Indian J Microbiol (July–Sept 2018) 58(3):345–352
https://doi.org/10.1007/s12088-018-0728-y

ORIGINAL RESEARCH ARTICLE

SIV-Specific Antibodies are Elicited by a Recombinant Fowlpox Virus Co-expressing SIV Gag and envT

Yilong Zhu[1,3] · Shouwen Du[3] · Yang Zhang[4] · Jingwei Liu[4] · Yan Guo[1] · Cunxia Liu[3] · Jieying Bai[3] · Maopeng Wang[3] · Fei Zhao[3] · Tingting Cao[3] · Wang Xu[3] · Bing Bai[1,3] · Kelong Zhang[3] · Yizhen Ma[1] · Chang Li[2,3] · Ningyi Jin[1,2,3]

Received: 10 October 2017 / Accepted: 20 April 2018 / Published online: 27 April 2018

Abstract Given the failures of past HIV-1 vaccine clinical trials, potential HIV-1 vaccine candidates should be rigorously screened in preclinical models including simian immunodeficiency virus (SIV) primate models and small animal models. In this study, we tested the immunogenicity of a recombinant fowlpox virus (rFPV) expressing the SIV gag and SIV envT ($rFPV_{sg–se}$) proteins in BALB/c mice, to establish a foundation for further development. $rFPV_{sg–se}$ was constructed through homologous recombination techniques and purified through plaque screening assays using *enhanced green fluorescent protein* as the reporter gene. The integration, transcription, and translation of the SIV genes were measured by PCR (genomic DNA), RT-PCR (RNA), Western-blot, respectively. The levels of SIV-specific antibodies were assessed by ELISA following a single immunization (n = 18/group) or a prime-boost strategy (n = 24/group) with $rFPV_{sg–se}$ and compared to FPV and PBS controls. Residual virus was measured in distant organs following immunization using PCR. SIV-specific IgG titers against gag and gp120 were detected following single vaccination and the prime-boost. As expected the titers were higher following the prime-boost approach. The levels of Gag- and gp120-specific antibodies were significantly higher than controls ($p < 0.01$) 14 days after the booster immunization. Residual $rFPV_{Sg–Se}$ was detected in the muscle at the site of injection, but not in distant organs, from day 1–7 post immunization. In summary, $rFPV_{sg–se}$ induced high levels of SIV-specific antibodies suggesting it may be a viable candidate for further development.

Keywords Recombinant fowlpox virus · SIV gag–envT · SIV-specific antibodies · Residual virus

Introduction

Despite the fact that human immunodeficiency virus-1 (HIV-1) was discovered more than 30 years ago, there is an urgent need for a safe and effective global HIV-1 vaccine. While HIV-1 vaccine clinical efficacy trials have not been stopped, only four vaccine concepts have been evaluated for protective efficacy in humans [1, 2]. However, the tested vaccines ultimately failed to provide sufficient protection against HIV infection in clinical trials [3]. One of the lessons learned from these failures was that HIV-1 vaccines should be evaluated first in stringent preclinical studies [2].

Fowlpox virus (FPV) has been used as a live vector vaccines against infectious diseases and cancer in poultry, humans, and other mammals, and has been shown to be safe [4–6]. FPV undergoes a transient infection in which

Yilong Zhu and Shouwen Du contributed equally to this work.

✉ Chang Li
lichang78@163.com

✉ Ningyi Jin
ningyik@126.com

[1] Changchun University of Chinese Medicine, Changchun 130117, People's Republic of China

[2] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, People's Republic of China

[3] Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Institute of Military Veterinary, Academy of Military Medical Sciences, Changchun 130122, People's Republic of China

[4] Department of Neurosurgery, First Hospital, Jilin University, Changchun 130021, People's Republic of China

infection by progeny virus does not occur in mammalian cells, but the virus and target genes are still expressed in these cells. Thus, FPV is likely to be a promising vector for delivering HIV-1 antigen in a vaccine.

Prior clinical trials have provided insights regarding the optimal HIV-1 antigens for use in a vaccine. The RV144 HIV-1 vaccine study demonstrated a vaccine efficacy of 31.2% compared to placebo in Thailand [7], which was directly correlated with variable regions 1 and 2 (V1V2) of the HIV-1 envelope (Env) IgG antibodies and Env-specific IgA antibodies that were elicited [8]. This result suggested that Env proteins could be used to elicit broadly neutralizing antibodies against HIV-1 and/or simian immunodeficiency virus (SIV) [9–11]. However, broadly neutralizing antibodies are difficult to induce because of their unique characteristics [12]. Therefore, an alternative approach is to elicit antibodies that are similar to neutralizing antibodies and able to neutralize many HIV-1 strains [13–16]. In addition to Env, HIV-1 gag and SIV gag can also elicit high frequencies of gag-specific $CD8^+$ T cells and a long-lasting humoral response [17–19]. Therefore, the gag and env proteins would likely be the preferred antigen for use in a recombinant FPV (rFPV) vaccine candidate.

As previously noted, HIV-1 vaccine candidates should be extensively studied in stringent preclinical models prior to human efficacy studies. SIV infection in rhesus macaque monkeys is one such system which has been used for evaluating HIV-1 vaccine. Thus, the first step in developing an rFPV HIV-1 vaccine, is developing an rFPV vaccine expressing SIV antigens that can be experimentally tested in the preclinical models to estimate the immunogenicity and protective efficacy of the SIV/HIV vaccine candidates. However, the macaque model is limited in that the experiments are expensive, use outbred animals, and use relatively small numbers of animals. Therefore, the immunogenicity of candidate vaccines are often tested in small animal models prior to primate studies.

