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An LASV GPC pseudotyped virus based reporter system enables evaluation of vaccines in mice under non-BSL-4 conditions

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ABSTRACT

Lassa virus (LASV) causes a severe hemorrhagic fever endemic throughout western Africa. Because of the ability to cause lethal disease in humans, limited treatment options, and potential as a bioweapon, the need for vaccines to prevent LASV epidemic is urgent. However, LASV vaccine development has been hindered by the lack of appropriate small animal models for efficacy evaluation independent of biosafety level four (BSL-4) facilities. Here we generated an LASV-glycoprotein precursor (GPC)-pseudotyped Human immunodeficiency virus containing *firefly luciferase* (Fluc) reporter gene as surrogate to develop a bioluminescent-imaging-based BALB/c mouse model for one-round infection under non-BSL-4 conditions, in which the bioluminescent intensity of Fluc was utilized as endpoint when evaluating vaccine efficacy. Electron microscopy analysis demonstrated that LASV GPC pseudotyped virus appeared structurally similar to native virion. Meanwhile, we constructed DNA vaccine (pSV1.0-LASVGPC) and pseudoparticle-based vaccine (LASVpp) that displayed conformational GPC protein of LASV strain Josiah to vaccinate BALB/c mice using intramuscular electroporation and by intraperitoneal routes, respectively. Vaccinated mice in LASVpp alone and DNA prime + LASVpp boost schedules were protected against 100 AID_{50} of LASV pseudovirus challenge, and it was found that *in vivo* efficiencies correlated with their anti-LASV neutralizing activities and MCP-1 cytokine levels in serum sampled before infection. The bioluminescence pseudovirus infection model can be useful tool for the preliminary evaluation of immunogenicity and efficacy of vaccine candidates against LASV outside of BSL-4 containments, and the results with pseudoparticle-based vaccine provided very helpful information for LASV vaccine design.

1. Introduction

Lassa virus (LASV) is the causative agent of Lassa fever (LF) endemic in West Africa for more than 40 years [1,2]. It is now recognized as a major threat to global public health for its reappearance in Nigeria in 2016 at a high fatality rate of 34%[3] and the imported cases reports from around the globe by travelers [4,5]. Currently, the only treatment available is based on ribavirin, which is beneficial in reducing mortality if given early in the course of LF, and there are no licensed vaccines for preventing LASV infection [6].

It was suggested that a vaccine that could elicit appropriate immune response would be protective against LASV infection, and there is no evidence of repeat infection in LF survivors, suggesting an effective vaccine could be developed [7]. There are four replication-competent LASV vaccines, which are based on vaccinia virus [8], vesicular stomatitis virus (VSV) [9], Mopeia virus (MOPV) [10], and yellow fever virus [11] vectors. Although potent and promising, these vaccines were widely disputed because of safety concerns [12,13]. Recent years, the increasing attention has been received on the non-replicating gene-based approaches, including a Venezuelan equine encephalitis virus replicon [14] and a virus-like particle (VLP), which preformed antigenic subunits arrayed in a fashion mimicking the surfaces of virions and can trigger antibody responses against conformational epitopes of viral proteins [15].

The challenges preventing vaccine development related to the high pathogenicity of the live LASV virus, as well as the limited animal models available. The experimental animal models with

Abbreviations: LASV, lassa virus; LF, lassa fever; GPC, glycoprotein precursor; Fluc, firefly luciferase; LASVpp, pseudoparticle-based LASV vaccine; IV, intravenous; IP, intraperitoneal; NTAb, neutralizing antibody.

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observations of serious disease are limited to the use of guinea pigs and nonhuman primates, with high costs and limited availability [16,17]. As a category A pathogen, the experiments with pathogenic LASV must be performed in a Biosafety Level 4 (BSL-4) facilities [18], that called for the development of surrogate murine models to study LASV in a BSL-2/3 environment [19]. The LASV enters into cells by the mediation of two envelope glycoproteins (GP1 and GP2) after binding to the receptor of α-dystroglycan [20,21]. The mature GP1 and GP2 are derived by posttranslational processing of glycoprotein precursor (GPC) using host enzyme subtilase SKI-1/S1P for cleavage [22]. Therefore, the human immunodeficiency virus (HIV)–based pseudotyped virus containing LASV GPC gene was constructed to develop *in vitro* high-throughput screening protocol to identify entry inhibitors for LASV [23]. However, the low titer of these viruses substantially limits their wider applications, especially *in vivo*.

In this study, we developed a highly efficient pseudovirus production system and generated an LASV-GPC-pseudovirus to develop a bioluminescent-imaging-based mouse model for vaccine efficacy evaluation under non-BSL-4 conditions. Moreover, the protection efficacies of the replication-deficient LASV GPC pseudoparticle-based vaccine in combination with or without DNA vaccine were evaluated in the developed mouse model, and the correlation between the protection and immune response during LASV vaccination was also studied.

2. Materials and methods

2.1. Cells

HEK293 (American Type Culture Collection [ATCC], CRL-1573), 293T (ATCC, CRL-3216), 293FT (Invitrogen, Carlsbad, CA, USA, R70007), Hep G2 (ATCC, HB-8065), Vero E6 (ATCC, CRL-1586), Vero (ATCC, CCL-81), A549 (ATCC, CCL-185), BHK21 (ATCC, CCL-10), Hela (ATCC, CCL-2), and MDCK (ATCC, CCL-34) were maintained in Dulbecco's modified Eagle's medium (HyClone, South Logan, UT). K562 (ATCC, CCL-243) were grown in RPMI 1640 (HyClone) supplemented with 10% fetal bovine serum [FBS] (Gibco, Carlsbad, CA, USA) and 1% penicillin–streptomycin solution (Gibco).

