An Inactivated Rabies-based Lassa Fever Virus Vaccine Candidate, LASSARAB, Protects Nonhuman Primates from Lethal Disease

Matthias Schnell

matthias.schnell@jefferson.edu

Thomas Jefferson University  https://orcid.org/0000-0001-9040-9405

Gabrielle Scher

Thomas Jefferson University  https://orcid.org/0000-0002-7571-4791

Catherine Yankowski

Thomas Jefferson University

Drishya Kurup

Thomas Jefferson University  https://orcid.org/0000-0002-3959-7700

Nicole Joselyn

United States Army Medical Research Institute of Infectious Diseases

Eric Wilkinson

United States Army Medical Research Institute of Infectious Diseases

Jay Wells

United States Army Medical Research Institute of Infectious Diseases

Jesse Steffens

United States Army Medical Research Institute of Infectious Diseases

Ginger Lynn

United States Army Medical Research Institute of Infectious Diseases

Sean Vantongeren

United States Army Medical Research Institute of Infectious Diseases

Xiankun (Kevin) Zeng

USAMRIID  https://orcid.org/0000-0003-3526-8755

Nancy Twenhafel

United States Army Medical Research Institute of Infectious Diseases

Kathleen Cashman

United States Army Medical Research Institute of Infectious Diseases
Abstract

Lassa fever virus (LASV), a member of the Arenavirus family, is the etiological agent of Lassa fever, a severe hemorrhagic disease that causes considerable morbidity and mortality in the endemic areas of West Africa. LASV is a rodent-borne CDC Tier One biological threat agent and is on the World Health Organization’s (WHO) Priority Pathogen list. Currently, no FDA-licensed vaccines or specific therapeutics are available. Here, we describe the efficacy of a deactivated rabies virus (RABV)-based vaccine encoding the glycoprotein precursor (GPC) of LASV (LASSARAB). Nonhuman primates (NHPs) were administered a two-dose regimen of LASSARAB or an irrelevant RABV-based vaccine to serve as a negative control. NHPs immunized with LASSARAB developed strong humoral responses to LASV-GPC. Upon challenge, NHPs vaccinated with LASSARAB survived to the study endpoint, whereas NHPs in the control group did not. This study demonstrates that LASSARAB is a worthy candidate for continued development.

INTRODUCTION

Lassa fever virus (LASV) is one of many emerging biosafety level-4 (BSL-4) hemorrhagic viruses, for which no approved vaccine exists. LASV is endemic to West Africa\(^1\), where it is maintained by its rodent reservoir, *Mastomys natalensis*\(^2,3\). The virus is most frequently transmitted to humans when they come into proximity with infected rodents\(^3,4\). However, human-to-human transmission can occur, most often in nosocomial settings upon contact with contaminated bodily fluids\(^5,6\). It is estimated that between 300,000-500,000 people are infected with LASV annually\(^1\), with an overall case fatality rate (CFR) of 1–2\(^%\)\(^2\). In contrast, the CFR increases significantly for hospitalized patients, with one study reporting a 69% CFR in Sierra Leone\(^7\). The disease caused by LASV infection, Lassa fever (LF), is similar to other viral hemorrhagic fevers, starting with flu-like symptoms, such as fever, sore throat, and headache, and in severe cases, progressing to vascular leakage and multiple organ failure\(^8\). While many patients survive the disease, some develop severe sequela, with a third of patients developing sensorineural hearing loss that is permanent in some cases\(^9\). Given the severity of LF and lack of approved vaccines, LASV is categorized as a high priority pathogen by various United States (US) government agencies, the World Health Organization (WHO), and the Coalition for Epidemic Preparedness Innovations (CEPI).

LASV is an arenavirus, and thus has a bi-segmented, ambisense RNA genome that codes for four proteins. One of these proteins, the glycoprotein precursor (GPC), is proteolytically cleaved by a host protease into two glycoproteins (GP1 and GP2), which are present on the surface of the virion and used for attachment and entry into cells\(^10\). Given the easy accessibility of the glycoproteins to the immune system and its indispensable function in the LASV lifecycle, the GPC gene is an attractive target for LASV vaccine development. In fact, neutralizing antibodies targeting the glycoproteins were shown to protect both guinea pigs and non-human primates (NHPs) from lethal LASV challenge\(^11,12\). Additionally, several vaccine candidates targeting GPC were protective in various LASV challenge models\(^13–22\). However, many of these vaccine strategies have disadvantages. DNA vaccines have been successful in protecting...
guinea pigs and NHPs from lethal infection and have advanced to human clinical trials, but they are poorly immunogenic without the use of specialty delivery techniques, such as electroporation\textsuperscript{14,23–26}. RNA-based vaccines such as alphavirus RNA replicon vaccines require cold-chain storage. Live viral vectors have the potential to develop mutations, rendering them pathogenic, and are typically not suitable for immunization of vulnerable populations including immunocompromised individuals and pregnant patients. Thus, a need for the development of alternative vaccine strategies that mitigate these issues remains.

Rabies virus (RABV) is a promising vaccine platform that has advantages over the vaccine platforms described above. RABV has been successfully used as a vaccine platform for various pathogens\textsuperscript{27}, including LASV\textsuperscript{28} and a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine, CORAVAX™, that has been tested in a phase I human clinical trial\textsuperscript{29}. The rabies vaccine is administered as an inactivated vaccine, has a well-established safety profile, and provides long-term protective immune responses to the rabies antigens\textsuperscript{30}. RABV shares endemic regions with many pathogens, including LASV, which greatly increases the impact this bivalent vaccine could have in the affected areas. An inactivated RABV vectored vaccine can be lyophilized and remains stable when stored at various temperatures, including at 50˚C for up to two weeks\textsuperscript{31}. Therefore, the RABV platform is an excellent choice for a LASV vaccine.

The correlates of protection for LASV have not yet been defined. Studies investigating the mechanism of protection of various vaccines have produced different results. Both a live Mopiea-Lassa reassortment virus (ML29) and live recombinant vaccinia virus expressing LASV-GPC elicited robust cellular responses without humoral responses and were protective in a challenge model\textsuperscript{17,32}. In contrast, another protective live vaccine platform, recombinant vesicular stomatitis virus with its glycoprotein replaced with LASV-GPC (VSVDG/LVGPC), was shown to induce both cellular and humoral responses\textsuperscript{22}. Finally, we previously developed a chemically inactivated RABV-based vaccine expressing all the RABV viral proteins and the LASV-GPC (LASSARAB) that was shown to protect mice against challenge with a VSV-based surrogate virus for LASV through Fcg-receptor mediated functions of antibodies (i.e., antibody-dependent cellular cytotoxicity [ADCC] or phagocytosis [ADCP])\textsuperscript{28}. Thus, it seems that vaccine-mediated protection against LASV can occur through various mechanisms. While different platforms appear to elicit protection through diverse mechanisms, one commonality between the platforms is a poor neutralizing antibody response after vaccination\textsuperscript{17,22,28,32}. This is not surprising given the absence of neutralizing antibodies seen in many convalescent LASV patients\textsuperscript{33}.

