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Adenoviral vector-based vaccine is fully protective against lethal Lassa fever challenge in Hartley guinea pigs

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ABSTRACT

Lassa virus (LASV), the causative agent of Lassa fever (LF), was first identified in 1969. Since then, outbreaks in the endemic countries of Nigeria, Liberia, and Sierra Leone occur on an annual basis resulting in a case-fatality rate of 15–70% in hospitalized patients. There is currently no licensed vaccine and there are limited animal models to test vaccine efficacy. An estimated 37.7 million people are at risk of contracting LASV; therefore, there is an urgent need for the development of a safe, effective vaccine against LASV infection. The LF endemic countries are also inflicted with HIV, Ebola, and malaria infections. The safety in immunocompromised populations must be considered in LASV vaccine development. The novel adenovirus vector-based platform, Ad5 (E1-,E2b-) has been used in clinical trial protocols for treatment of immunocompromised individuals, has been shown to exhibit high stability, low safety risk in humans, and induces a strong cell-mediated and pro-inflammatory immune response even in the presence of pre-existing adenovirus immunity. To this nature, our lab has developed an Ad5 (E1-,E2b-) vector-based vaccine expressing the LASV-NP or LASV-GPC. We found that guinea pigs vaccinated with two doses of Ad5 (E1-,E2b-) LASV-NP and Ad5 (E1-,E2b-) LASV-GPC were protected against lethal LASV challenge. The Ad5 (E1-,E2b-) LASV-NP and LASV-GPC vaccine represents a potential vaccine candidate against LF.

1. Introduction

Lassa virus (LASV), a member of the *Arenavirdae* family, is the causative agent of Lassa fever (LF) [1]. LASV is endemic in West Africa and the hospitalized case-fatality rate ranges from 15 to 70% depending on the outbreak [2–5]. After a 2 to 21 day incubation period, LF begins with flu like symptoms before progressing to more severe symptoms such as facial edema, high fever, bleeding from mucosal and gastrointestinal tracts. Death is proceeded by shock and coma and typically occurs 14 days after the onset of symptoms [6,7]. The antiviral Ribavirin has been shown to be effective when administered early, but is not readily available in high incidence areas [8]. Approximately one-third of Lassa fever survivors develop sensorineural hearing loss which is often permanent [9]. An estimated 37.7 million people are at risk of contracting LASV, therefore, the development of a safe and effective vaccine is a crucial medical need [10].

Arenaviruses, including LASV, have bi-segmented negative single stranded RNA as their genomes. Each segment contains 2 open-reading frames of viral proteins in ambisense manner. Large (L)-segment codes RNA-dependent RNA polymerase (L) and Z protein, which plays a role of matrix protein in other enveloped-RNA viruses. Small (S)-segment codes nucleoprotein (NP), which is the most abundant protein in arenavirus infected cells, and glycoprotein precursor (GPC) [1]. GPC is cleaved into stable signal peptide (SSP), GP1 and GP2 [11,12]. GPC is the only membrane-anchored surface protein of arenaviruses and, therefore, the main target of neutralizing antibodies [13–15].

The immune response in severe and fatal cases of LF is considered immunosuppressive [16,17]. LASV primary targets are antigen-presenting cells (APCs) such as dendritic cells and macrophages. LASV infects APCs and fails to activate the cells resulting in

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a diminished T-cell response [18]. Neutralizing antibodies are not induced in fatal LF cases or survivors' until the convalescence stage [16,17,19]. Lassa fever survivors are able to produce a strong, early T cell response [20]. LASV NP or GPC-specific T-cells have been detected in LF survivors many years after infection [21–23]. Therefore, a vaccine that elicits not only antibodies but also a robust LASV-specific T-cell response is ideal.

The non-replicative adenovirus serotype 5 vector [Ad5 (E1-, E2b-)], which has deletions in the early 1 (E1), early 2b (E2b) and early 3 (E3) gene regions, has been shown to be safe and well tolerated in human clinical trials even in the presence of pre-existing immunity [24–28]. The novel Ad5 (E1-, E2b-) vector is able to infect dendritic cells resulting in the upregulation of co-stimulatory molecules and the presentation of antigens to the immune response. This ultimately leads to the induction of B-cells and T-cells against the transgene expressed protein resulting in the production of antibodies and specific-T-cell responses [29,30]. Additionally, the LF endemic countries are also inflicted with human immunodeficiency virus (HIV), human papilloma virus, tuberculosis, Ebola virus and malaria infections [16,31–34]. Safety in immunocompromised populations should be considered in LASV vaccine development. The adenovirus vector-based platform, Ad5 (E1-,E2b-) has been used in immunocompromised individuals, has been shown to exhibit high stability, low safety risk in humans, and induce a strong cell-mediated and pro-inflammatory immune responses even in the presence of pre-existing adenovirus immunity [24,30,35]. Therefore, based on the need for an effective T-cell response and the safety of the Ad5 (E1-, E2b-) vector, we have utilized this novel gene delivery Ad5 vector platform to develop a vaccine against LF [16,17,20]. Single-antigen vector vaccines expressing either the LASV GPC or LASV NP were constructed. In a lethal LF guinea pig challenge model, vaccination with both the vaccines were shown to be protective.

