Current Microbiology (2025) 82:334
https://doi.org/10.1007/s00284-025-04320-z

A New Vaccine Candidate Expressing JUNV GP1–GP2 Against Argentine Hemorrhagic Fever Based on Baculovirus Surface Display

Carla Tomatis[1,2] · Mara C. A. M. Aguiar[2] · Nancy Charo[2] · Silvia Aquila[1] · Pablo D. Thomas[1] · Paula N. Arrías[3] · Matías L. Pidre[3] · Víctor Romanowski[3] · Laura Riera[4] · Julia Brignone[4] · Andrea Maiza[4] · Eugenio Antonio Carrera Silva[2] · María Florencia Ferrer[1] · Ricardo Martín Gómez[1]

Received: 30 December 2024 / Accepted: 29 May 2025

Abstract

With the aim of developing a new vaccine candidate using a recombinant baculovirus (rBV) expressing the Junín virus (JUNV) GP1 and GP2 proteins on its surface, a pBacPAK9 vector containing the BV-GP64 signal peptide, the JUNV GP1–GP2 and the transmembrane domain of the vesicular stomatitis virus G (VSV-G) protein were synthesized. This plasmid was co-transfected with the bApGOZA bacmid into Sf9 cells. The rBV was recovered from the supernatant. Protein expression was confirmed with a human serum by Western blot and with an anti-GPC monoclonal antibody by IFI staining and virometry. In an ELISA test, mice immunized with rBV exhibited specific antibody levels that also showed neutralizing properties in a plaque reduction test compared to controls. Guinea pigs immunized with rBV and challenged with JUNV showed partial protection compared to the Candid#1 vaccine. We have developed an rBV surface display platform to validate a potential JUNV vaccine candidate.

Introduction

Argentine hemorrhagic fever (AHF), a serious disease caused by the Junín virus (JUNV), continues to be a major public health problem in Argentina. The high mortality rate of the disease, which is between 20 and 40% without treatment, underlines its severity [1, 2].

JUNV belongs to the genus *Mammarenavirus*, a group of viruses that mainly infect rodents and are restricted to certain geographic regions [3]. An exception is the lymphocytic choriomeningitis virus (LCMV), which infects *Mus musculus* and spreads worldwide. In contrast, most mammarenaviruses are restricted to specific geographic regions, which gives them an endemic character [4].

In their natural rodent hosts, mammarenaviruses often cause persistent asymptomatic infections. However, these viruses can occasionally be transmitted to humans and cause severe hemorrhagic fevers (HF). In addition to JUNV, other mammarenaviruses associated with HF are Machupo (MACV) and Chapare (CHPV) in Bolivia and Sabiá (SABV) in Brazil and Lassa (LASV) in Africa. In contrast, some mammarenaviruses such as Tacaribe (TCRV), Pichindé (PICV), and Mopeia virus (MOPV) do not normally cause disease [2]. Mammarenaviruses pose a significant threat as emerging pathogens. Human activities such as deforestation and urbanization lead to increased contact with wild rodents in new environments. This increased interaction increases the risk of future outbreaks and the discovery of new mammarenavirus isolates [4].

JUNV has a bisegmented, single-stranded RNA genome that uses an ambisense coding strategy in both segments. The L segment codes for the RNA-dependent RNA polymerase (L) and the matrix protein (Z). The smaller S segment codes for the nucleoprotein (NP) and the glycoprotein

✉ María Florencia Ferrer
mflorenciaferrer@yahoo.com

✉ Ricardo Martín Gómez
rmg1426@gmail.com

1 Laboratorio de Patogénesis Viral, Instituto de Biotecnología y Biología Molecular (IBBM), CONICET-UNLP, Calle 49 y 115, 1900 La Plata, Buenos Aires, Argentina

2 Laboratorio de Trombosis Experimental, Instituto de Medicina Experimental (IMEX), Academia Nacional de Medicina, Buenos Aires, Argentina

3 Laboratorio de Virología Molecular, Instituto de Biotecnología y Biología Molecular (IBBM), CONICET-UNLP, La Plata, Buenos Aires, Argentina

4 Instituto Nacional de Enfermedades Virales Humanas (INEVH), ANLIS Malbrán, Pergamino, Argentina

Published online: 13 June 2025

precursor (GPC). After post-translational cleavage, GPC produces the mature virion glycoproteins GP1, GP2 and the stable signal peptide SSP. SSP plays a crucial role in the formation of the spikes that adorn the viral surface [5].

