medium was replaced with fresh medium and incubated for 48 h. The culture supernatants containing the pseudovirus were harvested, filtered (0.45 μM pore-size) and stored in 1ml aliquots at −80 °C until use. 50% tissue culture infectious dose ($TCID_{50}$) of CHIKV pseudovirus batch was determined. Briefly, 293T cells were incubated with serial dilutions of the CHIKV pseudovirus in a 96-well culture plates, and after 48 h incubation, the titer was determined using Bright-Glo luciferase reagent (Promega, Madison, WI). The $TCID_{50}$ was calculated using the Reed-Muench method. All works involving pseudotyped CHIKV were performed in a biosafety level 2 (BSL-2) facility at the National Institutes of Food and Drug control, Beijing, China.

2.4. In vitro neutralization with pseudotyped virus

For the high-throughput *in vitro* neutralization assay, pseudotyped virus (pHIV-CHIKV-Fluc) was incubated with serial dilutions serum samples from guinea pigs or mice immunized with pSV1.0-CHIKV for 1 h at 37 °C, then mixed with 293T cells in 96-well plate and subsequently incubated for 48 h. The infectivity of pHIV-CHIKV-Fluc was determined by measuring the bioluminescence as described previously in HIV pseudovirus based neutralization assay (PBNA) [16,17]. The 50% inhibition dilution (ID_{50}) was defined as the neutralization antibodies titer of the serum [18].

2.5. Animals

The mice and guinea pigs used in the study were housed and handled strictly in accordance with the guidelines set by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC, Frederick, MD). The study protocol was approved by the Animal Care and Use Committee in National Institutes for Food and Drug Control (NIFDC, Beijing, China). Guinea pigs and Four-week-old BALB/c, C57BL/6 and NIH mice were obtained from the Institute for Laboratory Animal Resources, NIFDC (Beijing, China).

2.6. Protection effect of CHIKV DNA vaccine

Groups of eight BALB/c mice with or without 10 μg recombinant murine GM-CSF (Amoytop, Xiamen, China) were immunized with 100 μg pSV1.0-CHIKV DNA vaccine [19], and followed or not followed by electroporation and the mice were immunized three times at a two-week interval, while control group (n = 8) with equal volume of phosphate buffer saline. After the last immunization, mice were then challenged with 200 AID_{50} of pHIV–CHIKV-Fluc via intravenous (IV) injection after ten days of boost. Bioluminescence data was collected at indicated time points. The value of emitted light intensities in vaccinated groups and control group was compared and used to evaluate the protective potency. Meanwhile, mouse serum samples were collected on the day before vaccination and one week after each immunization or pseudovirus challenge. Neutralizing antibodies for mouse sera were quantified by *in vitro* PBNA, and analyzed for protection effect.

2.7. Bioluminescent imaging analysis

Bioluminescent imaging (BLI) was performed with the IVIS Lumina Series III Imaging System (PerkinElmer, Baltimore, MD). Mice were anaesthetized by pelltobarbitalum natricum (75 mg/kg body weight) by intraperitoneal (IP) injection and luminescence was measured 10 min after an IP injection of the substrate, D-luciferin (75 mg/kg body weight, Xenogen-Caliper Corp, Alameda, CA). Living Image software (Caliper Life Sciences, Baltimore, MD) was used to measure the luciferase activities and the signals emitted from different ROIs in the body were measured and presented as the total flux in photons/s.

2.8. Statistical analysis

All graphs and data were generated and analysed with the GraphPad Prism 5.0 software (GraphPad, San Diego, CA). Results were presented as means and standard deviations (SD). The statistical significance comparisons were performed by nonparametric one-way ANOVA or Student *t*-test. P values of <0.05 were consid-

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