Therefore, in this study we constructed an rFPV vaccine expressing the SIV *gag* and SIV *envT* genes to evaluate the immunogenicity of the rFPV-gag/env vaccine in BALB/c mice to provide a foundation for evaluating the vaccine construct in the macaque model.

Materials and Methods

Plasmid, Virus, Cells and Animals

pVR-SIV gag and pVR-SIV envT were kindly provided by Xia Feng at the Chinese Center for Disease Control and Prevention. The *gag* and *envT* genes belong to the SIV/-mac239 (Genebank accession #M33262), *envT* was constructed by truncating env.

The rFPV vaccine was constructed from the Chinese FPV vaccine strain FPV282E4 and an FPV shuttle vector. The plasmid pVAX-Cre and FPV shuttle vectors were constructed previously. FPV282E4 was purchased from the Animal Pharmaceutical Factory of Nanjing (Nanjing, China). Baby hamster kidney (BHK) cells were cultured in DMEM with 10% fetal bovine serum and 1% penicillin (10,000 U/mL) / streptomycin (10,000 μg/mL) solution. Eight-day-old specific-pathogen free chickens were used to prepare the chick embryo fibroblast (CEF) cells that were purchased from Meiliyaweitong Experimental Animal Technology Co. Ltd (Beijing, China).

Six-week-old, female BALB/c mice (Experimental Animal Center, Academy of Military Medical Sciences of PLA, Beijing, China) were housed in an animal facility.

Construction of Recombinant Plasmids

The FPV shuttle vector pT3eGFP150 (4816 bp) containing the left (TKL) and right (TKR) halves of the *TK* gene, a double-gene expression cassette, and *EGFP* reporter gene was constructed in our laboratory [20]. The 1.5 kb SIV *gag* and 2.1 kb SIV *envT* genes were amplified and cloned into the multiple cloning site (MCS) 1 and MCS 2 respectively, to produce pT3eGFP150-SIV gag-SIV envT (pT3eGFP–Sg–Se).

Isolation of $rFPV_{Sg–Se}$ from CEF Cells

CEF cells were infected with FPV282E4 at a multiplicity of infection (MOI) of 1 for 2 h. The cells were then transfected with 1 μg of the plasmid pT3eGFP150–Sg–Se using Lipofectamine 2000 (Invitrogen, US). The infected cells expressing EGFP were picked out under a fluorescence microscope and used for additional rounds of infection. After 12 rounds of plaque screening, the purified virus was termed $rFPV_{Sg–Se}$. $rFPV_{Sg–Se}$ was then characterized by PCR, RT-PCR, and Western-blot.

Characterization of $rFPV_{Sg–Se}$

The genomic DNA (gDNA) and total RNA from cells infected with $rFPV_{Sg–Se}$ were extracted to use as templates for amplifying the SIV *gag*, SIV *envT*, FPV-*P4b,* and *TK* genes by PCR and RT-PCR. The PCR reaction conditions were 95 °C 5 min, 30 cycles of 95 °C 30 s, 60 °C 30 s and 72 °C 2 min 10 s, and a final extension at 72 °C for 10 min. The primers utilized are shown in Table 1. The SIV *gag* and SIV *envT* genes were inserted into the FPV genome such that the *TK* gene was broken and blocked, therefore the *TK* gene was used as a selection marker to purify $rFPV_{Sg–Se}$. The *P4b* gene encoding the virion

Table 1 Primer sequences used for PCR and the expected lengths of the amplified fragments

Genes	Sequence (5′–3′)	Length (bp)
SIV *gag*[a]	F:CCCTTAAGGCCACCATGGGAGTGAGGAACTC (Afl II)	1533
	R:CCGCGGCCGCTCACTGGTCTCCTCCGAAC (Not I)	
SIV *envT*[a]	F: CCGGTACCGCCACCATGGGATGCCTGGGAAAC (Kpn I)	2160
	R: CCGTCGACTCACTGCCTCAGCTTAGCCAG (Sal I)	
TK[a]	F:GGACGCGTATTGATTCACACCGTATTACAGAGG	1006
	R:CGCCCGGGTTCTCCTAATAAGTTACACCGTTTG	
P4b[a,b]	F:GGACGCGTCAGCAGGTGCTAAACAACAA	578
	R:GGCTGCAGCGGTAGCTTAACGCCGAATA	
SIV *gag*[b]	F: CCGAGACCATGCCTAAGACC	429
	R: AGTACCGGCTGAAGCACATC	
SIV *envT*[b]	F:TCGGATTCAACGGAACCAGG	433
	R:TGGTTAGCGGTGTTCCTGTC	

Target sites for restriction enzyme digestion are indicated by underlined text

[a]PCR primers used for identifying $rFPV_{Sg–Se}$

[b]PCR primers used for detecting residual $rFPV_{Sg–Se}$

nucleoprotein (75 kDa), which is widely found in FPV, was used to identify FPV [21].