We previously demonstrated that when LASSARAB was administered to NHPs, it elicited strong antibody responses to both LASV-GPC and RABV glycoprotein (G) for up to a year post immunization\textsuperscript{34}. In the current study, our goal was to test the vaccine efficacy of LASSARAB in a lethal LASV NHP challenge model. To this end, we immunized NHPs with LASSARAB or CORAVAX™ (an irrelevant RABV-based vaccine) as a negative control and challenged the NHPs at day 70 postimmunization with LASV. All the LASSARAB immunized NHPs survived the LASV challenge, while controls developed severe clinical
symptoms and reached euthanasia criteria before the end of the study. These results indicate that LASSARAB is a good candidate for LASV vaccine clinical trials.

RESULTS

LASSARAB induces a strong humoral response before challenge

Twelve cynomolgus macaques were used in this study: six (two males and four females) immunized with LASSARAB and six (four males and two females) immunized with CORAVAX™, a rabies-vectored SARS-CoV-2 vaccine (Fig. 1). Three of the NHPs were previously immunized with the RABV vaccine before the start of this study, and thus were placed into the CORAVAX™ group. The remaining NHPs were randomly assigned into the vaccine groups. NHPs were administered 150 µg of each vaccine, adjuvanted with 15 µg of Monophosphoryl lipid A, 3D(6A)-PHAD, in a 2% stable emulsion (PHAD-SE). Immunizations were given on days 0 and 28 as outlined in Fig. 1b & c. The antibody responses were first measured against LASV-GPC and RABV-G. As seen previously, antibody responses against both antigens could be seen starting on day 14 postimmunization, with a peak titer at day 42 postimmunization for both LASV-GPC and RABV-G (Fig. 2). Only NHPs receiving LASSARAB developed antibody responses against LASV-GPC, while both groups of NHPs developed binding antibody responses against RABV-G as demonstrated through both 50% effective concentration (EC₅₀) antibody titers (Fig. 2a) and antibody endpoint titers (Fig. 2b).

In our previous studies, we adjuvanted LASSARAB with glucopyranosyl lipid A in SE (GLA-SE), which is a similar adjuvant to PHAD-SE but is not commercially available. To determine whether these two adjuvants could be used interchangeably with LASSARAB, we performed an immunogenicity experiment in mice (Figure S1). Groups of ten mice were immunized with either LASSARAB or FILORAB1, a rabies-based Ebola virus vaccine, and five mice from each group received GLA-SE adjuvant, with the other five receiving PHAD-SE adjuvant. There was no difference between adjuvants in the EC₅₀ of anti-LASV immunoglobulin G (IgG) antibody titers at any point postimmunization (Figure S1b). Both adjuvants are toll-like receptor (TLR)-4 agonists and thus should elicit a Th1-biased immune response. Both LASSARAB groups regardless of adjuvant elicited an antibody response with a bias towards a Th1 response as indicated by an isotype ratio of IgG2c/IgG1 greater than 1 (Figure S1c-e). The mice immunized with FILORAB1 did not show any immune responses against LASV-GPC. Thus, the immune responses elicited by LASSARAB adjuvanted with PHAD-SE are comparable to those with GLA-SE adjuvant.

Functionality of antibodies induced by LASSARAB immunization
It was previously demonstrated that the anti-LASV-GPC antibodies elicited by LASSARAB immunization in mice were non-neutralizing with Fc-mediated functions$^{28}$. To confirm whether LASSARAB immunization of NHPs produced antibodies with non-neutralizing Fc functions, a series of functional antibody assays were run. First, a virus neutralization assay (VNA) using a VSV reporter virus pseudotyped with LASV-GPC (ppVSV-DG-GPC) was performed. Neither sera from the LASSARAB immunized NHPs, nor from the CORAVAX™ immunized control NHPs showed neutralizing activity against ppVSV-DG-GPC, while human monoclonal 37.7H had strong neutralizing activity as previously described$^{28,39}$ (Fig. 3a). To determine whether these antibodies had Fc-mediated functions, an ADCC assay was performed using cells infected with VSV expressing LASV-GPC (VSV-DG-LASV-GPC) and treated with a mixture of NHP sera and a Jurkat reporter cell line expressing human Fcg receptor IIIa (FcgRIIIa). The human FcgRIIIa ADCC activation Reporter Bioassay measures the binding of the Fc portion of the antibody to the human FcgRIIIa of the effector cells (Jurkat), which results in a quantifiable luminescence signal from the nuclear factor of an activated T-cell (NFAT) pathway. Activation of the luciferase activity in the effector cells was used as an indicator of ADCC activity. Sera from NHPs immunized with LASSARAB showed strong ADCC activity towards VSV-DG-LASV-GPC infected cells, whereas sera from control NHPs immunized with CORAVAX™ only showed background signal activity (Fig. 3b). These data indicate that, as in mice, NHPs immunized with LASSARAB produce non-neutralizing antibodies with Fc-mediated functions.

Whereas, the role of neutralizing antibodies for protection in LASV is unclear, high titers of neutralizing antibodies are the correlate of protection for RABV$^{40}$. To measure RABV neutralizing antibody titers, we performed the well-established rapid fluorescent focus inhibition test (RFFIT), where a neutralizing titer of 0.5 international units (IU)/mL or more is considered protective by the WHO definition. Starting on day 14 postimmunization, all NHPs regardless of which vaccine they received had RABV-neutralizing antibody titers well above the 0.5 IU/mL threshold, which increased throughout the course of the experiment (Fig. 3c). Additionally, we confirmed that the three NHPs in the control CORAVAX™ group were previously immunized against RABV before the start of the study, as demonstrated by the anti-RABV antibody titers at day 0 (Fig. 2b, 3c).

**LASSARAB protects NHPs from lethal LASV challenge**

On day 70 post immunization, the NHPs were challenged with 1000 plaque forming units (PFU) of LASV Josiah strain$^{41}$. While the CORAVAX™ immunized NHPs reached endpoint criteria between days 10 and 19 postchallenge (pc), none of the LASSARAB immunized NHPs reached endpoint criteria at any point during the study (Fig. 4a). In addition to requiring euthanasia, all CORAVAX™ NHPs demonstrated high clinical scores, weight loss, and an elevated temperature followed by a drastic drop throughout the course of the challenge (Fig. 4b-d). In contrast, only one LASSARAB NHP displayed clinical symptoms that were mild and resolved quickly (Fig. 4b). Additionally, all LASSARAB immunized NHPs maintained weight throughout the challenge and only had a brief increase in body temperature (Fig. 4c-d).

**LASSARAB immunization reduces viral loads**
To quantitate viremia postchallenge, viral loads were measured in serum by plaque assay (actively replicating LASV) and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR, presence of viral RNA). All CORAVAX™-immunized NHPs had measurable viral replication starting on day 6 pc that persisted until euthanasia (Fig. 5a). However, two LASSARAB-immunized NHPs did not have detectable replicating LASV in the blood and the other four NHPs had low viral loads that were rapidly cleared (Fig. 5a). Both LASSARAB and CORAVAX™-immunized NHPs had detectable viral RNA in the blood starting at day 3 pc (Fig. 5b). Viral RNA was cleared from the blood in LASSARAB-vaccinated NHPs by day 21 for four NHPs and day 28 for the remaining two NHPs. By contrast, viral RNA persisted in the blood of CORAVAX™-vaccinated NHPs throughout the course of LASV infection (Fig. 5b). Thus, vaccination with LASSARAB greatly reduced viral loads compared to vaccination with CORAVAX™.