2. Materials and methods

2.1. Cells and viruses

Vero, Vero E6, and E.C7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, and L-glutamine. LASV strain LF2384, which was isolated from a fatal LF case during a 2012 outbreak in Sierra Leone, was propagated in Vero cells and virus-containing cell culture supernatant was stored in an −80 °C freezer until use [36,37]. All work with infectious LASV was performed in biosafety level 4 (BSL-4) facility in Galveston National Laboratory (GNL), The University of Texas Medical Branch (UTMB) in accordance with institutional guidelines.

2.2. Ad5 (E1-, E2b-) vector based vaccine expressing LASV-GPC or -NP

The Ad5 (E1-, E2b-) was generated as previously described [27–29,38,39]. The LASV NP or LASV GPC single vectored vaccines were constructed utilizing the GPC and NP gene sequence of LASV Josiah strain. Purified Ad5 (E1-, E2b-) LASV-GPC or -NP infectious unit (IU) was determined by Foci forming unit (FFU) assay on E.C7 cell monolayers. Briefly, 100 μl of 10-fold dilution of viral stock with 10% FBS DMEM was inoculated into monolayer of E.C7 cells in 24-well plates, and incubated in a CO_2 incubator for 1 h at 37 °C. After washing inoculum out, 10% FBS DMEM was added to each well. The cells were fixed with 100% methanol 40–48 hpi. The goat anti-hexon-HRP conjugated antibody (1:500) (Pierce) was incubated on cells for 1 h at 37 °C then stained using the ImmPACT DAB Peroxidase Substrate Kit (Vector) according to manufactures instructions.

2.3. Western blot

Western blot analysis was performed at previously described [40]. Briefly, E.C7 cells were transfected with the Ad5 (E1-, E2b-) GPC or NP constructs using X-tremeGene 9 (Roche). Cell lysates were prepared at 24 h post-transfection with 2x Laemmli sample buffer (BioRad) with 5% β-Mercaptoethanol then boiled at 95 °C for 5 min. The protein samples were electrophoresed by SDS-PAGE, and then transferred to PVDF membrane using Mini Trans-Blot Electrophoretic Transfer Cell apparatus according to manufactures instructions (Bio-Rad). The membranes were incubated with the anti-NP monoclonal antibody NA05-AG12 (1:1000) (BEI Resources) or anti-GP-2 monoclonal antibody (1:1000) (ProSci) overnight at 4 °C and with appropriate secondary antibodies conjugated with HRP (1:3000) (Cell Signaling) for 1 h at room temperature. Proteins were visualized with ECL-2 Western Blotting Detection Reagents (Thermo Scientific) according to the manufacturer's instruction.

2.4. Animal experiments

Five- to 7-week-old female Hartley guinea pigs were purchased from Charles River. All animals were housed in ABSL2 and ABSL4 facilities in GNL, UTMB. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee at UTMB and were carried out according to the National Institutes of Health guidelines. Measuring of body temperature and weight were performed with subcutaneously implanted BMDS IPTT-300 transponders and a DAS-6007 transponder reader (Bio Medic Data Systems). Guinea pigs were intramuscularly vaccinated with mixed adenovirus vector expressing LASV GPC or NP ($1.0x10^{10}$ IU each), or adenovirus vector expressing H1N1 subtype influenza A virus hemagglutinin as control in 100 μl of PBS at 56 and 40 days before challenge. Guinea pigs were intraperitoneally inoculated with 8.0×10^4 PFU of LASV strain LF2384 in 100 μl of PBS, and monitored daily for 21 days after inoculation. Animals were humanely euthanized once they showed neurological symptom, were not able to access their food or water, or lost more than 15% of their body weight. Blood and tissues (brain, lung, liver, spleen, and kidney) were collected for virological and pathological study.

