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Development of reverse genetic tools to study Chapare and Machupo viruses

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Abstract

Arenaviruses are highly pathogenic viruses that pose a serious public health threat. Chapare virus (CHAV) and Machupo virus (MACV), two New World arenaviruses, cause hemorrhagic fevers with case fatality rates of up to 45%. Research on therapeutic drug targets and vaccines for these viruses is limited because biosafety level 4 containment is required for handling them. In this study, we developed reverse genetics systems, including minigenomes and recombinant viruses, that will facilitate the study of these pathogens. The minigenome system is based on the S segment of CHAV or MACV genomes expressing the fluorescent reporter gene ZsGreen (ZsG). We also generated recombinant CHAV and MACV with and without the ZsG reporter gene. As a proof-of-concept study, we used both minigenomes and recombinant viruses to test the inhibitory effects of previously reported antiviral compounds. The new reverse genetics system described here will facilitate future therapeutic studies for these two life-threatening arenaviruses.

Keywords

Arenaviruses; Chapare virus; Machupo virus; Reverse genetics; Antiviral; Hemorrhagic fever

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CRediT authorship contribution statement

Shilpi Jain: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing. **Punya Shrivastava-Ranjan:** Methodology, Validation, Formal analysis, Writing – review & editing. **Mike Flint:** Resources, Writing – review & editing. **Joel M. Montgomery:** Resources, Supervision, Funding acquisition. **Christina F. Spiropoulou:** Resources, Supervision, Funding acquisition. **César G. Albariño:** Investigation, Methodology, Validation, Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Supervision.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2023.109888.

1. Introduction

Arenaviruses are enveloped, single-stranded, negative-strand RNA viruses belonging to the family *Arenaviridae*. The family is classified into 4 genera: *Hartmanivirus, Mammarenavirus, Reptarenavirus*, and *Antennavirus*. The bisegmented genome of mammarenaviruses codes for 4 viral proteins using the ambisense strategy. Each gene of the 2 segments is separated by a non-coding intergenic region (IGR) comprising stem-loop structures. The large (L) segment codes for RNA-dependent RNA polymerase (L polymerase) and small zinc-finger matrix protein Z. The small (S) segment codes for glycoprotein precursor (GPC) and nucleoprotein (N) (Howley et al., 2021).

Chapare virus (CHAV) and Machupo virus (MACV) are two closely related New World arenaviruses responsible for the hemorrhagic fever diseases, Chapare hemorrhagic fever (CHHF) and Bolivian hemorrhagic fever (BHF), respectively. MACV was identified as the cause of BHF in 1963 when it was isolated from the spleen of a fatal case in Bolivia (Johnson et al., 1966; Mackenzie et al., 1964). Since that first report, several other MACV outbreaks have been documented. Person-to-person transmission has been documented in different MACV outbreaks (Peters et al., 1974; Patterson et al., 2014a). In 2003, CHAV was found to be the cause of an outbreak of CHHF in Bolivia (Delgado et al., 2008). Sixteen years after its discovery, CHAV was associated with another outbreak of CHHF. Recently, human-to-human transmission was reported in CHAV (Escalera-Antezana et al., 2020; Loayza Mafayle et al., 2022). Both CHAV and MACV are endemic to Bolivia with a case fatality rate of up to 45% (Silva-Ramos et al., 2021).

Due to the need for BSL-4 containment to work with CHAV and MACV, studies of these viruses are limited. The minigenome and the recombinant infectious virus with the fluorescent reporter are thus useful tools for studying and combating infections associated with these mammarenaviruses. There are several reports on the development of recombinant arenaviruses like Junin virus (Albariño et al., 2009), Lujo virus (Bergeron et al., 2012), LASV (Albariño et al., 2011), lymphocytic choriomeningitis virus (LCMV) (Sanchez and de la Torre, 2006), Pichinde virus (Lan et al., 2009), MACV (Patterson et al., 2014c), Tacaribe virus (Ye et al., 2020), and attenuated arenaviruses for vaccine production (Emonet et al., 2011b; Golden et al., 2017; Koma et al., 2016; Martinez-Sobrido and de la Torre, 2016; Ortiz-Riano et al., 2013; Patterson et al., 2014b).

In this report, we describe new reverse genetic systems for CHAV and MACV, including minigenomes and recombinant viruses expressing or not a fluorescent reporter gene. As a proof-of-concept, we first tested a limited set of previously described antiviral compounds using minigenomes at biosafety level 2 (BSL-2) conditions. Afterwards, we efficiently generated recombinant CHAV and MACV, and used them to test the same set of antiviral compounds in our BSL-4 labs. The new systems described here will facilitate the study of these viruses and aid future research of antivirals against these emerging pathogens.

2. Materials and methods

2.1. Cell culture and biosafety

Huh-7 (Apath LLC), BSR-T7/5 (generous gift from K. Conzelmann, Ludwig-Maximilians-University), and Vero-E6 cells (CDC Core Facility) were propagated in Dulbecco's modified Eagle medium (DMEM; Gibco) supplemented with 1 × non-essential amino acids, 5% (v/v) fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin (Gibco). All cells were incubated at 37 °C in 5% CO_2. All the work with wild-type and recombinant viruses was done in a BSL-4 facility at the Centers for Disease Control and Prevention (CDC, USA). The minigenome experiments were performed in a BSL-2 laboratory. This work was approved by CDC Institutional Biosafety Committee (IBC).