**Humoral responses are boosted postchallenge**

Antibody responses were measured to LASV-GPC following LASV-exposure to determine how LASSARAB vaccination impacted the humoral responses after challenge. Two of the CORAVAX™ control NHPs did not develop LASV-GPC-specific antibodies before euthanasia, while the other four developed only low antibody titers starting on day 10 pc (Fig. 6a). In contrast, all the LASSARAB-immunized NHPs had low antibody titers on days 0–6 pc and substantially increased antibody titers starting at day 10 pc, peaking at day 14 pc (Fig. 6a). These data indicate that LASSARAB immunization primed the immune system, resulting in this rapid recall response in antibody titers postchallenge.

To understand what role, if any, neutralizing antibodies played among LASSARAB vaccinates in LASV infection, a microneutralization assay was performed. We determined that LASV-Josiah neutralizing antibodies were present in the sera of LASSARAB vaccinates but not CORAVAX™ vaccinates by day 10 pc (Fig. 6b). Neutralizing antibodies persisted in the sera of all LASSARAB vaccinates through day 28 pc (Fig. 6b), albeit levels varied. Specifically, at days 10 and 14, LASSARAB vaccinated NHPs had NEUT50 titers of 1:100 (n = 2 cynomolgus macaques), 1:31.6 (n = 2), and 1:10 (n = 2). At days 21 and 28, NEUT50 titers were 1:100 (n = 4 cynomolgus macaques), 1:31.6 (n = 1), and 1:10 (n = 1). These data suggest that sera neutralizing antibody levels may peak between days 14 to 21 pc in LASSARAB vaccinates. Among the LASSARAB vaccinates, LOG10 (IC50) values ranged from a minimum of < 0 to a maximum of 2.39 (Supplementary Table 1). For four of six NHPs, the neutralizing-antibody activity was greatest at day 14 postchallenge. For one NHP, the most potent neutralizing-antibody activity was observed on day 21. Lastly, one NHP had only slight neutralizing activity with no observable change in potency between days 10, 14, 21, and 28 (LOG10 (IC50) < 0). Only 2 of the 6 NHPs showed a slight decrease in the neutralizing-antibody response between days 14 and 21, and 21 and 28, respectively.

**Immunization with LASSARAB mitigates changes in hematology and blood chemistry**

It is well established that LASV infection causes drastic changes to various cell populations and blood chemistry in both humans and NHPs. While changes to blood cell populations were similar between LASSARAB and CORAVAX™-vaccinated NHPs at the beginning of the challenge, these changes were
mostly normalized in LASSARAB vaccinated NHPs starting around day 10 pc (Supplementary Fig. 2). Most notably in the LASSARAB group, platelets returned to baseline levels, and neutrophils, which typically expand and cause damage in LASV infection\(^{43,45}\), were maintained at consistently low levels throughout the course of the study (Supplementary Fig. 2).

In terms of blood chemistry, LASSARAB-immunized NHPs maintained normal levels of all analytes tested throughout the course of the challenge (Supplementary Fig. 3), unlike CORAVAX™-immunized NHPs, which had significantly increased levels of analytes, indicative of liver and kidney damage (ALT, AST, ALP, GGT, and BUN), as well as significant decreases in analytes, indicative of vascular leakage (CA, ALB, and TP). (Supplementary Fig. 3).

**Acute pathology is limited by LASSARAB immunization**

Significant LASV histologic findings in NHPs at necropsy are presented in Table 2. The primary histopathologic diagnoses observed in the NHPs include the following: meningoencephalitis, inflammation and vasculitis of the choroid plexus, inflammation in the heart, necrotizing hepatitis, vasculitis with or without surrounding inflammation, interstitial pneumonia with alveolitis and vasculitis, and lymphoid hyperplasia in one or more lymph nodes and spleen. The most severe of these lesions, as indicated in Table 2, are restricted to the control NHP. Severe lesions observed in multiple tissue types of the control NHP are shown in Fig. 7a-f. These lesions are severe, widespread, and typical of fatal Lassa fever in NHPs\(^{43}\). Interestingly, a proliferative and necrotizing arteritis was observed in 5/6 of vaccinated survivors, similar to what has been previously described (Fig. 7g-j)\(^{46}\). Importantly, the proliferative and necrotizing polyarteritis (polyarteritis nodosa) noted in 5/6 survivors is histologically distinct from the vasculitis and/or arteritis noted in all the control animals that succumbed during the acute phase of LASV infection. Figure 7g-j shows these proliferative and necrotizing polyarteritis lesions observed in multiple tissues of vaccinated survivors. These lesions were marked by thickening of medium muscular arteries in numerous organ systems including the brain, meninges, lung, heart, liver, kidneys, uterus, testicles, and epididymis. This has been previously documented in LASV surviving macaques and guinea pigs\(^{46,47}\). Polyarteritis is a form of systemic necrotizing vasculitis that typically affects medium-sized muscular arteries and can result in secondary tissue ischemia and organ failure. Cashman et al. proposed an immune-mediated response in surviving LASV-infected macaques as primary etiology in previous studies\(^{46}\). In this study, most survivors were found to have the same proliferative and necrotizing polyarteritis to some degree. In the surviving NHP, the arteritis affected primarily medium-sized muscular arteries. These findings are similar to polyarteritis nodosa described in animals and humans.

To assess the potential presence of viral antigen in tissues at the study endpoint, we performed immunohistochemistry (IHC) analysis. Figure 8a-d demonstrates the presence of LASV antigen at high levels in multiple tissues, including brain, liver, lung, and spleen in control NHP. We mainly noted IHC positivity in lymphoid tissues (BALT and GALT and germinal center) and observed arterial smooth muscle cells within vasculitis lesions in multiple organ systems of LASSARAB-vaccinated NHPs (Fig. 8e-I).
strong positivity by IHC in the vaccinated survivors supported further investigation, thus we performed in-situ hybridization (ISH) and transmission electron microscopy (TEM) techniques on selected tissues to determine if the viral antigen present represented a persistent infection of actively replicating virus in these tissues. The ISH probe targets the L segment of LASV. A strong ISH signal was present in the tissues tested, including lung, spleen, lymph node, and brain (Fig. 8m-p). Unfortunately, the reagents used for ISH analysis can damage tissues, making identification of individual cell types that are positive for LASV-L segment RNA difficult. As a final experiment, we subjected selected tissues to visualization by transmission electron microscopy to determine if whole LASV virions were present in the areas that were positive for L segment RNA (data not shown). No LASV virions were identified in the examined sections of spleen and lymph node by TEM. The most consistent finding within the germinal centers was significant fibrin deposition. Fibrin was abundant within the germinal centers surrounding lymphocytes. Based on the TEM results that did not detect any LASV virions in the spleen and lymph node, the strong IHC and ISH positivity within these areas of the spleen and lymph nodes of surviving animals is most likely due to viral fragments and antigen, possibly trapped within the fibrin, not whole, intact and replicating virus. Additionally, we did not perform TEM on muscular arteries; however, based on these results, fibrin deposition may be present in these locations, causing the positive IHC signal.