2.5. Enzyme-linked immunosorbent assay (ELISA)

LASV GPC or NP were used for ELISA antigens. GPC or NP open reading frame of LASV Josiah strain were cloned into pCAGGS expression plasmid. The plasmids were transfected into HEK293T cells and the cells were collected at 72 h-post transfection and washed with PBS. LASV GPC was extracted by Mem-PER Plus Membrane Protein Extraction Kit (Thermo Scientific) according to manufacturer instruction. LASV NP was extracted with cell lysis buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 0.5% Triton X-100) [41]. Empty vector transfected cells were used as negative control. ELISA plates were coated with 1:100 diluted antigens at 4 °C overnight. After discarded antigens, plates were blocked with PBS containing 0.05% Tween20 and 3% skim milk for 1 h at room temperature. After washing with PBS containing 0.05% Tween20 (PBST), plates were incubated with serial diluted sera at 4 °C overnight. Plates were washed with PBST 3 times and then 1:10000 diluted Goat Anti-Guinea pig IgG H&L (HRP) (Abcam) was added and incubated 1 h at room temperature. After 3 times wash with PBST, the reaction was visualized by adding 3,3′,5,5′-tetramethylbenzidineLiquid Substrate, Supersensitive, for ELISA (Sigma) and stopped with 1 M phosphoric acid. The optical density at 450 nm (OD450) was measured and standardized with OD450 of negative control antigens. The cut-off OD value was 0.1.

2.6. Detection of neutralizing antibody

Neutralizing antibody was detected by plaque reduction neutralizing test. LASV was diluted with 2% FBS DMEM to yield 80 PFU, and mixed with serial-diluted heat-inactivated serum. After 30 min incubation in 37 °C, mixture was inoculated into monolayer of Vero cells in 12-well plates, and incubated in a CO_2 for 30 min at 37 °C. After washing inoculum out, MEM with 0.6% Tragacanth (Sigma) and 2% FBS was added as overlay. After incubation for 5–6 days, cells were fixed with 10% formalin and plaques were visualized by crystal violet staining. Antibody titer was presented as 50% plaque reduction titer ($PRNT_{50}$).

2.7. Virus titration

Tissue samples were homogenized by a TissueLyser system (Qiagen) to yield 10% homogenate in Phosphate buffered saline (PBS). Blood was collected in EDTA tubes. Plaque assay was performed to detect virus from tissue homogenates and whole blood samples. Briefly, 100 μl of 10-fold diluted samples with 2% FBS DMEM was inoculated into monolayer of Vero E6 cells in 12-well plates, and incubated in a CO_2 incubator for 30 min at 37 °C. After washing inoculum out, MEM with 0.6% Tragacanth (Sigma) and 2% FBS was added as overlay. After incubation for 5–6 days, cells were fixed with 10% formalin and plaques were visualized by crystal violet staining.

2.8. Histology

Tissues were collected at the time of euthanasia and fixed in 10% buffered formalin for at least 21 days. The tissues were then trimmed and embedded in paraffin. Thin sections (5.0 μM) of the brain, liver, lung, spleen, and kidney were stained with hematoxylin and eosin.

2.9. Statistical analysis

Statistical analyses were performed with GraphPad Prism Software. The geometric mean of neutralizing antibody titers and statistically significant differences in the mean weight, mean temperature, ELISA data between groups of animals were determined by Student's *t*-test (*: $p < 0.05$). Log-rank (Mantel-Cox) test was used for survival curve comparison.

3. Results

3.1. Ad5 (E1-, E2b-) vector based vaccine construction and target protein expression

The novel Ad5 (E1-, E2b-) platform has been constructed using various transgenes and tested in clinical trials of patients having colorectal cancer, prostate cancer, HER2 positive breast cancer and has been shown to be absent of Serious Adverse Events (SAE). The Ad5 (E1-, E2b-) carrying various transgenes has been tested in animals for protection against HIV, influenza A virus infection [24,26,27,30,38] and HPV infection (unpublished). The deletion in the structural E1 gene and E2b gene render the vector non-replicative and deletions in the E3 gene allow the vector to be effective even in the presence of pre-existing Ad5 immunity [25,29]. The LASV GPC or LASV NP were inserted into the Ad5 (E1-, E2b-) vector based platform (Fig. 1A). The product was then amplified in E.C7 cells, (HEK293 cells) which constitutively express