2.2. Minigenome studies

The CHAV and MACV minigenomes were constructed by cloning the ZsGreen1 (ZsG) ORF between the 5′ UTR of the N gene and the 3′ UTR of the GPC gene (Suppl. Fig. 1). The primary transcription of both minigenomes is driven by the human RNA polymerase I (PolI) promoter and terminator (Emonet et al., 2011a; Martinez-Sobrido et al., 2016; Ortiz-Riano et al., 2013). The viral proteins required for replication and transcription, L and N, were expressed from pCDNA3 plasmids, and co-transfected with the minigenome.

2.3. Rescue of recombinant viruses

Recombinant CHAV and MACV were generated based on the available sequences of the CHAV prototype strain 200,001,071 (GenBank accession numbers EU260464 and EU260463) and the MACV strain 200,002,427 (GenBank accession numbers AY960327, AY935527, and AY924204) (Cajimat et al., 2009). Basically, the S and L segments of CHAV and MACV were amplified by RT-PCR, and cloned in a previously described T7 transcription vector (Albariño et al., 2009, 2011; Bergeron et al., 2012; Gerrard et al., 2007). The final plasmid constructions were designed to transcribe the full-length antigenomic copies of CHAV and MACV S and L segments, and contained two nonviral nucleotides, 5′G and 3′C, required for the efficient function of the T7 promoter and the hepatitis delta virus ribozyme (Suppl. Fig. 1). For both recombinant viruses expressing the reporter, ZsG was inserted upstream of the N gene, separated by the sequence of the self-cleaving peptide of the porcine teschovirus (P2A). To generate the stocks of the 4 recombinant viruses (rCHAV, rCHAV-ZsG, rMACV and rMACV-ZsG), BSR-T7/5 cells (Buchholz et al., 1999) were co-transfected with equimolar concentrations of the L and S full-length clones (with or without ZsG). Subsequently, the supernatants were blind passaged twice onto Vero-E6 cells. The working stocks were titered using a standard $TCID_{50}$ technique.

2.4. Antiviral compounds, screening, and data analysis

All antiviral compounds were obtained from either Selleckchem or Sigma-Aldrich except for T-705 (Favipiravir; BOC Sciences) and 2′-deoxy-2′-fluorocytidine (2′-DFC; Carbosynth). Antiviral properties of different compounds were screened using recombinant viruses and minigenomes as mentioned previously (Welch et al., 2016). All experiments with antivirals were done independently 2–3 times for confirmation. Compound concentrations inhibiting

50% ZsG expression (EC_{50}) were calculated by interpolating these values. Similarly, 50% cytotoxicity concentrations (CC_{50}) were determined for each compound. The selectivity index was calculated as the ratio of CC_{50} and EC_{50}.

2.5. NGS analysis of viral genomes

rCHAV-ZsG and rMACV-ZsG were passaged up to 5 times in Vero-E6 cells, viral RNA was extracted and analyzed by NGS. No changes in the coverage of the S genomic segment were detected at passages 1 to 5. The associated table indicates that no mutations were detected in the S genomic segments of each virus (Suppl. Fig. 2).

2.6. Immunofluorescence antibody staining

For immunofluorescence assays (IFA), Vero-E6 cells were seeded onto 6-well plates (Costar) and infected with the recombinant viruses at MOI of 0.1. Three days post infection, cells were fixed with 10% formalin for 30 min followed by permeabilization with 0.1% Triton X-100 in phosphate-buffered saline (PBS) for 15 min and blocking with 1% bovine serum albumin for 1 h. Either rabbit *anti*-CHAV nucleoprotein (GenScript) or rabbit polyvalent hyperimmune serum raised against South American arenaviruses (Guanarito, Machupo, and Sabiá viruses) were used as primary antibodies (Delgado et al., 2008; Loayza Mafayle et al., 2022) followed by 2 washes with PBS and treatment with secondary anti-rabbit 594 antibody. Cell nuclei were stained with NucBlue (Thermo Fisher).

3. Results

3.1. Development of minigenome assays

Minigenomes were constructed in a previously described RNA polymerase I transcription-driven vector (Welch et al., 2016) by cloning the fluorescent reporter gene ZsG between the 5′ and 3′ UTRs of the S RNA segment of the indicated virus (Suppl. Fig. 1). Minigenomes were designed to replicate after co-transfection with support plasmids expressing the N and L proteins under the control of a Pol-II promoter (Fig. 1A). As expected, both minigenomes exhibited replication activity when co-transfected with their corresponding helper plasmids in Huh-7 cells (Fig. 1B).

Since CHAV and MACV are closely related in the phylogenetic analysis, we tested both minigenomes with heterologous support plasmids. Interestingly, similar levels of replication were observed with all combinations of minigenomes and support plasmids (Fig. 1D). This result was not unexpected since CHAV and MACV L and N proteins exhibit a high degree of amino acids similarity (61% and 71%, respectively; Fig. 1C), and they share a conserved 20 terminal nucleotides of 5′ and 3’ UTRs (Suppl. Fig.1).

3.2. Generation and characterization of recombinant viruses

To further study CHAV and MACV, we developed recombinant CHAV and MACV viruses expressing or not a fluorescent reporter gene. First, we generated full-length clones of L and S segments corresponding to the CHAV prototype strain 200,001,071 and MACV strain 200,002,427 (Cajimat et al., 2009). Afterwards, we modified the full-length S clone of both viruses by inserting the reporter gene ZsG fused to the self-cleaving P2A peptide and N

gene (Fig. 2A). To rescue recombinant viruses, T7 RNA polymerase-driven transcription plasmids expressing L and S antigenomes were co-transfected into BSR-T7/5 cells as described previously (Albariño et al., 2009, 2011). After two blind passages on Vero-E6 cells, viral rescue was confirmed by IFA for rCHAV and rMACV (Delgado et al., 2008; Loayza Mafayle et al., 2022), or by ZsG detection for rCHAV-ZsG and rMAC-ZsG (Fig. 2B). Viral stocks were titered using a standard $TCID_{50}$ protocol and sequenced by NGS.