**DISCUSSION**

Given the severity of Lassa fever (LF) and lack of preventative countermeasures against it, there is a great need to develop a safe and effective LASV vaccine. We showed previously that an inactivated RABV-based vaccine targeting LASV-GPC, LASSARAB, was protective in a guinea pig LF challenge model and induced strong humoral responses in nonhuman primates (NHPs) up to one-year postimmunization. This study aimed to determine the protective efficacy of LASSARAB in NHPs. We demonstrated that LASSARAB could protect against severe disease and death in a lethal NHP model of LF.

Here, we showed induction of neutralizing-antibody responses against LASV Josiah in 6/6 LASSARAB-vaccinated NHPs by day 10 postchallenge (pc). Neutralizing antibodies are known to protect NHPs from lethal LASV when administered as a cocktail via intravenous infusion as late as day 8 postchallenge. In vaccinated NHPs, we did not detect neutralizing antibodies prior to day 10. However, we observed neutralizing antibodies starting at day 10 that peaked between days 14 to 21 pc. This response persisted at varying levels through day 28 pc in all LASSARAB vaccinates. Based on these results, it appears that neutralizing antibodies are only produced as a result of LASV challenge, not vaccination with LASSARAB, and thus are not playing a main role in vaccine-mediated protection against LASV challenge.

All LASSARAB-immunized NHPs survived challenge, with only one NHP demonstrating minor outward clinical symptoms and four NHPs showing transient viremia. One of the main targets of LASV infection is the liver, as indicated by a dramatic increase in liver enzymes. LASSARAB immunized NHPs all maintained normal blood chemistry levels compared to controls, indicating that these NHPs were protected from liver dysfunction. NHPs in the control group developed severe and widespread
histological lesions consistent with fatal LF infection as previously described for the cynomolgus macaque model\textsuperscript{43}. Despite the positive clinical outcome and lack of CBC and blood chemistry changes in the vaccinated NHPs, pathologic analysis revealed significant lesions in lymphoid tissue as well as smooth muscle layer of arteries in multiple organ systems that stained positive for LASV antigen. This resembles a systemic auto-immune vasculitis that has been previously described in NHPs and guinea pigs that survive LASV infection\textsuperscript{46,47}. The disease process for LASV in NHPs is somewhat protracted compared to other hemorrhagic fever viruses in which NHPs become morbid and require euthanasia 5–7 days after exposure. This study’s endpoint was 28 days after virus exposure. It is unknown if the pathology that was observed in vaccinated survivors at day 28 would have resolved or become less prevalent if the study endpoint was longer. Additional studies may shed light on this.

Other vaccine platforms targeting LASV-GPC have also shown protection in lethal NHP challenge models. These include a recombinant vaccinia virus expressing LASV-GPC\textsuperscript{48}, recombinant\textsuperscript{22} and modified\textsuperscript{49} VSVs expressing LASV-GPC, recombinant measles virus (MeV) expressing LASV-GPC and nucleoprotein (NP)\textsuperscript{15}, attenuated Mopeia virus expressing LASV-GPC\textsuperscript{19}, and a DNA vaccine of LASV-GPC\textsuperscript{14,23,24}. Although not sterilizing, the protective efficacy of LASSARAB is comparable to many of these other vaccine platforms\textsuperscript{15,22,48,49}, with 100% of LASSARAB-vaccinated NHPs surviving challenge and showing minimal clinical symptoms. While these other platforms are protective, the inactivated RABV platform has some advantages over these platforms. As mentioned above, the rabies vaccine has been in use for decades and is safe to administer to a variety of patient populations, including both immunocompromised and pregnant patients\textsuperscript{30}. The rabies vaccine has been shown to elicit long-term immunity in humans\textsuperscript{50} and appears to confer this longevity to foreign antigens\textsuperscript{34,51,52}, although further studies are required to determine the full extent of the durability of immune responses against foreign antigens. The areas in which LASV is endemic have warm climates and limited access to cold-chain storage. While most of the other vaccine platforms mentioned above require cold-chain storage to remain stable over time, the inactivated RABV platform has been shown to remain stable over a variety of temperatures for extended periods of time\textsuperscript{31}. The areas where LASV is endemic are also endemic to RABV, and thus LASSARAB can provide protection against both viruses. Importantly given the presence of RABV in these areas, we have shown that vector pre-immunity does not impact the ability of this platform to elicit immune responses against a foreign antigen\textsuperscript{52}, although this will need to be confirmed with LASSARAB. Finally, the rabies vaccine is already commercially available, which means that infrastructure already exists for large-scale production of medical grade material\textsuperscript{53}.

Our previous study demonstrated that LASSARAB protects through non-neutralizing antibodies\textsuperscript{28}. We confirmed that pre-challenge, NHPs immunized with LASSARAB also develop non-neutralizing antibodies with Fcg-receptor mediated functions, but not neutralizing antibodies. The dispensability of neutralizing antibodies for protection against LASV is in line with other vaccine strategies\textsuperscript{17,22,32} and is likely a result of the LASV glycoprotein glycan shield blocking antibodies from binding to neutralizing epitopes\textsuperscript{33}. However, non-neutralizing antibodies acting as the mechanism of protection contrasts other vaccine
strategies targeting GPC, in which vaccine-mediated cellular immunity was protective\textsuperscript{17,32}. Additionally, a gamma-irradiated whole LASV virion vaccine elicited strong antibody responses, but could not protect NHPs from LASV challenge\textsuperscript{54}. These disparities in protective mechanism are likely attributed to the type of platform used, given that our platform is an inactivated RABV, while the two that require cellular immunity are live viral vectors (Vaccinia and Mopeia viruses)\textsuperscript{17,32}, and the non-protective vaccine was inactivated LASV virions\textsuperscript{54}. Overall, this suggests that there is not one specific mechanism of protection for LASV vaccines, and the mechanism will have to be determined on a case-by-case basis.

We are currently setting up a phase I clinical trial of LASSARAB in the US. Nevertheless, other studies should be performed to support the use of LASSARAB in the clinic. An ideal vaccine candidate should be cross-protective against a variety of LASV strains. Three LASV vaccine candidates, the MeV-, VSV-, and modified VSV-based vaccines, have demonstrated cross-protection against heterologous strains of LASV in NHPs\textsuperscript{49,55,56}. These vaccines target LASV-GPC, demonstrating that immune responses against LASV-GPC are protective against various strains of LASV. LASSARAB also targets LASV-GPC, indicating that this vaccine has the potential for cross-protection; however, further studies are required to investigate this. In addition to a vaccine that can protect against various strains of LASV, another focus has been the development of vaccines that only require a single dose and provide rapid protection. All live viral-vectored LASV vaccines showed protection in NHPs after a single dose\textsuperscript{15,19,22,48,49}. Additionally, a VSV-based LASV vaccine was able to protect NHPs that were challenged either 3- or 7-days postimmunization\textsuperscript{56}. Given the advantage of a single dose vaccine regimen, additional studies should be conducted to determine whether LASSARAB can protect NHPs after a single dose immunization and how rapidly LASSARAB confers protection postimmunization.