A live viral vaccine, Candid#1 (C#1), is effective against AHF. Since its introduction several decades ago, AHF cases have decreased significantly. C#1 is produced using the FRhL-2 cell line (fetal lung of rhesus monkeys) and its attenuation has been demonstrated in guinea pigs [6]. Attenuated vaccines such as C#1 cannot be administered to pregnant or immunocompromised individuals. For this reason, the development of new vaccine candidates is desirable [7].

Baculoviruses (BVs) are insect viruses that have been used for decades to express recombinant heterologous proteins. Among the most commonly used is the *Autographa californica multicapsid nucleopolyhedrovirus* (AcMNPV). Several BV vaccines are already commercially available worldwide, and numerous others are in development or clinical trials [8]. Baculovirus display technology is widely used in the research and development of antibodies, vaccines and biopharmaceuticals (reviewed in [9]).

In this article, a vaccine candidate against AHF produced using a baculovirus surface display expression system is presented.

Materials and Methods

Virus and Cells

Baby hamster kidney (BHK)−21 and Vero cells were maintained in monolayers as previously described [10]. The attenuated Candid 1 strain and virulent P3790 of JUNV were obtained from the *Instituto Nacional de Enfermedades Virales Humanas* (INEVH). JUNV stocks and titration of infectivity were prepared as previously described [10]. High-Five cells derived from the *Trichoplusia ni* and *Spodoptera frugiperda* Sf9 cell line (ATCC CRL-1711) and *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV, WT) or recombinant baculovirus AcMNPV (rBV) were propagated in Grace's medium containing 10% fetal bovine serum (BioSer, Argentina) and titrated as previously described [11].

Production of Recombinant Baculovirus

Recombinant BV (rBV) was generated by homologous DNA recombination of the transfer vector pBacPAK9 GP64-GP1-GP2-VSV-G synthesized by Genscript (Hong Kong, China), which contains the essential gene ORF1629, with the bacmid bAcGOZA, which lacks this essential gene, using the reagent Cellfectin II, as described [11]. To obtain budding virions of baculovirus AcMNPV (WT) or rBV, Sf9 cells were infected at MOI 1- and 3-day postinfection (dpi) the supernatant was harvested and titrated.

DNA Isolation and PCR

For gene expression analysis, rBV were harvested and DNA extraction was performed as previously described [12]. PCR reactions were performed with 1 µl of DNA as described [10]. The primers used in this study were (5'−3') Bac1 (fwd) AACCATCTCGCAAATAAATA and GPC (Rev) AGTTTA CCCTGCCTGTCCG.

Western Blot Analysis

The expression of GP1–GP2 in rBV was analyzed by Western blot (WB). In brief, 15 ml containing 5×10^7 PFU WT and rBV were centrifuged at 16,000 g for one hour and resuspended in 100 µl. The presence of GP1–GP2 was tested with a human convalescent serum (1:500) and an anti-human IgG HRP (Biolegend) as previously described [11].

Immunofluorescence Staining

Immunofluorescence staining (IFI) was performed on HighFive cells grown on glass coverslips as previously described [13] using a mAb anti-GPC (clone GB03-BE08, BEI resources) and an anti-mouse immunoglobulin G (GenScript) coupled to FITC. Confocal images were acquired using a Leica TCS SP5 II microscope. This microscope was equipped with a HCX PL APO CS 63.0 × 1.40 oil ultraviolet objective, a helium–neon laser with a wavelength of 543 nm, an argon ion laser with a wavelength of 488 nm, and the software LAS AF version 2.2.1 4842.

Flow Cytometry Analysis

The expression of GP1–GP2 in rBV was analyzed by flow cytometry. In brief, 5×10^6 PFU rBV were centrifuged at 16,000*g* for one hour and fixed with 0.1% PFA. The presence of GP1–GP2 was tested using the described mAb anti-GPC and an anti-mouse IgG labeled with Alexa Fluor 647 (Biolegend). DNA was stained with DAPI. Recombinant BV with GFP expression on the surface was used for initial recruitment and to confirm specificity. Analyses were performed using a FACS Canto I (Becton Dickinson, USA) or Partec-Sysmex CyFlow flow cytometer (Görlitz, Germany). All analyses were performed with FlowJo software (Tree Star).