Although all 4 recombinant viruses were rescued with high efficiency (100%, 12/12 positive wells), we noticed that rCHAV-ZsG and rMACV-ZsG exhibited somewhat low titers as compared to those of rCHAV and rMACV (~10^5 vŝ10^6 $TCID_{50}$/mL, respectively) in different repeats of the rescue experiments. To further characterize the 4 recombinant viruses, we analyzed their growth kinetics in Vero-E6 cells at MOI of 0.01 for 5 days post infection (dpi). As shown in Fig. 2C, the 4 recombinant viruses exhibited a similar growth pattern and reached the highest titers at 3 dpi. Modest growth attenuation was observed for the ZsG-expressing viruses. This phenotypic characteristic is due to the incomplete cleavage of the self-cleaving peptide, resulting in a rather lower level of free available NP downstream. We have previously reported a similar phenomenon for different recombinant arenaviruses and filoviruses expressing reporter genes (Albariño et al., 2015; Welch et al., 2016). Furthermore, the stability of the reporter gene was confirmed up to 5 successive passages post-recovery of both recombinant viruses (Suppl. Fig. 2).

3.3. Antivirals screening

Antivirals were screened in Huh7 cells as Huh7 cells, a human hepatocyte-derived cell line, that is commonly used to screen antiviral compounds (Lo et al., 2021; McMullan et al., 2019; Welch et al., 2017). To evaluate the suitability to screen antiviral compounds in BSL-2 containment, we tested a limited set of previously described viral replication inhibitors on CHAV and MACV minigenomes. The Z' score for CHAPV and MACV minigenomes were calculated to be 0.60 and 0.62, respectively, indicating the suitability of minigenomes for HTS. As shown in Fig. 3A, 6-azaUridine (6-AzaU) and 2′-DFC, which were previously shown to block replication of the Lassa virus (LASV) minigenome (Welch et al., 2016), efficiently inhibited both CHAV and MACV minigenomes. In contrast, ribavirin only inhibited both minigenomes at high concentration, while favipiravir (T705) did not exhibit inhibitory effects (Fig. 3C). Furthermore, replication of both minigenomes was inhibited by baloxavir acid, although with moderate cytotoxicity (Fig. 3C). Interestingly, baloxavir was reported to block the replication of influenza virus by downregulating the CAP-snatching activity of the viral polymerase (Noshi et al., 2018), and thus, suggesting a similar effect on arenavirus transcription and replication (Howley et al., 2021).

The same set of antiviral compounds were further tested using the reporter-expressing viruses, rCHAV-ZsG and rMACV-ZsG. Concentration-response curves were generated with the compounds in Huh-7 cells infected with either rCHAV-ZsG or rMACV-ZsG at MOI of 0.3 (Fig. 3B). After 72 h, infection was measured by quantifying ZsG fluorescence levels. The Z′ scores were 0.72 for rCHAV/ZsG and 0.68 for rMACV/ZsG, indicating the suitability of both the reporter viruses for HTS of antiviral compounds. Comparably to the results of the minigenome assay (Fig. 3A), 6-AzaU, 2′-dFC, baloxavir, and ribavirin

exhibited antiviral activity against both viruses (Fig. 3B). A reduction in fluorescence was observed in cells treated with either of the 4 compounds, with maximum reduction seen in 2′-DFC treated cells, with EC_{50} values of 0.3 μM (rCHAV-ZsG) and 0.2 μM (rMACV-ZsG) (Fig. 3C). Interestingly, T705 inhibited both reporter-expressing viruses, but it did not inhibit the corresponding minigenomes, which could be attributed to the overexpression of the viral polymerase in the minigenome system. Similarly, we hypothesized that the used concentration of 2′DFC was sufficient to inhibit the virus replication, but not the minigenome replication, due to an overexpression of L in the latter system.

To confirm the suitability of reporter-expressing viruses as surrogates for the wild-type counterparts, we tested 2′-dFC, baloxavir, and T705 against wild-type rCHAV and rMACV. Similarly, to the reporter-expressing viruses, all 3 compounds inhibited the recombinant wild-type viruses in a dose-dependent manner (Suppl. Fig. 3).

4. Discussion

Outbreaks of mammarenaviruses have serious public health implications, especially in regions where the viruses are endemic (Silva-Ramos et al., 2021). The recent outbreak of CHAV in 2019, with documented human-to-human transmission, signifies the importance of studying these highly pathogenic viruses (Loayza Mafayle et al., 2022). The most effective way to prevent viral diseases is through vaccination, but no vaccines are approved for CHAV and MACV; in fact, the only approved vaccine for an arenavirus, Candid #1, is against Junin virus (JUNV) (Ambrosio et al., 2005; Maiztegui et al., 1998). Reverse genetics has been extensively used to generate attenuated variants as vaccine candidates (Emonet et al., 2011b; Golden et al., 2017; Koma et al., 2016; Martinez-Sobrido and de la Torre, 2016; Ortiz-Riano et al., 2013; Patterson et al., 2014b). However, their efficacy was only proved in animal models (Koma et al., 2016; Olschlager and Flatz, 2013; Silva-Ramos et al., 2021). In principle, our system could be used to generate attenuated CHAV and MACV variants, as potential vaccine candidates, but extensive studies will be needed to confirm the attenuation phenotypes.

Treatment options for highly pathogenic mammarenaviruses remain limited. Besides the clinical use of convalescent plasma, broad-spectrum antiviral nucleoside analogs such as ribavirin and favirpiravir (T-705) have also demonstrated a relative success in treating LASV patients (Raabe et al., 2017). While ribavirin is still recommended for treatment of diverse arenaviral infections, severe concerns were recently raised regarding to its partially effectivity and significant side effects (Cheng et al., 2022; Eberhardt et al., 2019; Salam et al., 2022).