Considering the characteristics of FPV, we need to test the target proteins whether could be expressed effectively in $rFPV_{Sg–Se}$-infected BHK cells. Therefore, BHK cells were infected with $rFPV_{Sg–Se}$ at an MOI of 5 for 72 h, and total protein was extracted from $rFPV_{Sg–Se}$-infected cells to probe for expression of the SIV gag and SIV envT proteins by Western-blot. A rabbit anti-p27 (SIV/mac 239) antibody, rabbit anti-gp120/160 (SIV/mac239) antibody, and mouse anti-β-actin antibody were used as the primary antibodies to identify the SIV gag, SIV envT and β-actin proteins, respectively. HRP-conjugated goat anti-rabbit lgG and goat anti-mouse IgG were used as secondary antibodies. FPV-infected cells were used as the negative control, and β-actin served as the internal control.

To assess the stability of the SIV *gag* and SIV *envT* insertions, $rFPV_{Sg–Se}$ was passaged 20 times, and the genomic DNA (gDNA), RNA, and total protein were extracted from the 1st, 5th, 10th, 15th, and 20th passages at the genetic (PCR, RT-PCR) and protein (Western-blot) levels.

Single Immunization

Fifty-four (54) female BABL/c mice were divided evenly into three groups (n = 18). One group was immunized with 1×10^6 plaque forming units (PFU) of $rFPV_{Sg–Se}$ ($rFPV_{Sg–Se}$) by the intramuscular route, one group received 1×10^6 PFU of FPV282E4 (FPV282E4), and one received 100 μL of PBS (PBS). Blood samples were harvested at 1, 7, 14, 21, 28, and 35 days post immunization. The serum was collected and stored at − 80 °C until it was used in ELISAs to detect the levels of SIV- and vector-specific antibodies. The experimental design is shown in Fig. 2a.

Prime-Boost Immunization

Seventy-two (72) female BALB/c mice were divided into three experimental groups (n = 24). Animals in the $rFPV_{Sg–Se}$ group were primed with 1×10^7 PFU of $rFPV_{Sg–Se}$ in 100 μL of PBS by the intramuscular route at day 0 and were boosted with the same dosage at day 21. The second group received 1×10^6 PFU of FPV282E4 (FPV282E4) at days 0 and 21, and the third group received 100 μL of PBS on days 0 and 21. Serum sample were collected at days 1, 7, 14, 21, 28, 35, 42, and 49 post immunization to detect SIV and vector-specific antibodies by ELISA. The experimental design is shown in Fig. 3a.

ELISA Analysis and Residual Virus Detection

SIV-specific antibody responses to heterologous SIV-mac239 gag and gp120 (env) were detected by ELISA. For the ELISA, the SIV gag and gp120 proteins were utilized as capture antibodies, serum samples from immunized mice were the primary antibody (diluted in 1:20 in PBS) added to the wells, and a peroxidase-conjugated goat anti-mouse IgG antibody was used as the second antibody (diluted 3000-fold in PBS), and the optical density (OD) was detected at 492 nm. A standard curve was constructed using the same approach. Vector-specific antibodies against inactivated FPV282E4 (1×10^6 PFU/ 96 well plate) were detected in the same manner.

gDNA was isolated from the hearts, livers, spleens, lungs, kidneys, brains, and muscles of mice primed with