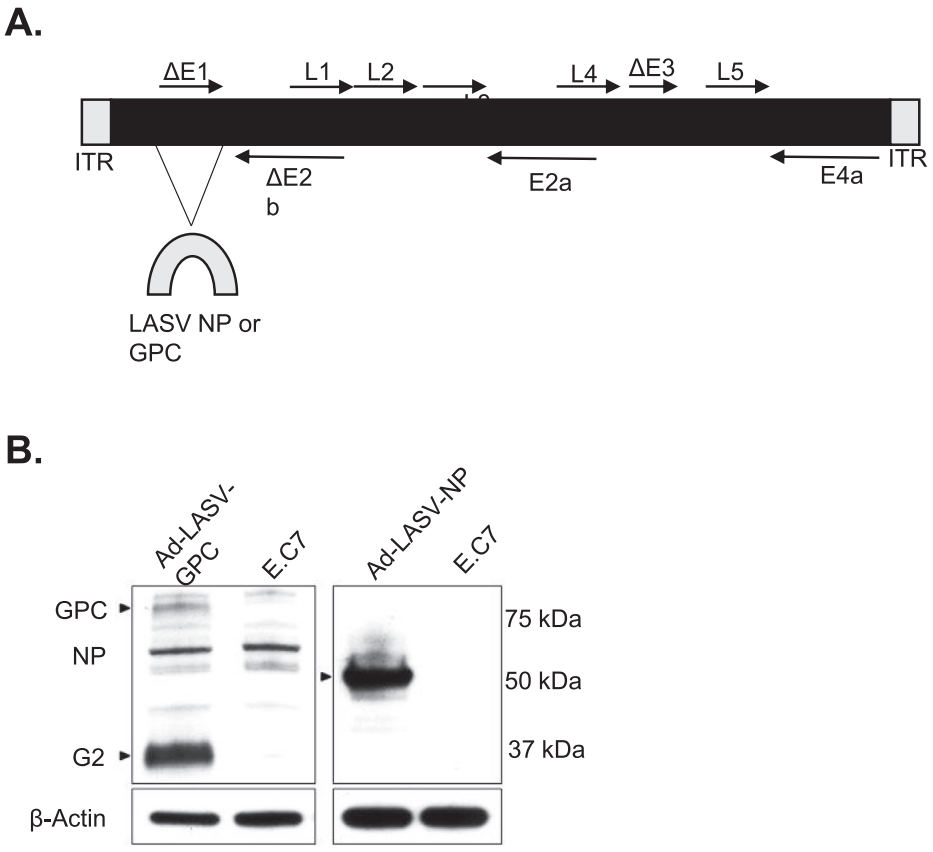

Fig. 1. Schematic of Ad5 (E1-,E2b-) expressing LASV GPC or NP. (A) Schematic of the adenovirus genome with mutations in the E1, E3 and E2b domains. (B) Western blotting of GPC and NP.

the Ad polymerase and preterminal protein, before undergoing concentration and purification [27–29,38,39]. The protein expression of LASV GPC or NP in E.C7 cells transfected with Ad5 (E1-, E2b-) LASV-GPC or -NP vector were confirmed via western blot (Fig. 1B).

3.2. Vaccination against LASV sing the Ad5 (E1-, E2b-) vaccines

The Ad5 (E1-, E2b-) single vector vaccines expressing LASV NP or LASV GPC were tested in a lethal guinea pig challenge model to determine their efficacy and immunogenicity. According to our experimental design (Fig. 2), guinea pigs were immunized twice on days −56 and day −46 with 1×10^{10} infectious units (IU) of both the single vectored Ad5 (E1-, E2b-) LASV-GPV and -NP vaccines (n = 8). As a control, the mock vaccinated group were immunized on day −56 and day −40 with $1x10^{10}$ IU of Ad5 (E1-, E2b-)-H1-HA (n = 6). None of the guinea pigs showed clinical symptoms nor abnormal change of body temperature or weight after vaccination (Fig. 3A and 3B), although transient weight and temperature changes in first 24–72 h after vaccination were not monitored because the data was not available. There are no significant differences between groups at any time points in body or temperature changes.

3.3. Antibody response after vaccination

To determine the antibody level after vaccination, enzyme-linked immunosorbent assay (ELISA) against the LASV GP and the LASV NP was performed using sera collected at day −6. All guinea pigs vaccinated with Ad5 (E1-, E2b-) LASV GPC or -NC exhibited antibodies to the GP (Fig. 4A) and NP (Fig. 4B); whereas, none of the mock-vaccinated group expressed antibodies against LASV. ELISA titer of sera from vaccinated animals against LASV GPC and NP were 1:6400 to 1:102400 and 1:25600 to 1:102400, respectively (Fig. 4C).

3.4. Protection effect of vaccination against LASV challenge

Vaccinated guinea pigs were inoculated with a lethal dose (over 5000 LD_{50}) of the fatal LASV clinical isolate LF2834 [37,42]. All guinea pigs vaccinated with Ad5 (E1-, E2b-) LASV-GPV and -NP survived the challenge and did not exhibit any signs of disease (Fig. 5A). The Ad5 (E1-, E2b-) H1-HA vaccinated animals became febrile, more than 40 °C, at 8 days post inoculation (d.p.i.) (Fig. 5B) and began losing weight at 6 d.p.i. (Fig. 5C). The guinea pigs began developing classic disease symptoms such as lethargy and loss of appetite at 11 d.p.i. Prior to death, guinea pigs developed hypothermia and hind leg paralysis. All animals succumbed to disease at 13–17 d.p.i. (Fig. 5A). The Ad5 (E1-, E2b-) LASV-GPV and -NP vaccinated guinea pig temperature remained steady throughout the course of the study (Fig. 5B) and continued to gain weight after challenge (Fig. 5C).