Since no FDA-approved drugs are currently licensed to specifically treat these pathogens, discovery of new and safe antiviral options is urgently needed. In this regard, recombinant viruses expressing reporter genes have been successful used in high-throughput screening assays (Cai et al., 2018; Herring et al., 2021; Lee et al., 2008; Welch et al., 2016, 2017, 2021).

In this study, we developed CHAV and MACV minigenomes expressing ZsG that could be used to study the mechanisms of viral transcription-replication, and to screen antiviral compounds. Interestingly, we found a similar minigenome activity when supported by the homologous and the heterologous support proteins (N and L). This finding confirms the conservation of the replication machinery between these two related arenaviruses.

Furthermore, we generated recombinant CHAV and MACV, along with recombinant viruses expressing a fluorescent reporter gene (ZsG). Expression of ZsG allows for direct and rapid quantification of infected cells, while avoiding a time-consuming immunofluorescence detection of the virus. These reporter-expressing viruses were used as suitable surrogates for wild-type viruses and made the system optimal for rapid screening of antiviral compounds.

In conclusion, the set of reverse genetic tools (including minigenomes and recombinant viruses expressing a reporter gene) described in this report will facilitate the identification of antiviral compounds efficiently, and could also be used to study the transcription-replication mechanisms of these high-consequence pathogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Albariño CG, Bergeron E, Erickson BR, Khristova ML, Rollin PE, Nichol ST, 2009. Efficient reverse genetics generation of infectious Junin viruses differing in glycoprotein processing. J. Virol 83, 5606–5614. [PubMed: 19321606]

Albariño CG, Bird BH, Chakrabarti AK, Dodd KA, Erickson BR, Nichol ST, 2011. Efficient rescue of recombinant Lassa virus reveals the influence of S segment noncoding regions on virus replication and virulence. J. Virol 85, 4020–4024. [PubMed: 21307206]

Albariño CG, Wiggleton Guerrero L, Lo MK, Nichol ST, Towner JS, 2015. Development of a reverse genetics system to generate a recombinant Ebola virus Makona expressing a green fluorescent protein. Virology 484, 259–264. [PubMed: 26122472]

Ambrosio AM, Riera LM, Saavedra Mdel C, Sottosanti MJ, 2005. [Preclinical assay of candid #1 vaccine against Argentine Hemorrhagic Fever made in Argentina]. Medicina 65, 329–332. [PubMed: 16193711]

Bergeron E, Chakrabarti AK, Bird BH, Dodd KA, McMullan LK, Spiropoulou CF, Nichol ST, Albarino CG, 2012. Reverse genetics recovery of Lujo virus and role of virus RNA secondary structures in efficient virus growth. J. Virol 86, 10759–10765. [PubMed: 22837210]

Buchholz UJ, Finke S, Conzelmann KK, 1999. Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the

human RSV leader region acts as a functional BRSV genome promoter. J. Virol 73, 251–259. [PubMed: 9847328]

Cai Y, Iwasaki M, Beitzel BF, Yu S, Postnikova EN, Cubitt B, DeWald LE, Radoshitzky SR, Bollinger L, Jahrling PB, Palacios GF, de la Torre JC, Kuhn JH, 2018. Recombinant Lassa virus expressing green fluorescent protein as a tool for high-throughput drug screens and neutralizing antibody assays. Viruses 10.

Cajimat MN, Milazzo ML, Rollin PE, Nichol ST, Bowen MD, Ksiazek TG, Fulhorst CF, 2009. Genetic diversity among Bolivian arenaviruses. Virus Res 140, 24–31. [PubMed: 19041349]

Cheng HY, French CE, Salam AP, Dawson S, McAleenan A, McGuinness LA, Savovic J, Horby PW, Sterne JAC, 2022. Lack of evidence for ribavirin treatment of Lassa fever in systematic review of published and unpublished studies (1). Emerg. Infect. Dis 28, 1559–1568. [PubMed: 35876478]

Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST, 2008. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. PLoS Pathog 4, e1000047. [PubMed: 18421377]

Eberhardt KA, Mischlinger J, Jordan S, Groger M, Gunther S, Ramharter M, 2019. Ribavirin for the treatment of Lassa fever: a systematic review and meta-analysis. Int. J. Infect. Dis 87, 15–20. [PubMed: 31357056]

Emonet SE, Urata S, de la Torre JC, 2011a. Arenavirus reverse genetics: new approaches for the investigation of arenavirus biology and development of antiviral strategies. Virology 411, 416–425. [PubMed: 21324503]

Emonet SF, Seregin AV, Yun NE, Poussard AL, Walker AG, de la Torre JC, Paessler S, 2011b. Rescue from cloned cDNAs and in vivo characterization of recombinant pathogenic Romero and live-attenuated Candid #1 strains of Junin virus, the causative agent of Argentine hemorrhagic fever disease. J. Virol 85, 1473–1483. [PubMed: 21123388]

Escalera-Antezana JP, Rodriguez-Villena OJ, Arancibia-Alba AW, Alvarado-Arnez LE, Bonilla-Aldana DK, Rodriguez-Morales AJ, 2020. Clinical features of fatal cases of Chapare virus hemorrhagic fever originating from rural La Paz, Bolivia, 2019: a cluster analysis. Trav. Med. Infect. Dis 36, 101589.