Humoral Immune Response

Six-week-old female BALB/c mice were obtained from the animal facility of the *Instituto de Medicina Experimental* (IMEX). The animals were free of pathogens and kept in a

special pathogen-free environment. All animal experiments were performed according to the guidelines and approved protocol of the Institutional Animal Care and Use Committee (CICUAL) of IMEX. To evaluate the humoral immune response induced by rBV, we performed immunization tests with mice. Five mice in each experimental group were lightly anesthetized with CO_2 and immunized intramuscularly (IM) twice at two-week intervals with 50-ml PBS containing 1×10^7 PFU rBV ($n = 6$) or 100-µl PBS alone ($n = 3$) as negative controls. Immunization was repeated three times at two-week intervals. Ten days after the second and third immunization, mouse sera were collected to investigate the presence of reactive JUNV-specific antibodies using a previously described ELISA test [14]. This test is used for diagnosis in humans and determines whether a serum is positive or not. The results are presented as the delta (OD difference) between specific plate Ig and irrelevant Ig. A standard serum neutralization test with Vero cells was performed as described [15]. The 50% plaque reduction neutralization antibody titer (PRNT50) was defined as the reciprocal of the highest serum dilution indicating a 50% reduction in plaque counts compared to viral control counts. IgG subtypes 1–3 were determined by ELISA as previously described [16].

Viral Challenge

Female guinea pigs (*Cavia porcellus*) weighing 250–400 g were purchased from HG (www.hgilardoni.com.ar). The animals were free of pathogens and maintained in a special pathogen-free environment at the *Instituto Nacional de Enfermedades Virales Humanas* (INEVH), ANLIS Malbrán, Pergamino. All animal experiments were performed according to the guidelines and approved protocol of the INEVH (form 0034-VVd15-02). All personnel were vaccinated.

To evaluate protection against virulent JUNV infection, guinea pigs immunized with rBV were compared with those immunized with WT-BV, with C#1 serving as a positive control. The JUNV challenge of the immunized guinea pigs was performed as follows: Groups of animals ($n = 5$) were immunized once with a standard dose of C#1 vaccine or, in the case of rBV and WT-BV, twice with 1×10^7 PFU IM 2 weeks apart. After the last immunization, a reboost with 1×10^5 PFU was performed 2 weeks later. Ten days later, the guinea pigs were infected subcutaneously (SC) with a lethal dose 50 (LD_{50}) of virulent JUNV (100 PFU). The LD_{50} was previously determined in guinea pigs.

Statistical Analysis

All results are expressed as mean values ± SEMs. To determine significant differences between the means of two groups, a paired Student *t* test was performed, with *p* values below 0.05 considered statistically significant. When multiple groups were compared, a one- or two-way analysis of variance (ANOVA) followed by Tukey or Fisher multiple comparison test was used to determine significant differences between groups.

Results

Design of the Vaccine Candidate

We first constructed a vector containing the signal peptide of BV-GP64 and the C-terminal domain of the VSV-G protein, with the JUNV GP1–GP2 positioned in the center (Fig. 1a). The recombinant gene was 1353 bp long and yielded a chimeric protein with a molecular weight of 45–48 KDa. The VSV-G protein was used to ensure uniform distribution of the chimeric protein on the surface of the budding virion (Fig. 1b).

rBV Characterization

To produce rBV, Sf9 cells were infected with rBV at a multiplicity of infection (MOI) of 1. After 3 rounds of amplification, we detected the recombinant gene in the DNA extracted from rBV by PCR using a primer set covering BV DNA and JUNV GP1 (Fig. 2a). The presence of the recombinant antigen at the protein level was confirmed by WB (Fig. 2b). HighFive cells grown on glass coverslips were then infected with rBV or WT-BV (MOI 1). 3 dpi cells were washed and then fixed with 4% PFA. We confirmed the expression of the chimeric protein by IFI analysis using an anti-GPC (Fig. 2c). In addition, cell surface expression of JUNV GP1–GP2 in rBV was analyzed by flow cytometry. Chimeric GPC was detected with the described mAb anti-GPC (clone GB03-BE08) and an anti-mouse AF647 (Biolegend), while DNA was detected with DAPI (Fig. 2d).