2.2. Pseudotyped virus

The replication-incompetent HIV pseudotyped with LASV GPC (strain *Josiah*) containing *firefly luciferase* (Fluc) was generated as previously described [24]. Briefly, the pcDNA3.1-opti-LASV-GPC and pSG3.Δenv.cmv.Fluc were co-transfected into 293T or 293FT cells using transfection reagents, including lipofectamine 2000 (Invitrogen, 11668019), lipofectamine 3000 (Invitrogen, L3000015), linear PEI (Alfa Aesar, 43896), Neofect (Neofect biotech), TruboFect (Thermo Scientific, R0531), VigoFect (Vigrous Biotechnology, T001). The culture supernatant was centrifuged at 210g for 5 min, filtered through a 0.45 μM pore-size filter, and concentrated with a 30-kDa ultrafiltration centrifugal tube (Millipore, Boston, MA, USA). pcDNA3.1-opti-LASV-GPC (nucleotide sequence 55–1530; Genbank accession no. HQ688672) was optimized for *Homo sapiens* and synthesized by GENEWIZ company (Suzhou, China).

2.3. Electron microscopy

The pHIV/LVGPC/Fluc was centrifuged at 14,000 rpm at 4 °C for 2 h and resuspended in phosphate buffer saline [PBS] (HyClone, SH30256.01B). Then, the purified LASV pseudovirus was adsorbed onto carbon-coated copper grids, stained with 0.2% phosphotungstic acid, and examined under electron microscope JEM-1400 (JEOL, Tokyo, Japan).

2.4. Western blot

The pcDNA3.1-opti-LASV-GPC plasmid transfected cells, the resuspended LASV pseudovirus and the mock cells were degenerated at 92 °C for 10 min with SDS-PAGE sample loading buffer. The membranes were incubated for 1 h with anti-GP (Lassa virus) (Immune Technology, IT-008-001), followed by incubation with HRP Goat anti-Rabbit IgG (Abcam, ab6721). The LASV GPs were visualized by ECL Western Blotting Substrate (Thermo, 32209, Waltham, MA, USA).

2.5. DNA and LASVpp vaccines

The DNA vector pDRVISV1.0 was kindly provided by Dr. Yiming Shao, Chinese Center for Disease Control and Prevention [25]. The codon-optimized GPC gene used in DNA (pDRVISV1.0-LVGPC) and LASV GPC pseudoparticle (LASVpp) vaccine construction was same as that in pseudovirus construction. Generation of non-replicative LASVpp vaccine was performed as the construction of pHIV/LVGPC/Fluc. In brief, the pcDNA3.1-opti-LASV-GPC and pSG3.Δenv were co-transfected into 293T cells using PEI (Alfa Aesar, 43896). The $TCID_{50}$ of LASVpp were detected on TZM-bl cells.

2.6. Animal experiments

Mice were handled in accordance with the institutional (National Institutes for Food and Drug Control, NIFDC, Beijing, China) guidelines for laboratory animal care and use, and the Animal Care and Use Committee at the NIFDC approved the study protocol. For the infection model construction, four-week-old BALB/c, C57BL/6, NIH, and KM mice were infected with 1.67×10^8 $TCID_{50}$ of pHIV/LVGPC/Fluc *via* intravenous (IV) route. And ten-week-old BALB/c mice were also infected via intraperitoneal (IP) route, then monitored for bioluminescent signals at different time points. As for the protection effect evaluation of LASV vaccines, four-week-old BALB/c mice were immunized with LASV DNA alone, LASVpp alone, and DNA prime/LASVpp boost vaccines at 0, 2 and 4 weeks, followed by the IP challenge of 100 AID_{50} of pHIV/LVGPC/Fluc at 6 weeks. The vaccinated group (n = 8) were IM injected with 20 μg of granulocyte-macrophage colony stimulating factor [GM-CSF] twice at 1 day interval, then inoculated with 50 μg of DNA vaccine (suspended in 100ul of PBS) delivered by electroporation [26]. The LASVpp vaccine ($(1 \times 10^8$ $TCID_{50}$ per mouse) was injected by IP route. Blood samples were collected at the day before challenge for neutralizing antibodies titration and cytokine quantitation.

2.7. In vitro neutralization assay

250 $TCID_{50}$ of pHIV/LVGPC/Fluc was incubated with serial 3-fold dilution of serum started at 10-fold dilution for 1 h at 37 °C and then mixed with HEK293T cells (5000 cells/well) in a 96-well plate and incubated for 48 h. The infectivity of pHIV/LVGPC/Fluc was determined by measuring the bioluminescence with the Bright-Glo luciferase reagent and Glomax 96 microplate luminometer (Promega, Madison, WI, USA), as described previously [27].

2.8. Bioluminescence imaging (BLI)

Bioluminescence image was acquired and analyzed with the IVIS Lumina II Imaging System (Xenogen, Baltimore, MD, USA), as described previously [28]. Briefly, mice were anaesthetized with Nembutal (100 mg/kg body weight) and inoculated with substrate

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