Gerrard SR, Bird BH, Albariño CG, Nichol ST, 2007. The NSm proteins of Rift Valley fever virus are dispensable for maturation, replication and infection. Virology 359, 459–465. [PubMed: 17070883]

Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW, 2017. An attenuated Machupo virus with a disrupted L-segment intergenic region protects Guinea pigs against lethal Guanarito virus infection. Sci. Rep 7, 4679. [PubMed: 28680057]

Herring S, Oda JM, Wagoner J, Kirchmeier D, O'Connor A, Nelson EA, Huang Q, Liang Y, DeWald LE, Johansen LM, Glass PJ, Olinger GG, Ianevski A, Aittokallio T, Paine MF, Fink SL, White JM, Polyak SJ, 2021. Inhibition of arenaviruses by combinations of orally available approved drugs. Antimicrob. Agents Chemother 65.

Howley PM, Knipe DM, Whelan SPJ, 2021. Fields Virology, seventh ed. Wolters Kluwer, Philadelphia.

Johnson KM, Kuns ML, Mackenzie RB, Webb PA, Yunker CE, 1966. Isolation of Machupo virus from wild rodent Calomys callosus. Am. J. Trop. Med. Hyg 15, 103–106. [PubMed: 5901620]

Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S, 2016. Machupo virus expressing GPC of the Candid#1 vaccine strain of Junin virus is highly attenuated and immunogenic. J. Virol 90, 1290–1297. [PubMed: 26581982]

Lan S, McLay Schelde L, Wang J, Kumar N, Ly H, Liang Y, 2009. Development of infectious clones for virulent and avirulent pichinde viruses: a model virus to study arenavirus-induced hemorrhagic fevers. J. Virol 83, 6357–6362. [PubMed: 19386714]

Lee AM, Rojek JM, Gundersen A, Stroher U, Juteau JM, Vaillant A, Kunz S, 2008. Inhibition of cellular entry of lymphocytic choriomeningitis virus by amphipathic DNA polymers. Virology 372, 107–117. [PubMed: 18022208]

Lo MK, Shrivastava-Ranjan P, Chatterjee P, Flint M, Beadle JR, Valiaeva N, Murphy J, Schooley RT, Hostetler KY, Montgomery JM, Spiropoulou CF, 2021. Broad-spectrum in vitro antiviral activity

of ODBG-P-RVn: an orally-available, lipid-modified monophosphate prodrug of remdesivir parent nucleoside (GS-441524). Microbiol. Spectr 9, e0153721. [PubMed: 34817209]

Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Davalos Anajia A, Mendoza Loayza N, Montano AM, Morales Alvis FL, Revollo Guzman J, Sasias Martinez S, Alarcon De La Vega G, Medina Ramirez A, Molina Gutierrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Anez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson BR, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM, 2022. Chapare hemorrhagic fever and virus detection in rodents in Bolivia in 2019. N. Engl. J. Med 386, 2283–2294. [PubMed: 35704480]

Mackenzie RB, Beye HK, Valverde L, Garron H, 1964. Epidemic hemorrhagic fever in Bolivia. I. A preliminary report of the epidemiologic and clinical findings in a new epidemic area in South America. Am. J. Trop. Med. Hyg 13, 620–625. [PubMed: 14196061]

Maiztegui JI, McKee KT Jr., Barrera Oro JG, Harrison LH, Gibbs PH, Feuillade MR, Enria DA, Briggiler AM, Levis SC, Ambrosio AM, Halsey NA, Peters CJ, 1998. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. J. Infect. Dis 177, 277–283. [PubMed: 9466512]

Martinez-Sobrido L, Cheng BY, de la Torre JC, 2016. Reverse genetics approaches to control arenavirus. Methods Mol. Biol 1403, 313–351.

Martinez-Sobrido L, de la Torre JC, 2016. Reporter-expressing, replicating-competent recombinant arenaviruses. Viruses 8.

McMullan LK, Flint M, Chakrabarti A, Guerrero L, Lo MK, Porter D, Nichol ST, Spiropoulou CF, Albarino C, 2019. Characterisation of infectious Ebola virus from the ongoing outbreak to guide response activities in the Democratic Republic of the Congo: a phylogenetic and in vitro analysis. Lancet Infect. Dis 19, 1023–1032. [PubMed: 31300330]

Noshi T, Kitano M, Taniguchi K, Yamamoto A, Omoto S, Baba K, Hashimoto T, Ishida K, Kushima Y, Hattori K, Kawai M, Yoshida R, Kobayashi M, Yoshinaga T, Sato A, Okamatsu M, Sakoda Y, Kida H, Shishido T, Naito A, 2018. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. Antivir. Res 160, 109–117. [PubMed: 30316915]

Olschlager S, Flatz L, 2013. Vaccination strategies against highly pathogenic arenaviruses: the next steps toward clinical trials. PLoS Pathog 9, e1003212. [PubMed: 23592977]

Ortiz-Riano E, Cheng BYH, Carlos de la Torre J, Martinez-Sobrido L, 2013. Arenavirus reverse genetics for vaccine development. J. Gen. Virol 94, 1175–1188. [PubMed: 23364194]

Patterson M, Grant A, Paessler S, 2014a. Epidemiology and pathogenesis of Bolivian hemorrhagic fever. Current opinion in virology 5, 82–90. [PubMed: 24636947]

Patterson M, Koma T, Seregin A, Huang C, Miller M, Smith J, Yun N, Smith J, Paessler S, 2014b. A substitution in the transmembrane region of the glycoprotein leads to an unstable attenuation of Machupo virus. J. Virol 88, 10995–10999. [PubMed: 25031335]

Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S, 2014c. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (alphabeta/gamma) receptor double knockout mice. J. Virol 88, 1914–1923. [PubMed: 24284323]