Humoral Immune Response

JUNV-specific antibodies were detected in the serum of more than 30% of the mice in the rBV-immunized group 10 days after the second immunization (Fig. 3a) and in 100% of the mice after the third immunization (Fig. 3b). The serum neutralization test showed that significant levels of neutralizing antibodies were found in the serum of the rBV-immunized mice (Fig. 3b), with IgG1 showing significantly higher levels (Fig. 3c).

Protection Test

To evaluate protection against virulent JUNV infection, guinea pigs immunized with rBV were compared with those immunized with WT-BV, with C#1 serving as a

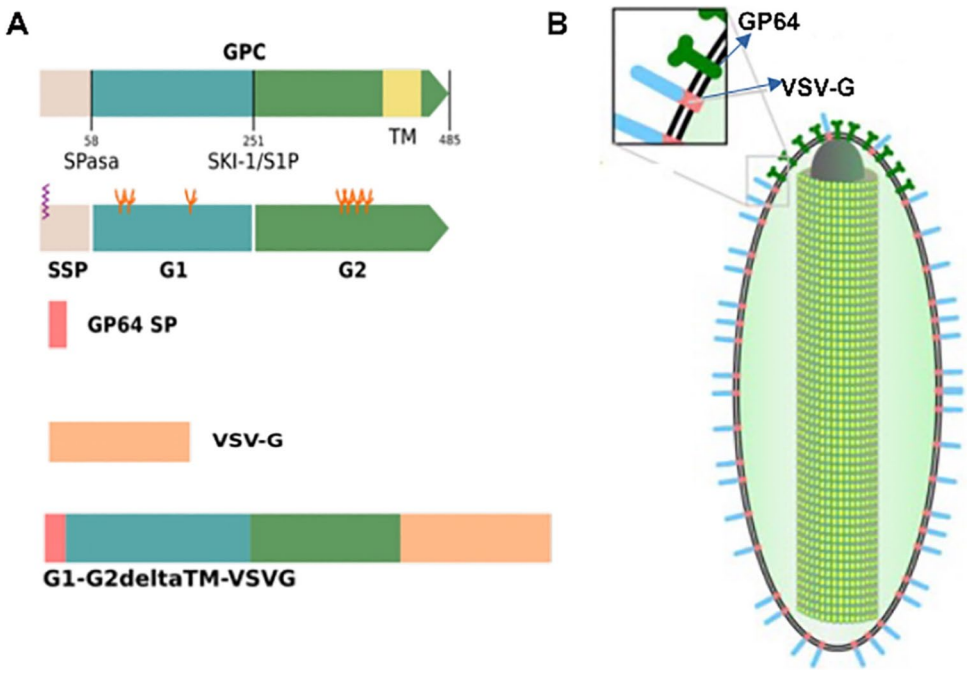

Fig. 1 Design of the recombinant BV. **a** Design of the chimeric protein leading to the exposure of the JUNV proteins GP1–GP2 on the surface of budding baculovirus using the signal peptide of the baculoviral GP64 protein (N-terminal) and the vesicular stomatitis virus G protein (C-terminal), resulting in recombinant baculovirus (rBV)-GP1-GP2-VSV-G. **b** The VSV-G protein enables homogeneous distribution on the surface of the budding baculovirus