Our study contains some limitations. Most notably, as a result of the SARS-CoV-2 pandemic, there was limited availability of NHPs at the time of this study. The NHPs we acquired for this study were young and small in size, which limited the amount of blood we were able to collect. Thus, we were unable to perform assays looking at cellular immunity elicited by LASSARAB or whether these responses play a role in LASSARAB-mediated protection. We previously showed that Fcg-receptor knockout mice immunized with LASSARAB were not protected from challenge with a VSV-based LASV surrogate challenge virus\textsuperscript{28}. While this indicates that the strong non-neutralizing antibody responses may play a role in the protection conferred by LASSARAB, we cannot exclude the possibility that neutralizing antibodies, as well as T cells, play a role in protection, especially given that these mechanistic studies have not yet been repeated in a challenge model with wildtype LASV. Future studies must determine whether LASSARAB induces a cellular response and what role (if any) these responses play in vaccine-mediated protection. LASSARAB is administered in a prime/boost immunization regimen, which is a significant limitation of this platform compared to other platforms that have shown protection after a single dose\textsuperscript{15,19,22,48,49}. As mentioned above, studies testing the protective efficacy of LASSARAB after immunization with a single dose are required to determine the extent of this limitation. It should also be noted that the vaccine did not prevent the development of systemic, immune-mediated, proliferative, and
necrotizing vasculitis in NHPs surviving to day 28 postchallenge. An additional study with a longer endpoint may be warranted to observe whether these pathologic lesions will resolve over time.

MATERIALS AND METHODS

Study Design

The goal of this study was to determine the protective efficacy of a rabies-based LASV vaccine, LASSARAB (vaccine design described in 28). Twelve NHPs were pre-screened to look for prior immunity to LASV-GPC and RABV-G. No NHPs had prior immunity to LASV-GPC, but three were previously immunized against RABV and were placed into the control group. The remaining nine NHPs were randomly assigned into two groups of six NHPs each. Each NHP was vaccinated at Alphagenesis (AGI, Yemassee, South Carolina, USA) by intramuscular (IM) administration of 150 µg LASSARAB or 150 µg of a negative control vaccine, CORAVAX™ (vaccine design described in 35), and both vaccines were adjuvanted with 15 µg of monophosphoryl lipid A, 3D(6A)-PHAD, in 2% stable emulsion (PHAD-SE). NHPs were immunized on days 0 and 28, and blood was taken on days −14, 0, 14, 28, 42, and 56 for immunological analysis. NHPs were shipped to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID, Fort Detrick, Maryland, USA) and moved into the ABSL-4 containment laboratory on day 63 in preparation for the challenge study. Study team members involved with daily observations, physical exams, and data acquisition were blinded to group assignment to eliminate bias. On day 70, Each NHP was administered a target dose of 1000 PFU of LASV via IM route. NHPs were evaluated daily for changes in clinical signs. Rectal temperatures and weights were obtained, and physical exams performed on all scheduled blood collection days (days 0, 3, 6, 10, 14, 21, and 28 postchallenge), as well as on the day of euthanasia. Animals surviving to day 98 (day 28 postchallenge) or deemed moribund based on clinical signs and euthanasia criteria were euthanized. Euthanasia determinations were made based on criteria in the IACUC-approved protocol. Each NHP received a clinical score at each observation as follows: 0 = animal is alert, responsive, and engaging in normal species-specific behavior; 1 = animal is exhibiting slightly diminished general activity, is subdued, but responding normally to external stimuli; 2 = animal is withdrawn, may have head down, upright fetal posture, hunched, and exhibiting reduced response to external stimuli; 3 = animal is prostrate but able to rise if stimulated, or is exhibiting dramatically reduced response to external stimuli; or 4 = animal is persistently prostrate, is severely or completely unresponsive. A clinical score of 4 was considered criteria for immediate humane euthanasia. At necropsy, tissues were collected, preserved, processed, and examined microscopically for each NHP. Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the AAALAC International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

Cells
BEAS-2B (ATCC® CRL-9609™), 293T (available from the Schnell laboratory), Vero CCL81 (ATCC® CCL81™), Vero E6 (ATCC®) and Vero 76 (ATCC®) cells were cultured with DMEM (Corning®) containing 5% fetal bovine serum (FBS) (Atlanta-Biological®) and 1% Penicillin-Streptomycin (P/S) (Gibco®). Mouse neuroblastoma (NA) (available from the Schnell laboratory) were cultured with RPMI (Corning®) containing 5% FBS and 1% P/S. Jurkat cells with the Fcg receptor IIIa and nanoluciferase reporter gene (Promega) were cultured with RPMI (Corning®) containing 10% FBS, 100 µg/mL hygromycin, and 500 µg/mL Geneticin. All cells were stored in incubators with 5% CO₂ at 37°C or 34°C for virus infected cells.

**Viruses**

RABV strain CVS-11 was produced on NA cells in the Schnell laboratory and is available upon request. The LASV Josiah stock used for this study was acquired and propagated by USAMRIID from the CDC in 1982 (CDC #800789). It was originally isolated during an outbreak in 1976 from the serum of a severely-ill patient in Sierra Leone, Africa.

**Immunization of Mice for Adjuvant Comparison**

C57BL/6 mice (Charles River) were immunized IM with 10 µg of LASSARAB or FILORAB1 (Rabies-based Ebola virus vaccine). The vaccines were adjuvanted with either glucopyranosyl lipid A in a squalene-in-oil emulsion (GLA-SE) or PHAD-SE at a dose of 5 µg GLA/PHAD and 2% SE. Each dose was administered as 50 µL in each hind leg (for a total of 100 µL). Mice were anesthetized with isoflurane and immunized on days 0 and 21. Serum was collected via retro-orbital bleeds on days 0 and 21, and the final bleed was on day 28. Mouse procedures were approved by the Thomas Jefferson University (TJU) Institutional Animal Care and Use Committee (IACUC).

**ELISA Antigen Production**

Both RABV-G and LASV-GPC were prepared as stripped antigens as previously described²⁸,³⁴,⁵⁷. In brief, BEAS-2B cells were infected with either recombinant VSV (rVSV)-DG-LASV-GPC or rVSV-DG-RABV-G-GFP in Opti-Pro SFM (Gibco). Viral supernatants were then concentrated, and sucrose purified, and glycoproteins were stripped using 2% OGP (Octyl-b-D-glucopyranoside) detergent.

**Enzyme-linked immunosorbent assay (ELISA)**

Mouse and NHP sera were analyzed by ELISA to look for anti-LASV-GPC or RABV-G immunoglobulin (Ig) G, as described elsewhere²⁸,³⁴. Briefly, plates were coated overnight with 50 ng/well of each antigen, then the following day blocked for 2 hr and incubated with mouse or NHP serum samples overnight. Next, plates were incubated with the respective secondary antibody at 25 ng/mL for 2 hr, and then o-phenylenediamine dihydrochloride substrate (Sigma-Aldrich) was added to each well for development.