Peters CJ, Kuehne RW, Mercado RR, Le Bow RH, Spertzel RO, Webb PA, 1974. Hemorrhagic fever in cochabamba, Bolivia, 1971. Am. J. Epidemiol 99, 425–433. [PubMed: 4601699]

Raabe VN, Kann G, Ribner BS, Morales A, Varkey JB, Mehta AK, Lyon GM, Vanairsdale S, Faber K, Becker S, Eickmann M, Strecker T, Brown S, Patel K, De Leuw P, Schuettfort G, Stephan C, Rabenau H, Klena JD, Rollin PE, McElroy A, Stroher U, Nichol S, Kraft CS, Wolf T, Emory Serious Communicable Diseases, U, 2017. Favipiravir and ribavirin treatment of epidemiologically linked cases of Lassa fever. Clin. Infect. Dis 65, 855–859. [PubMed: 29017278]

Salam AP, Duvignaud A, Jaspard M, Malvy D, Carroll M, Tarning J, Olliaro PL, Horby PW, 2022. Ribavirin for treating Lassa fever: a systematic review of preclinical studies and implications for human dosing. PLoS Neglected Trop. Dis 16, e0010289.

Sanchez AB, de la Torre JC, 2006. Rescue of the prototypic Arenavirus LCMV entirely from plasmid. Virology 350, 370–380. [PubMed: 16476461]

Silva-Ramos CR, Faccini-Martinez AA, Calixto OJ, Hidalgo M, 2021. Bolivian hemorrhagic fever: a narrative review. Trav. Med. Infect. Dis 40, 102001.

Welch SR, Guerrero LW, Chakrabarti AK, McMullan LK, Flint M, Bluemling GR, Painter GR, Nichol ST, Spiropoulou CF, Albarino CG, 2016. Lassa and Ebola virus inhibitors identified using minigenome and recombinant virus reporter systems. Antivir. Res 136, 9–18. [PubMed: 27771389]

Welch SR, Scholte FEM, Flint M, Chatterjee P, Nichol ST, Bergeron E, Spiropoulou CF, 2017. Identification of 2'-deoxy-2'-fluorocytidine as a potent inhibitor of Crimean-Congo hemorrhagic fever virus replication using a recombinant fluorescent reporter virus. Antivir. Res 147, 91–99. [PubMed: 29024765]

Welch SR, Spengler JR, Genzer SC, Chatterjee P, Flint M, Bergeron E, Montgomery JM, Nichol ST, Albarino CG, Spiropoulou CF, 2021. Screening and identification of Lujo virus inhibitors using a recombinant reporter virus platform. Viruses 13.

Ye C, de la Torre JC, Martinez-Sobrido L, 2020. Development of reverse genetics for the prototype new World Mammarenavirus Tacaribe virus. J. Virol 94.