3.5. Viral load in LASV-challenged guinea pigs

To assess the dissemination of LASV to organs and viremia after challenge, brain, lung, liver, spleen, kidney, and blood samples were collected from all the Ad5 (E1-,E2b-) LASV-GPC and -NP vaccinated and 4/6 Ad5 (E1-,E2b-) H1-HA vaccinated animals at euthanasia. All guinea pigs vaccinated with Ad5 (E1-, E2b-) LASV-GPC and -NP were euthanized at the end of study (day + 21) (Fig. 2), and 4/6 guinea pigs vaccinated with Ad5 (E1-, E2b-) H1-HA were euthanized at day + 13 and + 15 after reaching the humane endpoint criteria (Fig. 5A). LASV was readily detected in the spleen and the lung with the highest viral titers in the lung in all Ad5 (E1-, E2b-) H1-HA inoculated animals tested. LASV was also detected in the liver and kidney of 3/4 animals tested and the brain (Fig. 6A) and blood of 2/4 guinea pigs Ad5 (E1-, E2b-) H1-HA inoculated animals tested (Fig. 6B). No LASV was detectable in the brain, lung, liver, spleen, kidney or blood samples of the Ad5 (E1-, E2b-) LASV- GPC and -NP vaccinated guinea pigs (Fig. 6A and

Fig. 2. Schematic of experimental design. Schematic of immunization, challenge, and bleeding schedule.

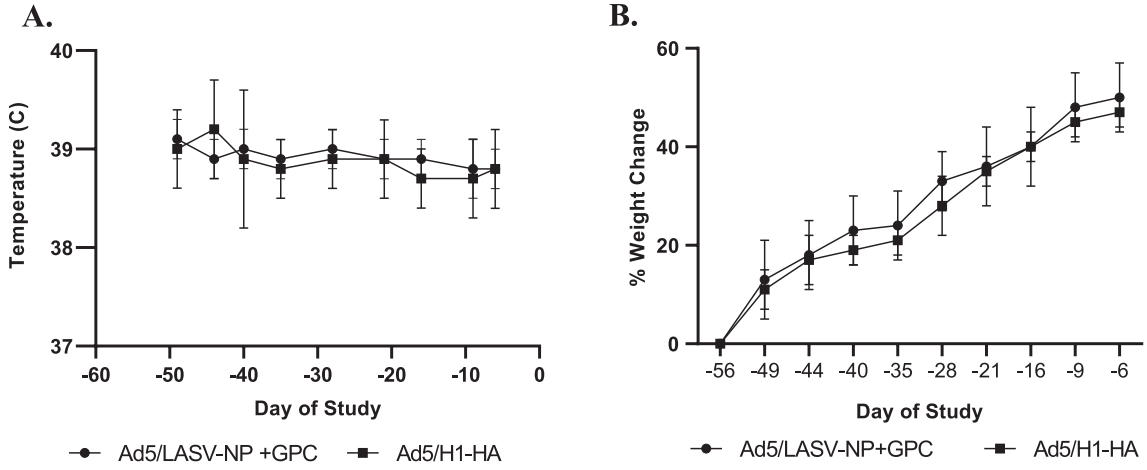

Fig. 3. Average temperature and weight change after vaccination. (A) Average temperature and (B) weight parameters after vaccination and prior to LASV challenge. There are no significant differences between groups at any time points in body weight or temperature changes.