Plates were read at the absorbance wavelengths of 630 nm and 490 nm, and the delta value was calculated by subtracting the 630 nm reading from the 490 nm reading. The delta values were used for analysis in GraphPad Prism 9 software. Both half-maximal effective concentration (EC₅₀) serum or antibody titer and endpoint cutoff values were calculated as previously described³⁴. Any samples
without a proper curve were considered to have no detectable antibodies, given that a full curve is required for calculating an accurate EC_{50} value.

**Rapid Fluorescent Focus Inhibition Test (RFFIT)**

Rabies virus neutralization was measured using the RFFIT assay as previously described\(^{58}\). Briefly, heat-inactivated NHP sera was incubated with RABV strain CVS-11 for 1 hr, overlaid onto NA cells for 2 hr, and then aspirated and replaced with fresh medium. After 24 hr total infection, cells were fixed and stained for RABV-N. 50% endpoint titers were determined using the Reed-Muench method and then, through comparison to the WHO standard, converted to international units (IU) per mL.

**Production of Pseudovirus**

VSV pseudovirus (ppVSV) was produced as previously described\(^{28}\). In brief, 293T cells were transfected with a pCAGGS plasmid containing LASV-GPC with X-tremeGENE9 (Sigma-Aldrich). After incubation overnight at 37°C, cells were infected with ppVSV-NL-GFP at a multiplicity of infection (MOI) of 1. Infected cells were incubated at 34°C until cells were 60–80% GFP positive, and then viral supernatant (termed ppVSV-DG-LASV-GPC-NL-GFP) was collected.

**LASV Pseudotype Virus Neutralization Assay (VNA)**

VNAs using the pseudovirus ppVSV-DG-LASV-GPC-NL-GFP was performed as described previously\(^{28}\). In brief, heat-inactivated NHP serum or positive control monoclonal antibody 37.7H (30 µg/mL starting dilution) was mixed with ppVSV-DG-LASV-GPC-NL-GFP for 2 hr and then overlaid onto Vero CCL81 cells and incubated overnight. The following day, cells were lysed and treated with NanoLuc substrate (Promega) as per the manufacturer's instructions.

Luminescence was read using the Omega Luminometer, with relative luminescence units (RLU) being normalized to the signal in wells without serum/antibody (100% infectivity). Any signal above 100% was reported as 100%.

**LASV Microneutralization Assay**

To determine the ability of serum to neutralize LASV Josiah host cell infection, we used a fluorescent microneutralization assay. The assay was performed in duplicate using 8 three-fold serial dilutions of sera starting at a 1:10 dilution in cell culture media containing 2% heat-inactivated FBS (MEM + 2% FBS) (Corning 10–010; GE Healthcare Hyclone). For negative and positive controls, we used serum from naïve and LASV convalescent NHPs. One day prior to starting the assay, we seeded Vero E6 cells (ATCC, Manassas, VA) at 2.5E4 cells/well in 96-well black clear-bottom plates (Greiner Bio-One). The following day, we mixed diluted serum with LASV Josiah, incubated at 37°C for 1 hour, and added the virus/serum mixture to the ATCC Vero E6 cells at a target MOI of 0.5. Unbound virus was removed after the 1-hour incubation at 37°C. Cells were washed once in Dulbecco's phosphate-buffered saline without calcium and magnesium (DPBS, MilliporeSigma), and cell culture media (MEM + 5% FBS + 1% P/S (Gibco Thermo Fisher Scientific, 15140122)) was added. Cells were fixed 48 hours after infection, washed 3 times with
DPBS (MilliporeSigma), permeabilized with 1% Triton X-100 (Bio-Rad), and blocked with Cell Staining Buffer (Biolegend). The number of infected cells was determined using LASV-GP-specific mouse monoclonal antibody (Clone L52-161-6), goat anti-mouse IgG (H&L) Alexa Fluor 568 F(ab’)2 fluorescently labeled secondary antibody (Invitrogen Thermo Fisher Scientific, A11019), and NucBlue Live ReadyProbes Reagent (Hoechst 33342) (Invitrogen Thermo Fisher Scientific). The percentage of infected cells was determined with the Cytation 5 (Agilent BioTek), using Gen 5.11 software. We determined the neutralization percentage for each serum sample at each dilution relative to untreated, virus-only control wells.

**Antibody-Dependent Cellular Cytotoxicity (ADCC) Assay**

Fc-mediated antibody effector functions were measured using an ADCC assay adapted from previously described methods\(^5^9\). Vero CCL81 cells were seeded in white, 96-well flat-bottomed plates at a density of 3E4 cells per well in the inner 60 wells of the plate and incubated overnight at 37°C. The following day, cells were infected with rVSV-DG-LASV-GPC at an MOI of 0.1 and incubated for 16 hr at 34°C. NHP sera samples were heat-inactivated for 30 min at 56°C. Serum was then serially diluted in a 2-fold dilution series at a starting dilution of 1:3.3 (for a final concentration of 1:10 once added to assay plates) in 96-well round-bottomed plates in assay buffer (RPMI 1640, 4% Low IgG Serum). The medium from the infected Vero CCL81 cells was removed and replaced by 25 µL of assay buffer and 25 µL of diluted NHP sera and incubated for 30 min at 34°C. Next, Jurkat cells expressing human Fcg receptor IIIa effector cells with an NFAT controlled nano-luciferase reporter gene (Promega) were added to the infected Vero CCL81 cells/sera plate in 25 µL at a 5:1 ratio of effector to target cells and incubated for 6 hr at 34°C. Next, plates were incubated at room temperature for 15 min, and then 75 µL of Bio-Glo Luciferase assay reagent (Promega) was added to each well in addition to 3 wells without cells or sera. After at least 5 min, plates were analyzed for luminescence on the Omega Luminometer. Fold induction was calculated using relative light units (RLU) with the following formula: \((RLU_{induced} – RLU_{background})/(RLU_{uninduced} – RLU_{background})\), with RLU induced being the NHP sera samples, RLU background being the plate without cells or sera, and RLU uninduced being the cells without sera. For each dilution, mean values and standard errors of the means (SEM) were graphed using a nonlinear regression curve on GraphPad Prism 9 software.

**Blood chemistries**

The General Chemistry 13 panel, which includes analytes alanine transaminase (ALT), serum albumin (ALB), alkaline phosphatase (ALP), amylase (AMY), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (CA), creatinine (CRE), gamma-glutamyl transferase (GGT), glucose (GLU), total bilirubin (TBIL), and total protein (TP) was run on Piccolo® blood chemistry analyzers (Abaxis).

**Hematology**

A VETSCAN® HM5 hematology analyzer was used to obtain complete blood counts throughout the study. The analytes included were white blood cells (WBC), neutrophils (NEU), eosinophils (EOS),
basophils (BAS), lymphocytes (LYM), monocytes (MON), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet counts (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW). Blood chemistries and hematology analyses were obtained contemporaneously during the in-life phase.