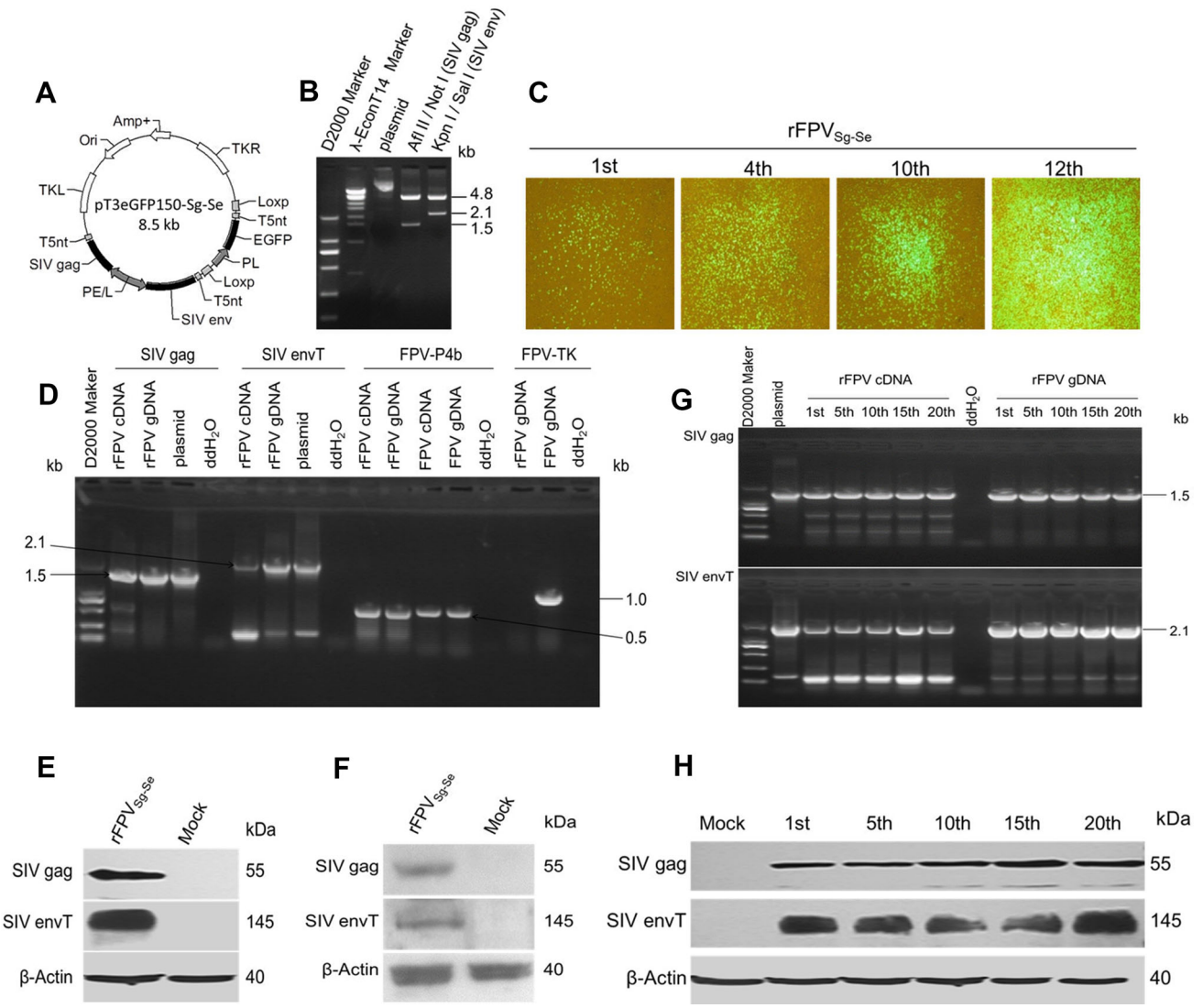

Fig. 1 Construction and characterization of the rFPV$_{sg-se}$ vaccine. **a** Schematic diagram of the strucutre of the plasmid pT3eGFP150–Sg–Se. **b** Identification of pT3eGFP150–Sg–Se by restriction enzyme digestion. **c** EGFP expression was used to screen for cells infected with rFPV$_{Sg-Se}$ under a fluorescent microscope at 72 h post-transfection, and after 4, 10, or 12 rounds of plaque screening. **d** Identification of rFPV$_{Sg-Se}$ by PCR. **e** Western bolt analysis of SIV gag and SIV envT in rFPV$_{Sg-Se}$-infected CEF cells compared to mock infection. **f** Western bolt analysis of SIV gag and SIV envT in rFPV$_{Sg-Se}$-infected BHK cells compared to mock infection. **g** The SIV *gag* and SIV *envT* genes were amplified from the gDNA and cDNA of rFPV$_{Sg-Se}$-infected CEF cells every five passage (1, 5, 10, 15 and 20) for twenty passages. **h** Protein expression levels of SIV gag and SIV envT were assessed by Western-blotting every five passages for twenty passages

rFPV$_{Sg-Se}$ on days 1, 3, 5, 7, and 14 post immunization for use as templates for amplifying the SIV *gag*, SIV *envT,* and *P4b* genes to detect residual rFPV$_{Sg-Se}$. The primers utilized are shown in Table 1.

Statistical Analysis

Statistical analyses were performed using the Graphpad Prism software version 5.0 (San Diego, CA, USA). Differences between groups with a p value < 0.05 were considered to be statistically significant. Data are presented as the mean ± standard deviation (SD).

Results

Construction and Characterization of the rFPV$_{sg-se}$ Vaccine

To construct rFPV$_{sg-se}$, we first generated the recombinant plasmid pT3eGFP150–Sg–Se (8509 bp) shown in Fig. 1a.

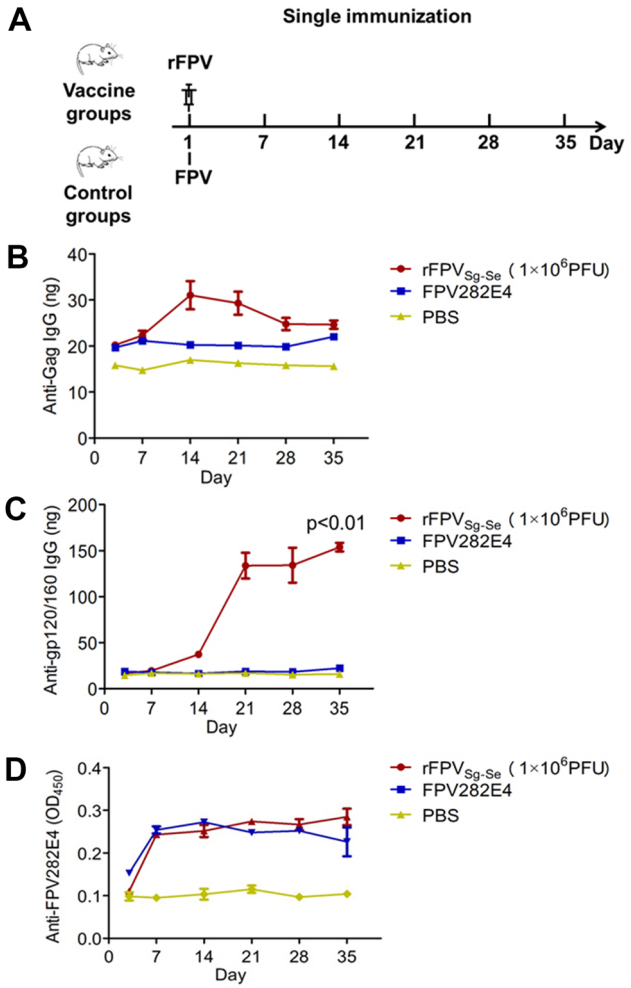

Fig. 2 Quantifying the serum antigen specific IgG titer in BALB/c mice following a single rFPV$_{sg-se}$ immunization. **a** Schematic of the experimental design. Female BALB/c mice were used (n = 18/group). The mice were divided into three groups and immunized with rFPV$_{sg-se}$, FPV282E4, or PBS. **b** SIV gag-specific IgG titer, **c** SIV gp120-specific IgG titer, and **d** vector-specific antibody levels were measured in the serum by ELISA