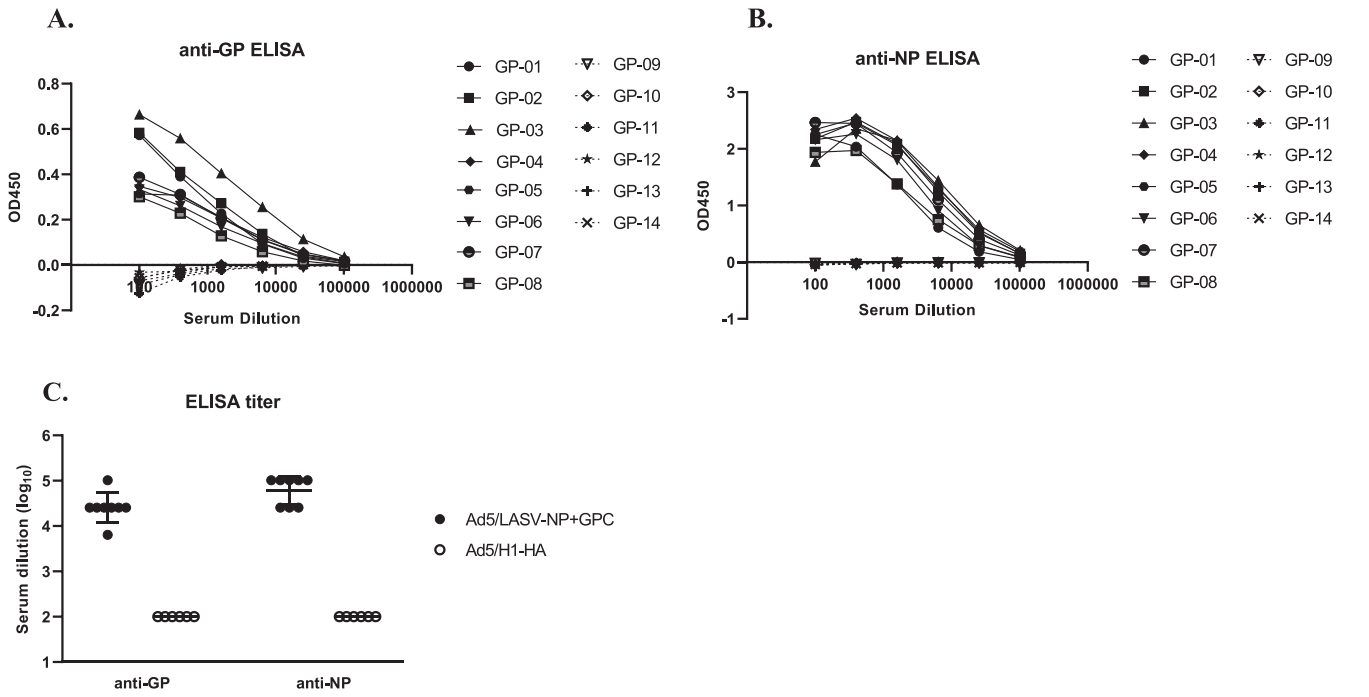

Fig. 4. Non-neutralizing antibody response after vaccination. The presences of (A) anti-GP, (B) anti-NP antibodies or (C) antibody titers were measured via ELISA from serum collected at Day -6. Statistical analysis between vaccinated and mock group was performed with student's *t*-test ($p < 0.05$). (A and B) Solid lines: Ad5 (E1-, E2b-) LASV-GPC and -NP. Broken lines: Ad5 (E1-, E2b-)-H1-HA. (C) Broken line indicated detection limit (1:<1 0 0).

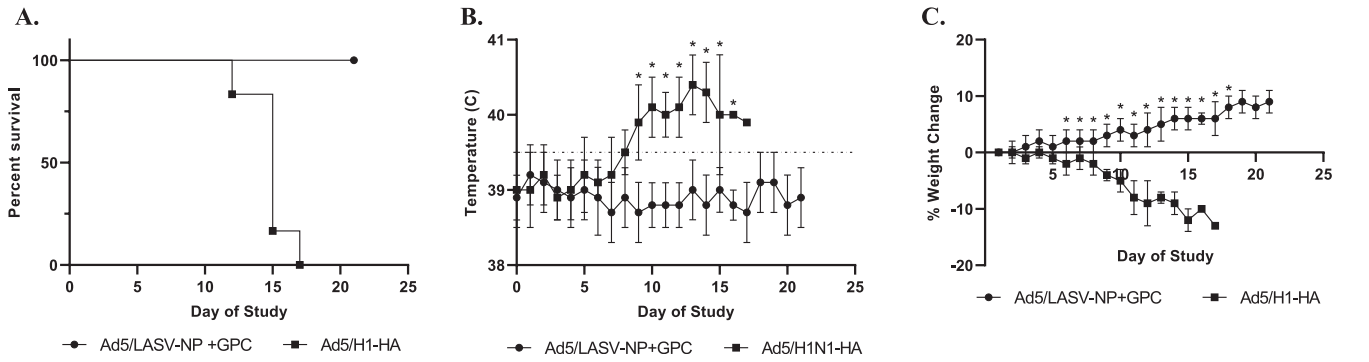

Fig. 5. Dynamics of weight and temperature change after lethal LF challenge. (A) Survival curve of guinea pigs following two doses of Ad5 (E1-,E2b-) LASV GPC and-NP vaccination and lethal LF challenge. (B) The average temperature after challenge. The dotted line represents the limit of normal guinea pig temperature range (*: $p < 0.05$). (C) The average weight change after challenge (*: $p < 0.05$). Survival curve was significantly different by Log-rank (Mantel-Cox) ($p < 0.001$).

B). Histology samples were taken from the liver, lung, kidney, brain and spleen. In the liver, lung, kidney, and brain no dramatic differences were observed between them. In the spleen, a depletion of neutrophils was observed in the red pulp of Ad5 (E1-,E2b-) H1-HA compared to Ad5 (E1-, E2b-) LASV- GPC and -NP vaccinated guinea pigs.