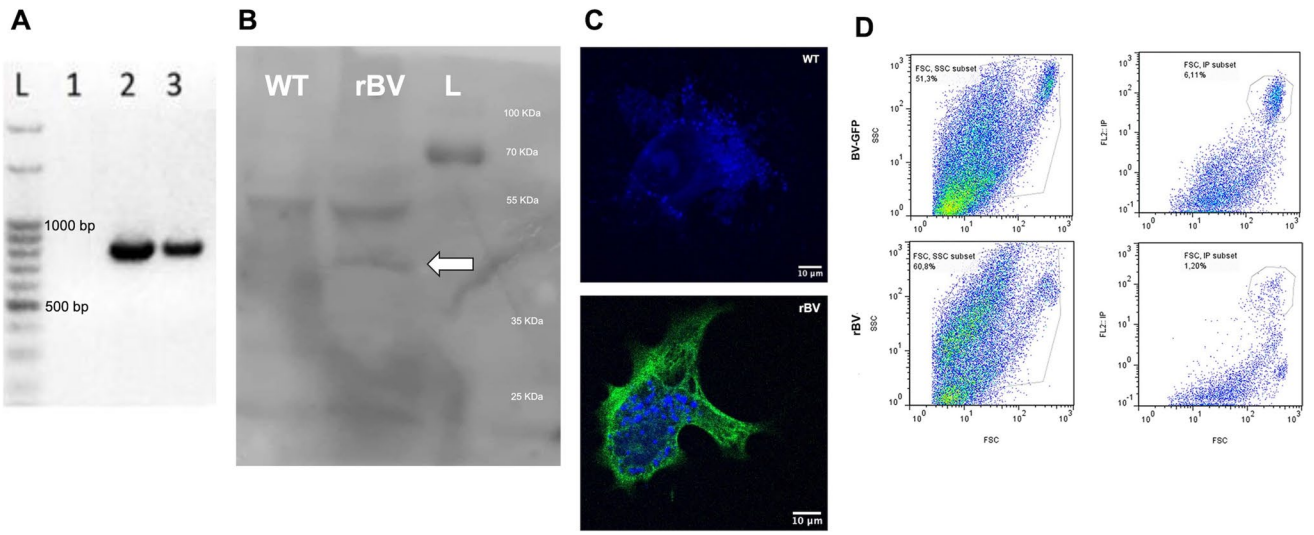

Fig. 2 Characterization of the recombinant BV. **a** PCR with a primer set covering the BV DNA and the JUNV GP1 ORF, using DNA extracted from rBV [13]. A 30-cycle PCR was performed with 1 µl of viral DNA and the primers (5'−3') Bac1 AACCATCTCGCAAAT AAATA and GPC reverse AGTTTACCCTGCCTGTCCG. (L: 100pb DNA ladder, lane 1: negative control, lane 2: pBacPAK9 BV-GP64-GP1-GP2-VSV-G as positive control, lane 3: rBV). As expected, a band above 800 bp was observed. **b** Representative Western blot analysis of semi-purified WT and rBV samples using a human convalescent serum. The arrow indicates the expected band size. **c** Fluorescence images of HighFive cells infected with BV-WT as negative control or rBV using an anti-GPC monoclonal antibody. DAPI staining of DNA shows multiple polyhedra inside the cell (BV-WT), while JUNV antigens can be detected on budding viruses and the cel surface (rBV). Images were taken with a Leica TCS SP5 II microscope. **d** Detection of JUNV antigens on the surface of recombinant BV was examined by flow cytometry (lower panel). Recombinant BV with GFP expression on the surface was used to confirm specificity (upper panel). DNA staining was performed with the dye DAPI to mark the gate in the BVs. Each experiment was repeated twice

positive control. The animals were observed for 30 days to monitor their survival rate. While 100% of the guinea pigs immunized with C#1 survived, all guinea pigs immunized with WT-BV died within 26 days. In contrast, 40% of