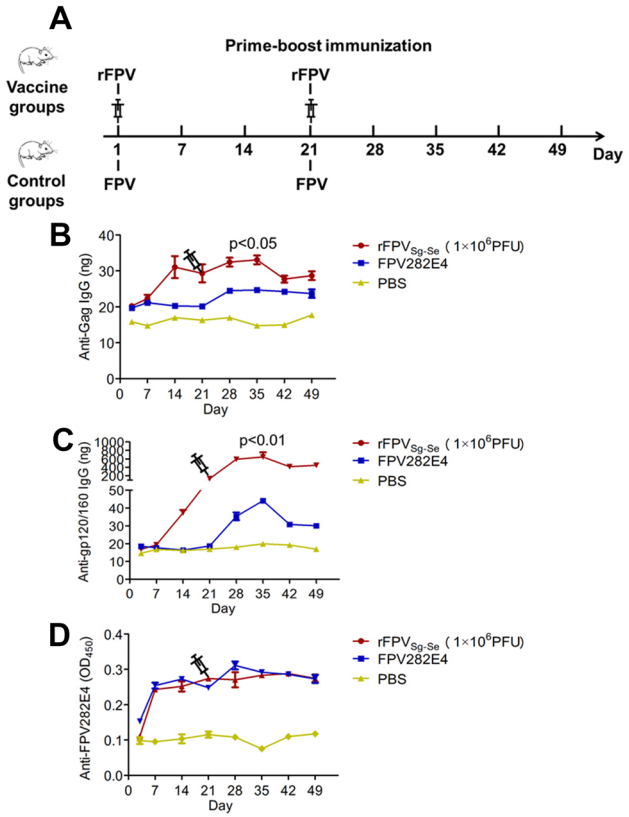

Fig. 3 Quantifying the serum antigen specific IgG titer in BALB/c mice following a prime-boost rFPV$_{sg-se}$ immunization. **a** Schematic of the experimental design. Female BALB/c mice were used (n = 24/group). The mice were divided into three groups and immunized with rFPV$_{sg-se}$, FPV282E4, or PBS. **b** SIV gag-specific IgG titers, **c** SIV gp120-specific IgG titers, and **d** vector-specific antibody levels were measured in the serum by ELISA

The successful construction of pT3eGFP150–Sg–Se was confirmed by restriction enzyme digestion using *Afl II*/*Not I* to identify the SIV *gag* insert (1533 bp) and *Kpn I*/*Sal I* to identify the SIV *envT* insert (2160 bp) as shown in Fig. 1b.

Having established the SIV genes were correctly inserted, we then infected CEF cells with FPV282E4 and transfected the infected cells with pT3eGFP150–Sg–Se. The rFPV$_{Sg-Se}$-infected cells were then selected based on expression of the *EGFP* reporter gene. Rounds of selection for rFPV$_{Sg-Se}$ continued until all of the plaques expressed *EGFP* (Fig. 1c). The SIV *gag* (1503 bp), SIV *envT* (2106 bp), *P4b* (578 bp), and *TK* (1006 bp) genes were amplified from rFPV-$_{sg-se}$ infected cells by PCR and RT-PCR (Fig. 1d), indicating that the foreign genes were integrated into the rFPV$_{Sg-Se}$ genome and transcribed. A pure stock of rFPV$_{Sg-Se}$, lacking contamination from FPV282E4, was obtained when the *TK* gene could not be amplified from gDNA or cDNA. The target proteins SIV gag (56 kDa) and SIV envT (120 kDa) could be identified in rFPV$_{Sg-Se}$-infected CEF and BHK cells by Western-blot (Fig. 1e, f).

To assess the stability of SIV gene expression, gDNA, RNA, and total protein was collected from rFPV$_{Sg-Se}$ infected cells every five passages for 20 passages to assess the integration, transcriptional, and translational levels of SIV gag and SIV envT by PCR, RT-PCR (Fig. 1g) and Western-blot (Fig. 1h). The results demonstrated that rFPV$_{Sg-Se}$ could be passaged at least 20 times and continue to stably express the inserted SIV genes.