3.6. Neutralizing antibody against LASV in vaccinated guinea pigs

To detect neutralizing antibody, serum samples were collected at pre-vaccination (day-56), post-vaccination (day-6), and the time of euthanasia (Final). As expected, no guinea pigs in the Ad5 (E1-, E2b-) H1-HA vaccinated group exhibited neutralizing antibodies to LASV at day -6 (Table 1). In the Ad5 (E1-, E2b-) LASV-GPC and -NP vaccinated group, however, 2/8 guinea pigs had a $PRNT_{50}$ at 1:10 dilution and one at 1:20 (Table 1). At the time of euthanasia, one guinea tested in the mock vaccine group had a $PRNT_{50}$ of 1:10 dilutions (Table 1). All guinea pigs expressed high neutralizing antibody titers at euthanasia (1:20 to 1:160) with a geometric mean of 1:61.6 (Table 1).

4. Discussion

Lassa virus can cause severe hemorrhagic fever illness with a high case fatality rate [3,4]. Approximately one-third of survivors develop sensorineural hearing loss, leading to an impact to their quality of life; thus, an effective and safe vaccine is of high medical need [9]. To this end, we have developed a LASV vaccine utilizing the novel and clinically tested Ad5 (E1-, E2b-) vector. The development of Ad5 vector vaccines has been hindered due to pre-existing immunity to Ad5. To subvert this, a novel Ad5 vector platform with deletions in the E1, E2, and E3 genes has been developed and shown to induce both an antibody and cell-mediated immune response even in the presence of pre-existing immunity [25,29,30]. Vaccines have been developed using this platform

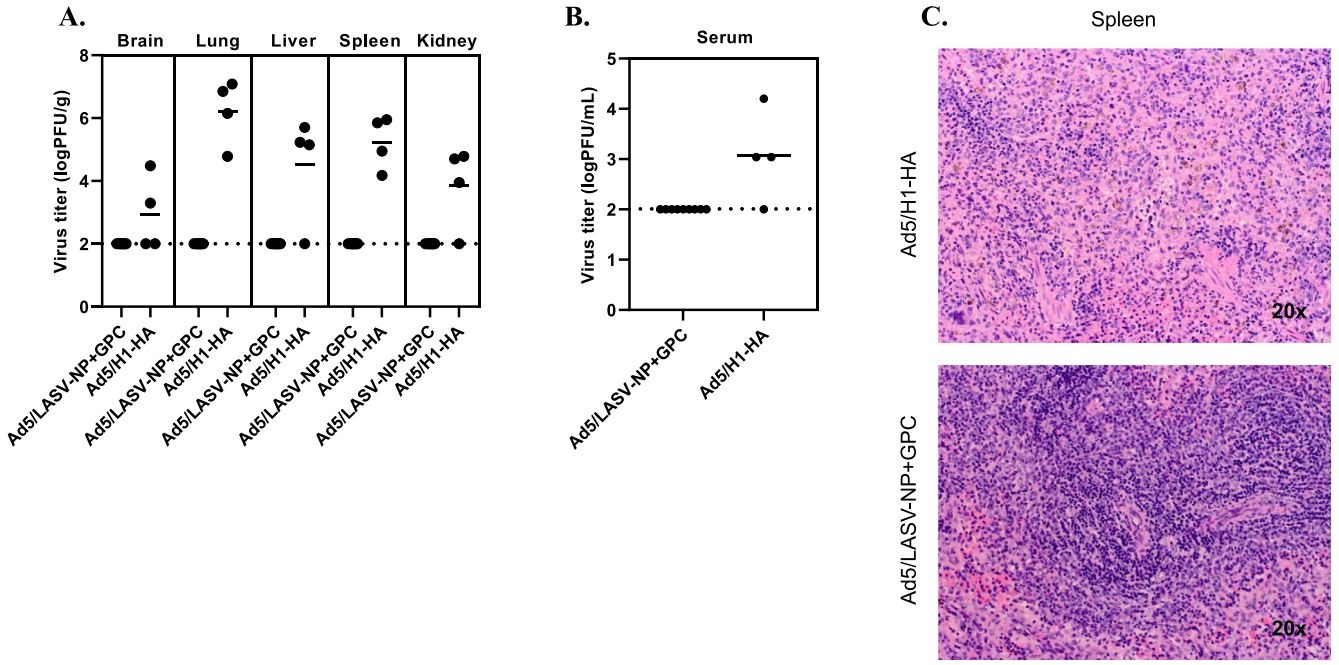

Fig. 6. Efficacy of Ad5(E1-,E2b-) LASV-GPC and -NP vaccine. (A) Organs or (B) blood were harvested at the time of euthanasia and titrated. Guinea pigs vaccinated with Ad5 (E1-, E2b-) LASV-GPC and -NP were euthanized at day + 21. Guinea pigs vaccinated with Ad5 (E1-, E2b-) H1-HA were euthanized at day + 13 or + 15. (C) H&E staining of the spleen at the time of euthanasia. Representative images shown.