**Viremia**

Serum viremia was measured by both standard plaque assay (which can enumerate replicating virus in samples) and polymerase chain reaction (PCR, which can enumerate viral particles present without regard to replication status). The standard plaque titration assay was performed on Vero 76 cells in accordance with (IAW) USAMRIID standard operating procedure (SOP) for LASV\(^{26}\). Briefly, required dilutions of each specimen were added to plates containing Vero 76 cells on assay day 0, in duplicate. The cells were stained with neutral red on assay day 4, and resulting viral plaque counts were obtained on assay day 5. Titers were calculated for each specimen based on all dilution series with countable plaques between 10 and 150. PCR was performed on Trizol-LS-inactivated plasma by first extracting RNA using a QIAamp® Viral RNA Mini Kit IAW USAMRIID SOP. The quantitative reverse transcription PCR (qRT-PCR) reaction used the Invitrogen™ SuperScript® II One-Step RT-PCR System with additional magnesium sulfate (MgSO\(_4\)) added to a final concentration of 3.0 mM. The sequence of the primer and probes for the LASV target gene are shown below in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Primer/Probe</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward primer (1 µM):</td>
<td>5′ - TGCTAGTACAGACAGTGCAATGAG – 3′</td>
</tr>
<tr>
<td>Reverse primer (1 µM):</td>
<td>5′ - TAGTGACATTCTTCCAGGAAGTGC – 3′</td>
</tr>
<tr>
<td>Probe (0.1 µM):</td>
<td>TGTTCATCACCTCTTC-MGBNFQ</td>
</tr>
</tbody>
</table>

Specimens were run in triplicate using an Applied Biosystems® 7500 Fast Dx instrument. The resulting data were expressed as cycle threshold (CT), which shows the number of PCR replication cycles in each sample that is required for the signal to exceed background levels. Limit of detection for this assay was determined to be 42 CT.

**Histopathology**

In preparation for histology analysis, tissue samples collected at necropsy were routinely processed and embedded in paraffin. The paraffin-embedded tissues were sectioned to 5 µm thick and placed on glass slides. The histology slides were deparaffined, stained with hematoxylin and eosin (H&E), coverslipped, and labeled in accordance with USAMRIID SOPs.

**Immunohistochemistry**
IHC was performed in accordance with USAMRIID SOPs and using the Dako Envision system (Dako Agilent Pathology Solutions, Carpinteria, CA, USA). Briefly, after deparaffinization, peroxidase blocking, and antigen retrieval, sections were covered with mouse anti-Lassa virus monoclonal antibody (clone 52-2074-7A, USAMRIID) at a dilution of 1:8000 and incubated at room temperature for 40 min. They were rinsed, and the peroxidase-labeled polymer (secondary antibody) was applied for 30 min. Slides were rinsed, and a brown chromogenic substrate 3,3’ Diaminobenzidine (DAB) solution (Dako Agilent Pathology Solutions) was applied for 8 min. The substrate-chromogen solution was rinsed off the slides, and slides were counterstained with hematoxylin and rinsed. The sections were dehydrated, cleared with xylene, and then coverslipped. The following severity scale was used for reporting: 0 = Negative: no cells in section are positive; 1 = < 10% of cells in section are positive (minimal); 2 = 11–25% of cells in section are positive (mild); 3 = 26–50% of cells in section are positive (moderate); 4 = 50–75% of cells in section are positive (marked); 5 = > 75% of cells in section are positive (severe).

**In-Situ Hybridization**

In-Situ Hybridization (ISH) was performed on select animals and select tissues. RNA ISH was performed using RNAscope® 2.5 HD RED kit according to the manufacturer’s instructions (Advanced Cell Diagnostics, Hayward, CA). Briefly, 20 ZZ probes set targeting to 466–1433 polymerase (L protein) of Lassa virus genome with GenBank accession number KM821901.1 were synthesized. After deparaffinization and peroxidase blocking, the sections were heated in antigen retrieval buffer and then digested by proteinase. The sections were covered with ISH probes and incubated at 40°C in hybridization oven for 2 hr. After rinsing, ISH signal was amplified using kit-provided Pre-amplifier and Amplifier conjugated to alkaline phosphatase and incubated with a Fast Red substrate solution for 8 min at room temperature. Sections were then stained with hematoxylin, air-dried, and cover slipped.

**Statistical Analysis**

GraphPad Prism 9 software was used for all statistical analysis. For ELISA and RFFIT assays, log transformed data were analyzed using the Mann Whitney test at each timepoint. Statistical differences in survival curves were determined using the log-rank Mantel-Cox test. Cell population, hematology parameters, and blood chemistry data were analyzed using multiple unpaired t-tests assuming a Gaussian distribution.

**Declarations**

**Acknowledgments:** KC would like to acknowledge husbandry personnel in the Veterinary Medicine Division at USAMRIID for excellent advice and daily care for the NHP assigned to this study. KC would also like to acknowledge the careful and detailed work of the USAMRIID pathology Division for preparation of the tissue sections and slides for analysis. Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facilities where this research was conducted are accredited by the Association for Assessment and Accreditation of Laboratory Animal
Care International and adhere to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011\textsuperscript{60}.

The authors would like to acknowledge Alphagenesis (Yemassee, South Carolina, USA) for immunizing and bleeding the NHPs prechallenge.

**Funding:** National Institutes of Health Contract HHSN272201700082C (MJS)

**Author contributions:**

Conceptualization: MJS

Methodology: MJS, KC, GS

Investigation: GS, CY, KC

Visualization: N/A

Funding acquisition: MJS


Postlife analysis: KC, EW, NJ, JW, NT

Project administration: MJS, DK

Supervision: MJS

Writing – original draft: GS and KC

Writing – review & editing: GS, MJS, KC

**Competing interests:** Authors declare that they have no competing interests.

**Data and materials availability:** All data are available in the main text or the supplementary materials.

**Disclaimer**

Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the AAALAC International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

**References**


https://doi.org:10.1038/s41541-022-00464-2


Tables

Table 2 is available in the Supplementary Files section.

Figures

Figure 1

Experimental design. (a) Schematic of vaccine constructs with all RABV and foreign proteins indicated. Red diamond indicates the attenuating mutation at amino acid 333 of RABV-G. N, nucleoprotein; P,
phosphoprotein; M, matrix protein; G, glycoprotein; L, RNA-dependent RNA polymerase; LASV-GPC, Lassa virus glycoprotein precursor; SARS-CoV-2 S1, severe acute respiratory syndrome coronavirus 2 spike protein subunit 1; ED31, 31 amino acids of the ectodomain; TM, transmembrane domain; CD, cytoplasmic domain. (b) Table outlining the vaccine groups and expected experimental outcomes. (c) Experimental timeline. Red droplets indicated blood draws, rhabdovirus and syringe indicate immunization, and the arenavirus represents LASV challenge. Figure made with BioRender.com.