Evaluating the Titer of SIV and FPV Specific Antibodies Elicited by Immunization with rFPV$_{Sg-Se}$

The ability of rFPV$_{Sg-Se}$ to elicit antibodies targeting SIV gag and gp120 was first assessed following a single

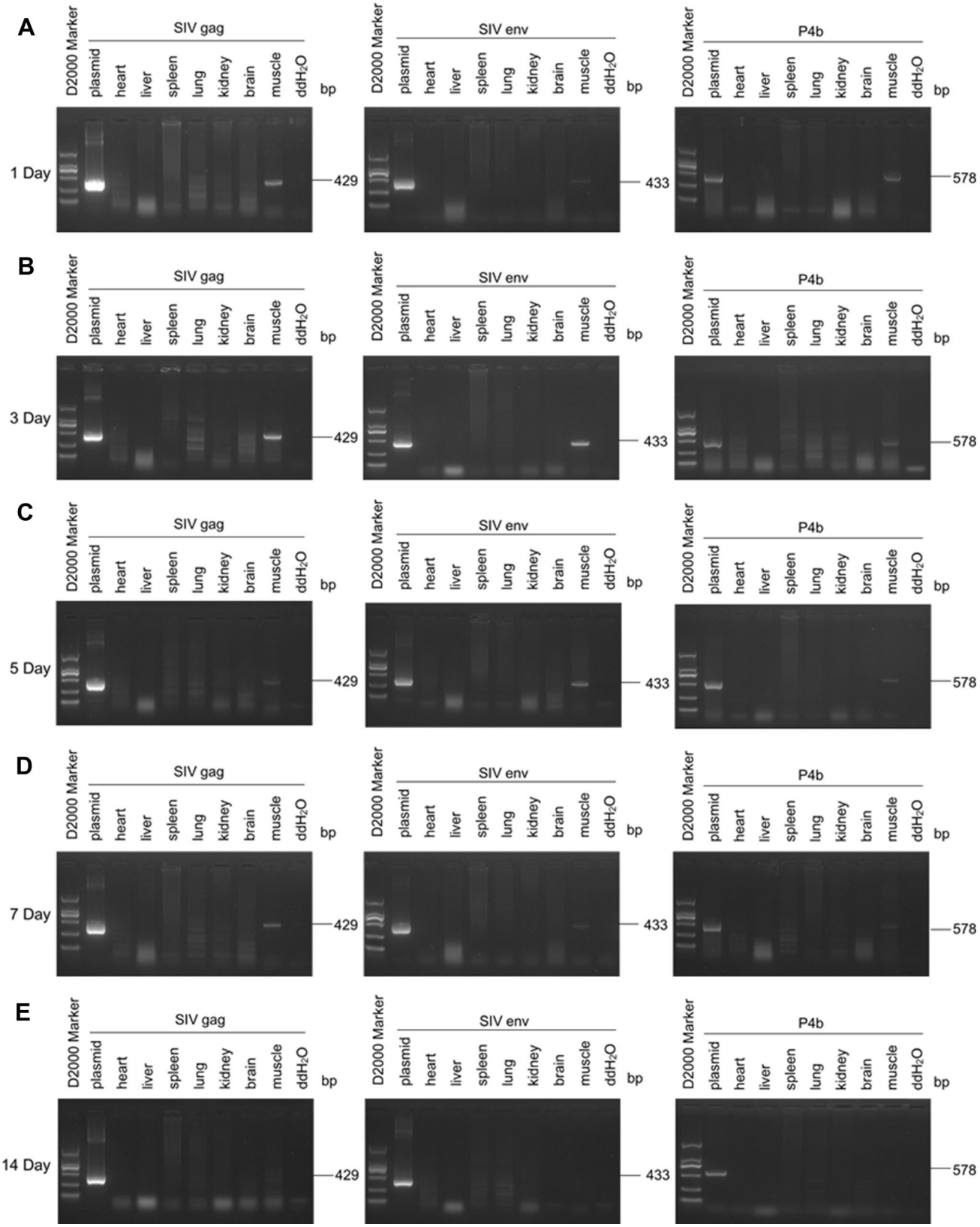

Fig. 4 Detection of residual virus in organs of $rFPV_{Sg-Se}$-immunized mice. Residual $rFPV_{Sg-Se}$ was detected by PCR in the heart, liver, spleen, lung, kidney, brain, and muscle of immunized mice at **a** 1, **b** 3, **c** 5, **d** 7 and **e** 14 days post immunization

immunization (Fig. 2a). The levels of SIV- and FPV specific IgG antibodies in the serum of immunized mice were measured by ELISA at 1, 7, 14, 21, 28, and 35 days post immunization. Following a single immunization, the levels of SIV gag- and gp120-specific antibodies in the $rFPV_{Sg–Se}$ immunized group were significantly higher than the other groups ($p < 0.05$, Fig. 2b, c). Vector-specific antibody levels were also markedly increased at day 7 post immunization in the $rFPV_{Sg–Se}$ and FPV groups, but plateaued from 14 to 35 days post immunization (Fig. 2d).

To test whether the antibody response could be boosted, we immunized mice twice on days 0 and 21. The serum antigen specific IgG titers were measured by ELISA on days 1, 7, 14, 21, 28, 35, 42, and 49 following the priming immunization (Fig. 3A). The levels of IgG antibodies specific to the SIV gag and gp120 proteins were further increased after boosting ($p < 0.01$, Fig. 3b, c). In contrast, the titer of vector-specific antibodies remained relatively stable after boosting (Fig. 3d). These results indicated that immunizing BALB/c mice with SIV gag and SIV gp120 in the FPV vector elicited an SIV-specific humoral response, and that a prime-boost strategy elicited higher titers of antibody than a single immunization.

Detection of Residual $rFPV_{Sg–Se}$ in Multiple Organs

As a safety measure and to demonstrate the transient nature of $rFPV_{Sg–Se}$ infection, residual $rFPV_{Sg–Se}$ was detected by amplifying the SIV *gag*, SIV *envT,* and *p4b* genes from the tissues and organs of $rFPV_{Sg–Se}$-immunized mice at days 1, 3, 5, 7 and 14 post immunization. $rFPV_{Sg–Se}$ detection are shown in Fig. 4a, e, respectively. $rFPV_{Sg–Se}$ was only detected in the muscle at 1, 3, 5, and 7 days post immunization. The preliminary results suggest that gene expression from 1×10^6 PFU of $rFPV_{Sg–Se}$ was cleared from the injection site by at most 14 days post immunization in BABL/c mice.