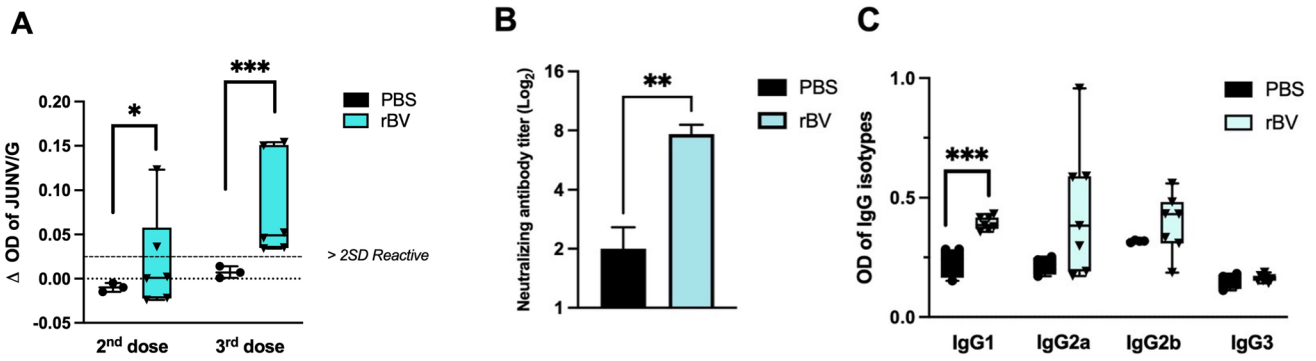

Fig. 3 Humoral immune response to recombinant BV. **a** Detection of specific antibodies against JUNV was performed by indirect ELISA in a serum dilution of 1:100 from the two immunized groups after the second and third immunization. The results are presented as delta (OD difference) between specific plate and irrelevant Ig. This test can be used to determine whether a serum is reactive or not. **b** The detection of neutralizing antibodies was investigated in mouse sera after the third immunization with PBS or rBV. Neutralizing antibody titers were measured using a plaque reduction serum neutralization assay. Significant differences between groups were determined using a two-way analysis of variance (ANOVA) followed by Fisher's LSD multiple comparison test. **c** Detection of IgG subtypes 1–3 was examined in mouse sera after the third immunization with PBS or rBV. IgG subtypes levels were measured using a commercial ELISA. Significant differences between groups were determined using a two-way analysis of variance (ANOVA) followed by Fisher's LSD multiple comparison test. Recombinant baculovirus (rBV, $n = 6$; PBS, $n = 3$). Statistical significance was set at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. All statistical analyses were performed using Prism 8 software (GraphPad)

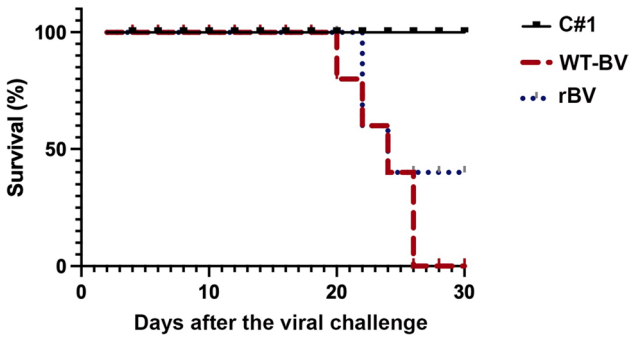

Fig. 4 Protection after a JUNV challenge. Survival curve of guinea pigs immunized with recombinant baculovirus (rBV, blue line), wild-type BV (WT-BV, red line, used as negative control), or C#1 (black line, used as positive control) and then infected subcutaneously with a fixed LD_{50} (100 PFU) of JUNV, strain P3790. The animals ($n = 5$) were observed daily for 30 days after virus infection

animals immunized with rBV survived 30 days after viral challenge (Fig. 4).

Discussion

The original baculovirus surface display method involved the expression of foreign proteins fused to the BV glycoprotein GP64 [17]. Later, the team proposed a new approach in which the foreign protein was expressed between the signal peptide of GP64 and the C-terminal transmembrane and cytoplasmic domains of the VSV-G protein. This was to achieve a more uniform distribution on the surface of the virion [18]. Our work has shown that this chimeric protein can be recognized by mouse monoclonal antibodies as well as by human and polyclonal antibodies, especially in the absence of the stable GPC signal peptide (SSP). The myristoylation of the GPC SSP and its additional role in the structure and transport of envelope glycoproteins emphasize the importance of our results for understanding the dynamics of envelope glycoproteins and may facilitate further research or therapeutic applications [5, 19]. However, a limitation of our study was that the amount of recombinant antigen on the budding BV could not be determined by a better method such as STORM/PALM [20].

Immunoglobulin G (IgG) exists in the form of four subclasses: IgG1, IgG2, IgG3, and IgG4. The distribution of the subclasses varies depending on whether the organism is in a state of homeostasis or whether this is disturbed. Viral infections primarily stimulate the IgG1 and IgG3 response [21]. We found a significant increase in IgG1, which is consistent with the results in patients with AHF [22].

The rBV vaccine candidate was successfully immunogenic, not only to induce specific antibodies, but also to generate partial protection in a JUNV challenge test without the use of adjuvants, thanks to the utilization of the immune-enhancing properties of BV [23]. The observation of only partial protection in our study could be due to several reasons. First, the recombinant protein might have lost natural epitopes because it was not naturally folded. Secondly, it could also be because the amount of immunogen used was insufficient. Interestingly, a recent study in a different disease model [13] showed 100% protection when a higher dose of rBV (10^8 PFU) was used. This highlights the need for

further research to optimize the amount of immunogen in future studies. Clarifying the relationship between dosage and protection could provide valuable insights for vaccine development.