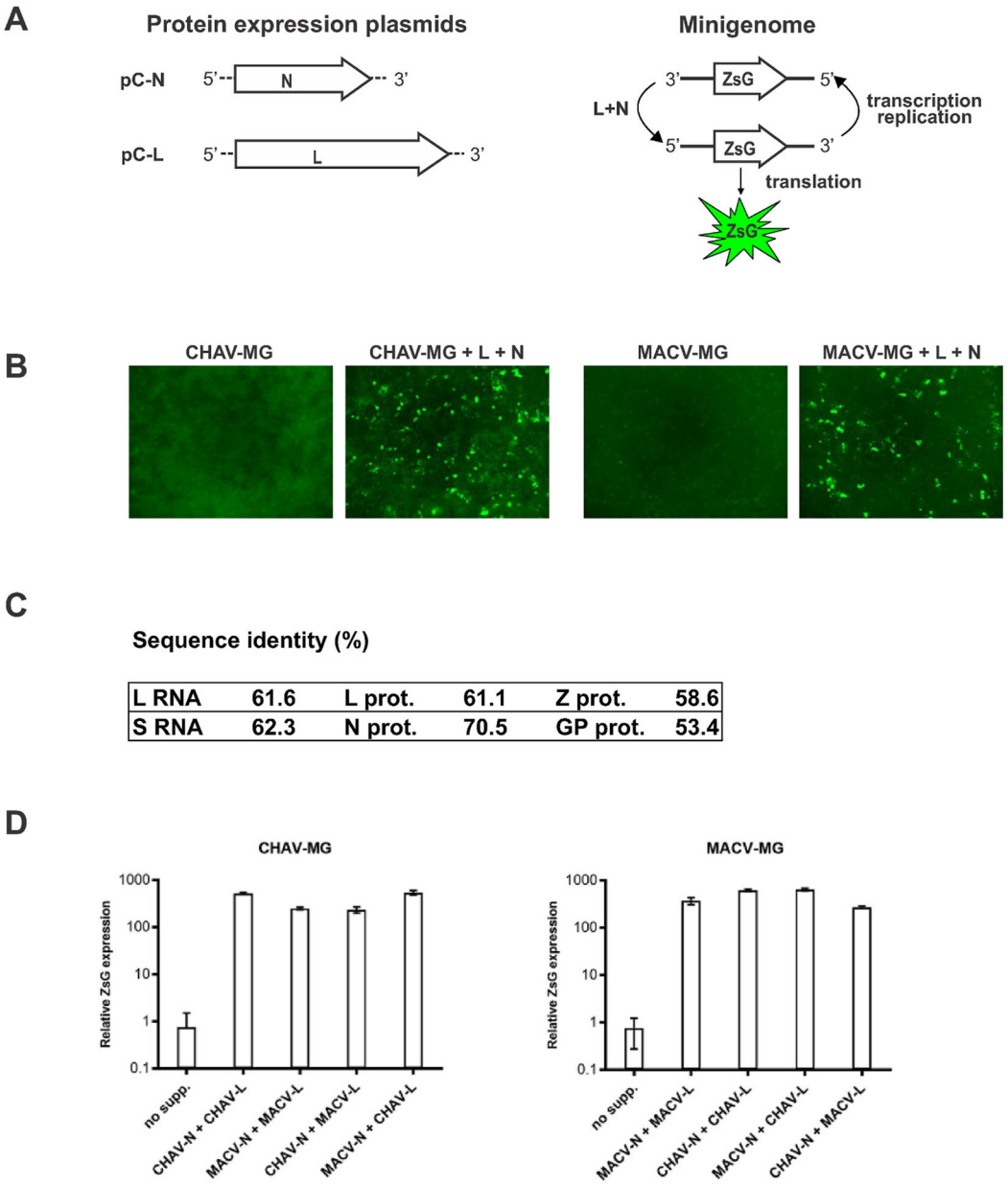


Fig. 1. Design and characterization of minigenomes.

(A) Schematics of Chapare and Machupo virus minigenomes and support plasmids (pC-N and pC-L). CHAV and MACV minigenomes contain the antigenome UTRs of the CHAV S segment and MACV S segment, respectively, in which the nucleocapsid protein (N) and glycoprotein precursor (GPC) coding sequences have been replaced with ZsGreen (ZsG). Plasmids expressing CHAV or MACV polymerase (L) and nucleoprotein (N) were provided in trans. **(B) Replication of CHAV and MACV minigenomes with or without their respective support plasmids.** Representative fluorescence images of Vero-E6 cells transfected with minigenomes of indicated viruses with (+L + N) or without support plasmids specific that minigenome. **(C) Sequence similarity between CHAV and MACV genes and proteins.** The table shows the percent nucleotide similarity between the

L and S segments of CHAV and MACV along with the percentage similarity between the different proteins of CHAV and MACV. **(D) Compatibility of CHAV and MACV proteins.** After transfection with the indicated combinations of support plasmids and minigenomes, minigenome replication was measured using BioTek Cytation for ZsG-positive cells. Relative ZsG expression was measured with values normalized to non-transfected cells. All experiments were done in quadruplicate.

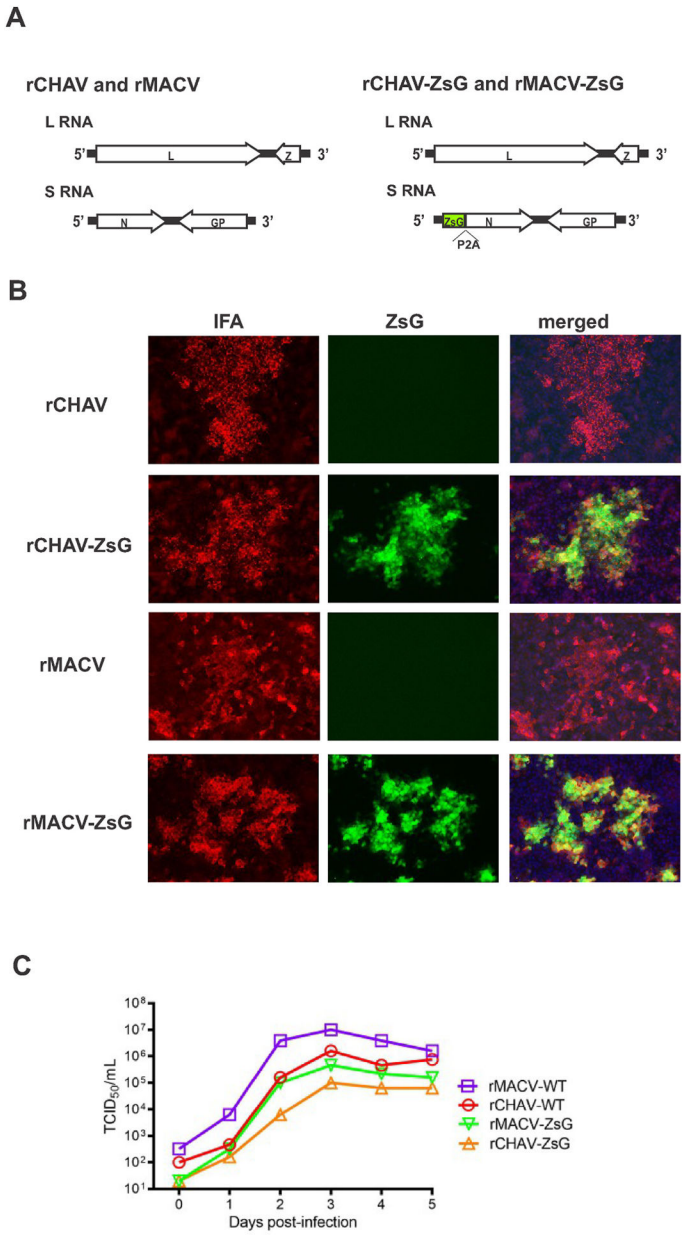
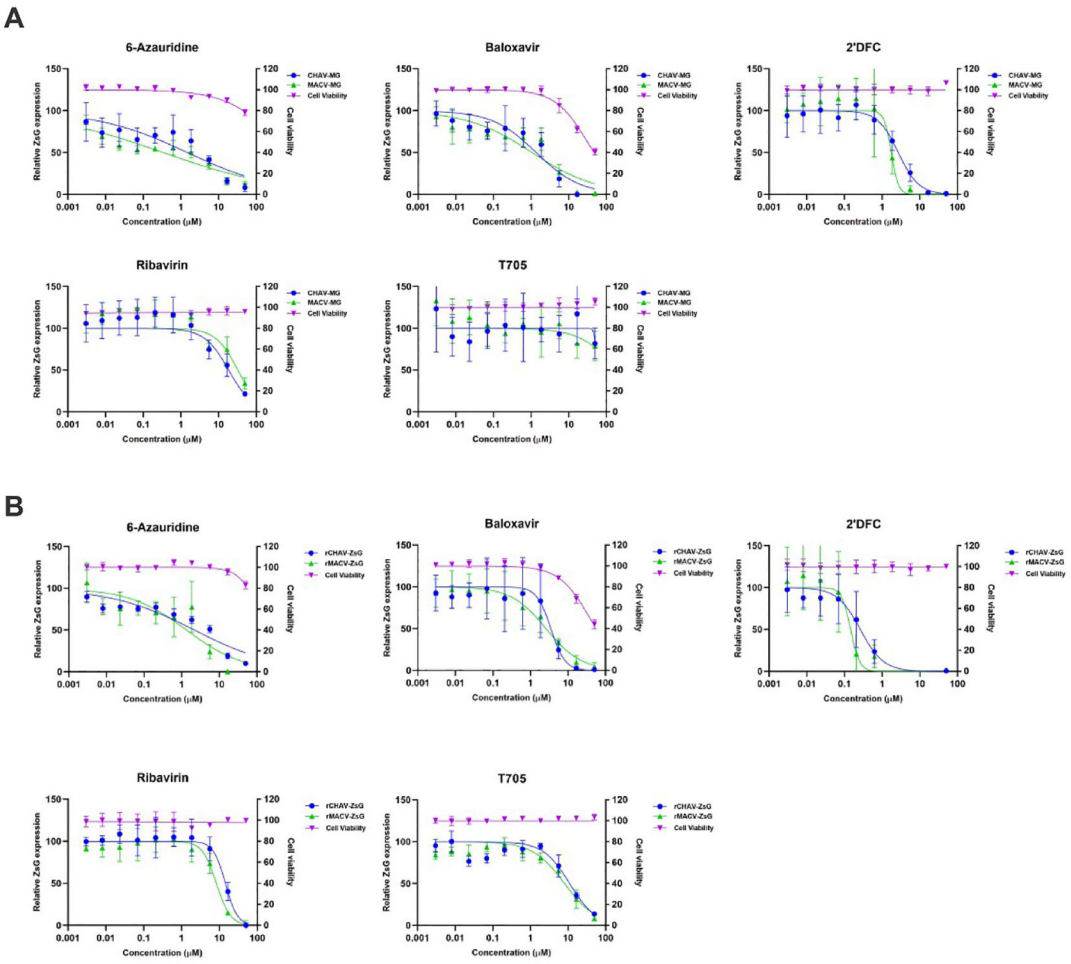