Discussion

To successfully elicit a durable, long-lasting humoral response to protect against HIV-1 and SIV infection over many years multiple immunizations are necessary, which may lead to immune tolerance if the same vaccine is used every time. Therefore, multiple vaccine platforms are necessary to use in combined immunization strategies to prevent HIV and SIV infection over many years.

To make the $rFPV_{Sg–Se}$ vaccine candidate tested in this study, we selected the *gag* and *env* genes which are well known to elicit a strong humoral and neutralizing antibody response in humans [22]. In early studies from our laboratory, BrdU selection has been used to construct recombinant vaccinia virus and rFPV vectors, this method generally has a low recombination efficiency, is labor intensive, and returns a high number of non-recombinant viruses during screening. Thus, we chose to use the EGFP reporter gene to construct the rFPV plasmid pT3eGFP150–Sg–Se. This method had the advantages that EGFP was only expressed in the recombinant viruses, and the screening process was visual based on easily observable green fluorescent plaques.

Immunization with $rFPV_{Sg–Se}$ elicited an antigen specific IgG response to SIVmac239 gag and gp120 proteins using a prime-boost strategy. We also observed an antibody response to the FPV vector, which did not increase following the booster immunization. These results indicated that vaccination using $rFPV_{sg–se}$ could be repeated at least once to increase antibody titer.

While we have shown that high levels of SIV-specific antibodies can be elicited by $rFPV_{Sg–Se}$, future studies are required to characterize the neutralizing antibody titer, cellular immune response, and protective efficacy of the vaccine. In addition, $rFPV_{Sg–Se}$ was not detected in the other organs by PCR, it is possible that rFPV does not bind to organs away from the injection site or that the low number of adsorbed-$rFPV_{Sg–Se}$ virions is not sufficient to detect by PCR. Therefore, we also need to further verify that rFPVsg–se is not widely disseminated and is transiently infecting the recipient. Our work did show that SIV gag- and SIV envT-specific antibodies were elicited strongly by $rFPV_{Sg–Se}$ in the BALB/c mouse model and provided a foundation for testing the immunogenicity and safety research of SIV/HIV rFPV candidate vaccines.

Acknowledgements This work was financially supported by grants from the National Natural Science Foundation of China (No. 31472197), Natural Science Foundation of Beijing (No. 5152023) and Foundation of State Key Laboratory of Pathogen and Biosecurity (No. SKLPBS1435).

References

1. Fauci AS, Marston HD (2014) Ending AIDS–is an HIV vaccine necessary? N Engl J Med 370(6):495–498. https://doi.org/10.1056/NEJMp1313771
2. Barouch DH, Michael NL (2014) Accelerating HIV-1 vaccine efficacy trials. Cell 159(5):969–972. https://doi.org/10.1016/j.cell.2014.10.046
3. Barouch DH (2013) The quest for an HIV-1 vaccine-moving forward. N Engl J Med 369(22):2073–2076. https://doi.org/10.1056/NEJMp1312711
4. Odunsi K, Matsuzaki J, Karbach J, Neumann A, Mhawech-Fauceglia P, Miller A, Beck A, Morrison CD, Ritter G, Godoy H, Lele S, duPont N, Edwards R, Shrikant P, Old LJ, Gnjatic S, Jager E (2012) Efficacy of vaccination with recombinant vaccinia and fowlpox vectors expressing NY-ESO-1 antigen in ovarian cancer and melanoma patients. Proc Natl Acad Sci USA 109(15):5797–5802. https://doi.org/10.1073/pnas.1117208109