In summary, we have shown that this recombinant chimeric BV-GP64-JUNV-GP1-GP2-VSV-G baculovirus can induce a humoral immune response specific for JUNV, including the production of neutralizing antibodies and partial protection upon viral challenge. These results encourage further studies to validate this vaccine candidate.

Acknowledgements We thank S. Tongiani, J. Guzman Cook, and L. Cayuela for technical assistance and L. Vasquez for assistance in media cell culture.

Author contributions Carla Tomatis contributed toward investigation. Mara C.A.M. Aguiar contributed toward investigation. Nancy Charo contributed toward investigation. Silvia Aquila contributed toward investigation. Pablo D. Thomas contributed toward investigation. Paula N. Arrías contributed toward investigation. Matías L. Pidre contributed toward methodology. Victor Romanowski contributed toward methodology. Laura Riera contributed toward investigation. Julia Brignone contributed toward investigation. Andrea Maiza contributed toward investigation. Eugenio A. Carrera Silva contributed toward investigation, data curation, methodology, and validation. María F. Ferrer contributed toward writing—review & editing, project administration, methodology, investigation, and conceptualization. Ricardo M. Gómez contributed toward writing—review & editing, writing—original draft, funding acquisition, and conceptualization.

Funding This work was supported by the *Agencia Nacional de Promoción Científica y Tecnológica* (ANPCyT), grants PCE 2018-0003 (in association with Synergium SA), PICT 2019-0767, and PICT 2020-2702, to RMG. The funders had no role in study design, data collection and interpretation, or in the decision to submit the work for publication.

Data Availability Data will be made available on request.

Declarations

COMPETING INTEREST The authors have no financial conflicts of interest.