Fig. 2. Design and characterization of recombinant viruses.

(A) Schematic representation of the genomes of recombinant CHAV and MACV with or without ZsG protein. Recombinant reporter viruses contain ZsG coding sequence fused to N via a porcine teschovirus-1 2A (P2A). **(B)** Immunofluorescence assay (IFA) was performed on Vero-E6 cells infected with different recombinant viruses using either *anti*-CHAV nucleoprotein antibody (for CHAV) or hyperimmune serum raised against South American arenaviruses (Guanarito, Machupo, and Sabiá viruses; for MACV). Representative IFA images (magnification 20 × ; EVOS microscope) of recombinant wild-type CHAV (rCHAV), recombinant reporter CHAV (rCHAV-ZsG), recombinant wild-type MACV (rMACV), and recombinant reporter (rMACV-ZsG). **(C)** Growth kinetics of different recombinant viruses in Vero-E6 cells.



C

Compound	CC_{50} (μM)	CHAV-MG		MACV-MG		rCHAV-ZsG		rMACV-ZsG	
		EC_{50} (μM)	SI_{50} (μM)	EC_{50} (μM)	SI_{50} (μM)	EC_{50} (μM)	SI_{50} (μM)	EC_{50} (μM)	SI_{50} (μM)
6-Azauridine	>50	1.5	>33	0.3	>167	2.2	>22.7	1.3	>39
Baloxavir	32	1.4	23	1.1	29	3.5	9	2.6	12
2'DFC	>50	2.7	>19	1.8	>28	0.3	>167	0.2	>250
Ribavirin	>50	18	>2.8	32	>1.6	14	>3.5	8	>6
T705	>50	55	>0.9	185	>0.3	11	>4.5	8	>6.3

Fig. 3. Screening antivirals using reverse genetics tools.

(A) Screening using minigenome assays. Concentration-response curves in Huh-7 cells transfected with CHAV (CHAV-MG, blue circles) and MACV (MACV-MG, green triangles) minigenomes and their respective support plasmids treated with nucleoside analogs. Reductions in ZsG fluorescence in infected cells were measured, with values normalized to mock-treated cells (DMSO only). For each concentration of antivirals, cell viability was measured via CellTiter-Glo assay (purple), and values were normalized to those in mock-treated cells. Each point on the graph represents the mean, and error bars indicate standard deviations of quadruplicate wells. **(B) Screening using recombinant viruses.** Concentration-response curves in Huh-7 cells infected with rCHAV-ZsG (blue circles) or rMACV-ZsG (green triangles) after treatment with various antiviral compounds. For each

concentration of antivirals, cell viability was measured via CellTiter-Glo assay (purple), and values were normalized to those in mock-treated cells. Each point on the graph represents mean and error bars indicate standard deviations of quadruplicate wells. **(C)** 50% effective inhibition concentration (EC_{50}), 50% cell cytotoxicity (CC_{50}), and selective index (SI) values for each compound against CHAV-MG, MACV-MG, rCHAV-ZsG, or rMACV-ZsG.