5. Arlen PM, Pazdur M, Skarupa L, Rauckhorst M, Gulley JL (2006) A randomized phase II study of docetaxel alone or in combination with PANVAC-V (vaccinia) and PANVAC-F (fowlpox) in patients with metastatic breast cancer (NCI 05-C-0229). Clin Breast Cancer 7(2):176–179. https://doi.org/10.3816/CBC.2006.n.032
6. Hemachandra A, Puls RL, Sirivichayakul S, Kerr S, Thantiworasit P, Ubolyam S, Cooper DA, Emery S, Phanuphak P, Kelleher A, Ruxrungtham K (2010) An HIV-1 clade A/E DNA prime, recombinant fowlpox virus boost vaccine is safe, but non-immunogenic in a randomized phase I/IIa trial in Thai volunteers at low risk of HIV infection. Hum Vaccines 6(10):835–840. https://doi.org/10.4161/hv.6.10.12635
7. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de Souza M, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH, Investigators M-T (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 Infection in Thailand. New Engl J Med 361(23):2209–2220. https://doi.org/10.1056/Nejmoa0908492
8. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang YD, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen XY, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH (2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. New Engl J Med 366(14):1275–1286
9. Patterson LJ, Daltabuit-Test M, Xiao P, Zhao J, Hu W, Wille-Reece U, Brocca-Cofano E, Kalyanaraman VS, Kalisz I, Whitney S, Lee EM, Pal R, Montefiori DC, Dandekar S, Seder R, Roederer M, Wiseman RW, Hirsch V, Robert-Guroff M (2011) Rapid SIV Env-specific mucosal and serum antibody induction augments cellular immunity in protecting immunized, elite-controller macaques against high dose heterologous SIV challenge. Virology 411(1):87–102. https://doi.org/10.1016/j.virol.2010.12.033
10. Li J, Valentin A, Kulkarni V, Rosati M, Beach RK, Alicea C, Hannaman D, Reed SG, Felber BK, Pavlakis GN (2013) HIV/SIV DNA vaccine combined with protein in a co-immunization protocol elicits highest humoral responses to envelope in mice and macaques. Vaccine 31(36):3747–3755. https://doi.org/10.1016/j.vaccine.2013.04.037
11. Kong L, de la Pena AT, Deller MC, Garces F, Sliepen K, Hua YZ, Stanfield RL, Sanders RW, Wilson IA (2015) Complete epitopes for vaccine design derived from a crystal structure of the broadly neutralizing antibodies PGT128 and 8ANC195 in complex with an HIV-1 Env trimer. Acta Crystallogr D 71:2099–2108. https://doi.org/10.1107/S1399004715013917
12. Fauci AS, Marston HD (2015) Toward an HIV vaccine: a scientific journey. Science 349(6246):386–387. https://doi.org/10.1126/science.aac6300
13. Barouch DH, Alter G, Broge T, Linde C, Ackerman ME, Brown EP, Borducchi EN, Smith KM, Nkolola JP, Liu J, Shields J, Parenteau L, Whitney JB, Abbink P, Ng'ang'a DM, Seaman MS, Lavine CL, Perry JR, Li W, Colantonio AD, Lewis MG, Chen B, Wenschuh H, Reimer U, Piatak M, Lifson JD, Handley SA, Virgin HW, Koutsoukos M, Lorin C, Voss G, Weijtens M, Pau MG, Schuitemaker H (2015) Protective efficacy of adenovirus/protein vaccines against SIV challenges in rhesus monkeys. Science 349(6245):320–324. https://doi.org/10.1126/science.aab3886
14. Zhang Y, Yuan TT, Li JJ, Zhang YY, Xu JQ, Shao YM, Chen ZW, Zhang MY (2013) The potential of the human immune system to develop broadly neutralizing HIV-1 antibodies: implications for vaccine development. Aids 27(16):2529–2539. https://doi.org/10.1097/Qad.0000000000000015
15. van Gils MJ, Sanders RW (2013) Broadly neutralizing antibodies against HIV-1: Templates for a vaccine. Virology 435(1):46–56. https://doi.org/10.1016/j.virol.2012.10.004
16. Azoitei ML, Ban YA, Kalyuzhny O, Guenaga J, Schroeter A, Porter J, Wyatt R, Schief WR (2014) Computational design of protein antigens that interact with the CDR H3 loop of HIV broadly neutralizing antibody 2F5. Proteins 82(10):2770–2782. https://doi.org/10.1002/prot.24641
17. Virnik K, Hockenbury M, Ni Y, Beren J, Pavlakis GN, Felber BK, Berkower I (2013) Live attenuated rubella vectors expressing SIV and HIV vaccine antigens replicate and elicit durable immune responses in rhesus macaques. Retrovirology 10:99. https://doi.org/10.1186/1742-4690-10-99
18. Liu J, Li H, Iampietro MJ, Barouch DH (2012) Accelerated heterologous adenovirus prime-boost SIV vaccine in neonatal rhesus monkeys. J Virol 86(15):7829–7835. https://doi.org/10.1128/JVI.00512-12
19. Goldoni AL, Maciel M, Rigato PO, Piubelli O, de Brito CA, Melo A, Marques ET, August JT, Duarte AJD, Sato MN (2011) Mucosal and systemic anti-GAG immunity induced by neonatal immunization with HIV LAMP/gag DNA vaccine in mice. Immunobiology 216(4):505–512. https://doi.org/10.1016/j.imbio.2010.08.007
20. Du S, Liu C, Zhu Y, Wang Y, Ren D, Wang M, Tan P, Li X, Tian M, Zhang Y, Li J, Zhao F, Li C, Jin N (2015) Construction and characterization of novel fowlpox virus shuttle vectors. Virus Res 197:59–66. https://doi.org/10.1016/j.virusres.2014.12.015
21. Scheiflinger F, Falkner FG, Dorner F (1997) Role of the fowlpox virus thymidine kinase gene for the growth of FPV recombinants in cell culture. Adv Virol 142(12):2421–2431
22. Garcia F, Bernaldo de Quiros JC, Gomez CE, Perdiguero B, Najera JL, Jimenez V, Garcia-Arriaza J, Guardo AC, Perez I, Diaz-Brito V, Conde MS, Gonzalez N, Alvarez A, Alcami J, Jimenez JL, Pich J, Arnaiz JA, Maleno MJ, Leon A, Munoz-Fernandez MA, Liljestrom P, Weber J, Pantaleo G, Gatell JM, Plana M, Esteban M (2011) Safety and immunogenicity of a modified pox vector-based HIV/AIDS vaccine candidate expressing Env, Gag, Pol and Nef proteins of HIV-1 subtype B (MVA-B) in healthy HIV-1-uninfected volunteers: a phase I clinical trial (RISVAC02). Vaccine 29(46):8309–8316. https://doi.org/10.1016/j.vaccine.2011.08.098