References

1. Gomez RM, de Jaquenod GC, Sanchez Vallduvi MM, Frik J, Ferrer MF, Schattner M (2011) Junin virus. A XXI century update. Microbes Infection Institut Pasteur 13(4):303–311. https://doi.org/10.1016/j.micinf.2010.12.006
2. Kerber R, Reindl S, Romanowski V, Gomez RM, Ogbaini-Emovon E, Gunther S, ter Meulen J (2015) Research efforts to control highly pathogenic arenaviruses: a summary of the progress and gaps. J Clin Virol 64:120–127. https://doi.org/10.1016/j.jcv.2014.12.004
3. Radoshitzky SR, Buchmeier MJ, Charrel RN, Gonzalez JJ, Gunther S, Hepojoki J, Kuhn JH, Lukashevich IS, Romanowski V, Salvato MS, Sironi M, Stenglein MD, Torre JC (2023) ICTV virus taxonomy profile: Arenaviridae 2023. J Gen Virol. https://doi.org/10.1099/jgv.0.001891
4. Charrel RN, de Lamballerie X (2010) Zoonotic aspects of arenavirus infections. Vet Microbiol 140(3–4):213–220. https://doi.org/10.1016/j.vetmic.2009.08.027
5. Bederka LH, Bonhomme CJ, Ling EL, Buchmeier MJ (2014) Arenavirus stable signal peptide is the keystone subunit for glycoprotein complex organization. MBio 5(6):e02063. https://doi.org/10.1128/mBio.02063-14
6. Ambrosio A, Saavedra M, Mariani M, Gamboa G, Maiza A (2011) Argentine hemorrhagic fever vaccines. Hum Vaccin 7(6):694–700
7. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J (2023) Vaccine candidates against arenavirus infections. Vaccines (Basel). https://doi.org/10.3390/vaccines11030635
8. Hong Q, Liu J, Wei Y, Wei X (2023) Application of baculovirus expression vector system (BEVS) in vaccine development. Vaccines (Basel). https://doi.org/10.3390/vaccines11071218
9. Rodriguez-Hernandez AP, Martinez-Flores D, Cruz-Resendiz A, Padilla-Flores T, Gonzalez-Flores R, Estrada K, Sampieri A, Camacho-Zarco AR, Vaca L (2023) Baculovirus display of peptides and proteins for medical applications. Viruses. https://doi.org/10.3390/v15020411
10. Perez Vidakovics MLA, Ure AE, Arrias PN, Romanowski V, Gomez RM (2019) Junin virus induces autophagy in human A549 cells. PLoS ONE 14(6):e0218730. https://doi.org/10.1371/journal.pone.0218730
11. Ferrer F, Zoth SC, Calamante G, Taboga O (2007) Induction of virus-neutralizing antibodies by immunization with Rachiplusia nu per os infected with a recombinant baculovirus expressing the E2 glycoprotein of bovine viral diarrhea virus. J Virol Methods 146(1–2):424–427. https://doi.org/10.1016/j.jviromet.2007.08.017
12. McCarthy CB, Romanowski V (2008) A simplified method for the extraction of baculoviral DNA for PCR analysis: a practical application. J Virol Methods 148(1–2):286–290. https://doi.org/10.1016/j.jviromet.2007.11.004
13. Tomatis C, Ferrer MF, Aquila S, Thomas PD, Arrias PN, Ferrelli L, Pidre M, Romanowski V, Carrera Silva EA, Gomez RM (2024) Baculovirus surface display of a chimeric E-NS1 protein of YFV protects against YFV infection. Vaccine 42(25):126045. https://doi.org/10.1016/j.vaccine.2024.06.013
14. Riera LM, Feuillade MR, Saavedra MC, Ambrosio AM (1997) Evaluation of an enzyme immunosorbent assay for the diagnosis of Argentine haemorrhagic fever. Acta Virol 41(6):305–310
15. Chambers TJ, Liang Y, Droll DA, Schlesinger JJ, Davidson AD, Wright PJ, Jiang X (2003) Yellow fever virus/dengue-2 virus and yellow fever virus/dengue-4 virus chimeras: biological characterization, immunogenicity, and protection against dengue encephalitis in the mouse model. J Virol 77(6):3655–3668. https://doi.org/10.1128/jvi.77.6.3655-3668.2003
16. Olivera N, Castuma CE, Hozbor D, Gaillard ME, Rumbo M, Gomez RM (2014) Immunization with the recombinant Cholera toxin B fused to Fimbria 2 protein protects against *Bordetella pertussis* infection. Biomed Res Int 2014:421486. https://doi.org/10.1155/2014/421486
17. Whitford M, Stewart S, Kuzio J, Faulkner P (1989) Identification and sequence analysis of a gene encoding gp67, an abundant envelope glycoprotein of the baculovirus *Autographa californica* nuclear polyhedrosis virus. J Virol 63(3):1393–1399. https://doi.org/10.1128/JVI.63.3.1393-1399.1989
18. Chapple SD, Jones IM (2002) Non-polar distribution of green fluorescent protein on the surface of *Autographa californica* nucleopolyhedrovirus using a heterologous membrane anchor. J Biotechnol 95(3):269–275. https://doi.org/10.1016/s0168-1656(02)00023-8
19. York J, Romanowski V, Lu M, Nunberg JH (2004) The signal peptide of the Junin arenavirus envelope glycoprotein is myristoylated and forms an essential subunit of the mature G1–G2 complex.

J Virol 78(19):10783–10792. https://doi.org/10.1128/JVI.78.19.10783-10792.2004
20. Martinez-Flores D, Rodriguez-Hernandez AP, Sampieri A, Cruz-Resendiz A, Tobias-Juarez I, Lara-Martinez R, Jimenez-Garcia LF, Vaca L (2024) Resolving the differential distribution of structural proteins in baculovirus using single-molecule localization microscopy. J Gen Virol. https://doi.org/10.1099/jgv.0.002054
21. Wasiluk T, Sredzinska M, Rogowska A, Zebrowska A, Boczkowska-Radziwon B, Stasiak-Barmuta A, Radziwon P (2023) Analysis of the IgG subclass profile and IgG sum-total discrepancy in COVID-19 convalescent plasma donors: a single-centre prospective cohort study. Transfus Apher Sci 62(1):103527. https://doi.org/10.1016/j.transci.2022.103527
22. del Carmen SM, Sottosanti JM, Riera L, Ambrosio AM (2003) IgG subclasses in human immune response to wild and attenuated (vaccine) Junin virus infection. J Med Virol 69(3):447–450. https://doi.org/10.1002/jmv.10308
23. Abe T, Takahashi H, Hamazaki H, Miyano-Kurosaki N, Matsuura Y, Takaku H (2003) Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. J Immunol 171(3):1133–1139. https://doi.org/10.4049/jimmunol.171.3.1133