Figure 2

**Humoral responses to LASSARAB.** Antibody responses against LASV-GPC and RABV-G were measured through enzyme-linked immunoassay (ELISA). (a) Antibody EC_{50} titers over time for LASV-GPC and RABV-G. (b) Antibody endpoint titers over time for LASV-GPC and RABV-G. Samples from each NHP were run in triplicate, and error bars are the mean with standard deviation (SD). The Mann Whitney nonparametric T test was used to determine statistical differences between groups at each time point. Where significance is not noted, samples have no significant difference. ****, <0.0001; ***, 0.0002; **, 0.0021; *, 0.0332; P > 0.05 ns, not significant. LOD, limit of detection.
Antibody functionality. Assays measuring neutralizing and non-neutralizing antibody functions. (a) Pseudotype virus neutralization assay (VNA) for LASV with sera from immunized NHPs and human monoclonal anti-LASV-GPC antibody 37.7H as a positive control. Error bars represent standard deviation. (b) Antibody-dependent cellular cytotoxicity (ADCC) assay for LASV with sera from immunized NHPs. Error bars represent standard error of the mean. (c) Rapid fluorescent focus inhibition test (RFFIT) to measure neutralizing antibodies against RABV (strain CVS-11). Neutralizing titers are represented as international units per mL (IU/mL). 0.5 IU/mL, the WHO threshold suggestive of protection, is indicated by the dotted line. NC, negative control; ns, not significant.
Figure 4

NHP survival and clinical signs. Clinical measurements of LASV disease throughout the NHP challenge study. (a) Kaplan-Meyer survival curves. Significance between groups was determined using the log-rank Mantel-Cox test (***P=0.0004) (b) Clinical scores for individual NHPs. (c) Changes in weight over time for individual NHPs. (d) Group average changes in body temperature over time.
**Figure 5**

**Viral loads.** (a) Serum Viremia postchallenge measured via plaque assay. Pfu, plaque forming units. (b) Plasma PCR CT values postchallenge measured by qRT-PCR. Limit of detection for this assay is 42 CT.
Humoral responses postchallenge. (a) Antibody responses against LASV-GPC postchallenge. Total IgG EC$_{50}$ antibody titers throughout the course of the challenge study. Error bars indicate standard deviation. Statistics at each timepoint were determined by the Mann Whitney non-parametric T test. Where significance is not noted, samples have no significant difference. ****, <0.0001; ***, 0.0002; **, 0.0021; *, 0.0332; P > 0.05 ns, not significant. (b) The serum neutralization levels were measured in a
microneutralization assay three-fold serial dilutions starting at 1:10. The bar graph shows longitudinally
the NEUT50 mean serum dilutions (log10) ± the standard deviation, with the x-axis representing the
sampling timepoint and y-axis, the log10 dilution of sera. Percent inhibition curves were plotted in
GraphPad Prism 9.4.0 with reciprocal serum dilution on the x-axis and percent inhibition on the y-axis,
with the dotted line at y=50 indicating the fifty percent inhibition level. The individual data points shown
for each cynomolgus macaque represent the mean ± standard deviation among two-replicate on days
10, 14, 21, and 28 postchallenge.

**Figure 7**

**Histopathology, in control (a-f) and vaccinated (g-j) NHP.** (a) Liver. Multifocal necrosis and loss of
hepatocytes (circled) that disrupt normal hepatic cord architecture were observed. (b) Brain. Within the
neuropil, there are increased numbers of glial cells or gliosis (circled), and the blood vessel (BV) wall is
disrupted by necrotic debris (arrow) and inflammatory cells. (c) Heart. The epicardium and myocardial
interstitium is expanded by inflammatory cells (arrows) composed of lymphocytes, macrophages, and
neutrophils. Note the increased clear space between myocardiocytes (asterisks), indicating edema. The
myocardiocytes appear normal. (d) Spleen. The arteries (A) are disrupted. The tunica intima, media, and
adventitia are disrupted and expanded by inflammatory cells (arrows). These cells are primarily
lymphocytes and neutrophils. (e) Lung. Interstitial pneumonia and most of the alveolar spaces (asterisk)
are filled with edema, fibrin, inflammatory cells (arrow), and hemorrhage. There is vasculitis present. The
blood vessel (BV) wall is ill-defined and expanded by edema, inflammation, and necrotic debris. (f) Kidney. Note the intact extremely thin tunica intima with endothelial cell nuclei present (arrows). Note the intact internal elastic lamina (squiggle arrow), the smooth muscle of the tunica media (TM), and few cells and abundant collagen fibers of the tunica adventitia (TA). (g) Testicle. Note the polyarteritis nodosa of the testicular arteries (A). The arteries have a nodular appearance, compress underlying testicle (arrows), and elevate the surface (arrowheads). (h) Testicle. Expansion of the tunica intima, proliferative infiltration of macrophages and neutrophils. The intact internal elastic lamina (squiggle arrow) is disrupted at the arrowhead. The tunica media contains necrotic debris (arrow), and the tunica adventitia is greatly expanded by inflammatory cells and clear space (edema). (i) Uterus. Note the polyarteritis nodosa of the uterine arteries (A) (circled). Note the tunica intima (TI) is expanded, proliferative, and infiltrated by macrophages, neutrophils, and necrotic debris (red arrow). (j) Uterus. The internal elastic lamina (squiggle arrow) remains intact in this section. The tunica adventitia (TA) is greatly expanded by inflammatory cells (black arrows).

Figure 8

**Immunohistochemistry and In Situ Hybridization in control (a-d) and vaccinated (e-p) NHP.**

(a) Brain, choroid plexus, and third ventricle. Strong IHC positivity in choroid plexus cuboidal cells (arrow) and ependymal cells of the third ventricle (arrowhead). (b) Liver. Strong LASV IHC positivity in
hepatocytes and endothelium of hepatic sinuses (arrows). (c) Lung. Strong LASV IHC positivity of endothelium of artery and interstitium. (d) Spleen. Strong LASV IHC positivity of endothelium of venous sinuses (arrow) of the red pulp. Additionally, there is positivity in the germinal centers of the white pulp (asterisk), likely FRCs or follicular dendritic cells FDCs. (e) Lung. Strong LASV IHC positivity in the germinal center of BALT. (f) Spleen. Strong LASV IHC positivity in the germinal centers of the white pulp. (g) Lung. Strong LASV IHC positivity in the smooth muscle of the tunica media of a muscular artery. (h) Kidney. Strong LASV IHC positivity in the smooth muscle of the tunica media of a muscular artery. (i) Small Intestine. Strong LASV IHC positivity in the germinal centers of GALT. (j) Inguinal Lymph Node. Strong LASV IHC positivity in the germinal centers of lymphoid follicles. (k) Testicle. LASV IHC positivity in the smooth muscle (arrow) of the tunica media (TM) of a muscular artery. Additionally, this artery displays proliferative and necrotizing arteritis. (l) Brain. There is strong LASV IHC positivity in the smooth muscle of the tunica media (arrows) of a meningeal muscular artery. (m) Lung. Strong LASV ISH positivity in the germinal center of BALT (arrow). (n) Spleen. Strong LASV ISH positivity in the germinal centers of the white pulp. (o) Inguinal Lymph Node. Strong LASV ISH positivity in the germinal centers of lymphoid follicles. (p) Brain. Strong LASV ISH positivity in the meningeal artery smooth muscle (arrows).

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.jpeg
- SupplementaryMaterialnatcom.docx