Table 1
$PRNT_{50}$ titer against LASV.

Vaccine	Animal ID	$PRNT_{50}$		
		day-56	day-6	Final
Ad5 (E1-, E2b-) LASV-GPC + NP	GP01	UD	UD	1:160
	GP02	UD	1:10	1:80
	GP03	UD	UD	1:160
	GP04	UD	UD	1:80
	GP05	UD	1:10	1:20
	GP06	UD	UD	1:160
	GP07	UD	1:10	1:20
	GP08	UD	UD	1:20
Ad5 (E1-, E2b-) H1-HA	GP09	UD	UD	NT
	GP10	UD	UD	<1:10
	GP11	UD	UD	<1:10
	GP12	UD	UD	NT
	GP13	UD	UD	1:10
	GP14	UD	UD	NT

UD: under detection limit (1:<10).
NT: not tested.

against infectious diseases caused by HIV-1 and influenza A virus, and immunotherapies for antigenic cancer targets [26,38,39,43].

As expected, all guinea pigs vaccinated herein with Ad5 (E1-, E2b-) LASV-GPC and -NP successfully produced antibodies against LASV GPC and NP after 2 doses (Fig. 3). However, only 37.5% of them had neutralizing antibodies although all the vaccinated guinea pigs were protected against lethal LASV challenge. This result indicates that neutralizing antibody existence before LASV infection is not essential for protection. Recently, Abreu-Mota et al. found that non-neutralizing antibodies against LASV GPC provide protection against LASV infection [44]. It is hypothesized that antibodies inducing antibody-dependent cellular cytotoxicity may play an important role for protection. Additionally, all survivors had neutralizing titer in their sera (Table 1). It is indicated that the 2-dose vaccination schedule induces an early neutralizing antibody response, leading to the elimination of LASV before development of symptoms.

An early, strong T-cell response has been shown to contribute to LF survival [19,20,45]. Antibodies to LASV are not induced during infection, even in survivors, until late in the covalence stage [23]. Vaccines that only induce an antibody-mediated response have been reported to lack protective against LF challenge [16]. Previous research has shown that the Ad5 (E1-, E2b-) vector elicits a robust, protective cell and antibody-mediated response following immunization [29,30]. The full characterization of a T-cell mediated response induced by the Ad5 (E1-,E2b-) single vector vaccines expressing LASV NP or LASV GPC are under investigation.

There are currently no vaccines under clinical development against LF; however, several vaccine candidates have been tested preclinically. Recombinant viral vector vaccines with mainly the GP have been successful in animal testing using the vesicular stomatitis virus (VSV), yellow fever 17D, Rabies, and Vaccinia (Lister and NYBH) [44,46]. The most promising and well characterized Lassa fever vaccine candidates are ML-29 and the VSV-LASV GPC. ML-

29 is a reassortment of the pathogenic LASV and non-pathogenic Arenavirus Mopeia virus (MOPV). This live attenuated vaccine candidate consists of the LASV S segment, which encodes the NP and GPC, and the MOPV L segment, encoding the Z protein and L protein. The current Ad5 platform has several advantages over the ML-29 and VSV-LASV GPC vaccine candidates [47]. Ad5 (E1-, E2b-) vector vaccines have been used safely in human clinical trials [21,27]. The vaccines are manufactured in a human E,C7 cell line at high dose levels.. Also, the Ad5 platform used here is non-replicating and can be used in immunocompromised individuals without concern of Ad5 infection [24,30]. This is important for the endemic region because there are a high number of HIV and other immunocompromised co-infections [31,32,34].

The Ad5 (E1-, E2b-) single vector vaccines expressing LASV NP or LASV GPC represent a promising vaccine candidate against Lassa fever. Further research will be needed to understand if the vaccine is fully protective with one dose, or vaccination target is only NP or GPC. More research is needed to be done to understand the cell-mediated immune response induced by the Ad5 (E1-, E2b-) single vector vaccines expressing LASV NP or LASV GPC to understand the correlates of protection. Non-the-less we believe that this LF vaccine should be tested in human clinical trials.

Author contributions

J.M. and E.J.M designed the study, performed in vivo and in vitro experiments, analyzed results, and in manuscript preparation. J.T. M. and R.S. assisted in in vivo experiments. A.V.S designed the vector. N.B. assisted in in vitro experiments. C.H. analyzed results and assisted in manuscript preparation. S.P. conceptualized the study, analyzed results and assisted in manuscript preparation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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