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Virus-like particle vaccines for poliovirus types 1, 2, and 3 with enhanced thermostability expressed in insect cells

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ABSTRACT

Poliovirus (PV) is a pathogen that causes poliomyelitis, which may lead to paralysis and fatality. Inactivated PV vaccines (IPVs) and live-attenuated oral PV vaccines (OPVs) are currently used to defend against PV worldwide. Vaccines must be developed in a PV-free environment given the biosafety issues associated with OPV and IPV production and to eradicate PV globally. In this study, PV1, PV2, and PV3 virus-like particles with enhanced thermostability (PV-sVLPs) were produced in large quantities by using a baculovirus expression vector system (BEVS). Mice immunized with PV-sVLPs generated antibodies with strong PV-neutralizing response. In addition, splenocytes collected from immunized mice expressed high levels of IFN-γ, IL-2, GM-CSF, IL-5, and IL-10 upon PV-sVLPs stimulation. These data suggest that PV-sVLPs can serve as vaccines against PV infection.

1. Introduction

Poliovirus (PV) is a human enterovirus that causes poliomyelitis. Three PV serotypes exist: PV1, PV2, and PV3. Although individuals with PV infections generally present mild symptoms, 0.1%–1% of these individuals may become paralyzed (poliomyelitis) if their central nervous systems become infected by PV. Two types of polio vaccines, inactivated PV vaccines (IPVs) and live-attenuated oral polio vaccines (OPVs), have been developed since the 1950s. The application of these vaccines has dramatically reduced the number of PV infections such that in 1988, the WHO announced its ambitious plan to eradicate polio [1]. In September 2015, the Global Polio Eradication Initiative (GPEI) declared its intent to eradicate wild-type PV2. Although the final goal of global wild-type polio eradication is imminent, immunization against PV must be continued for the next several decades given the possibility of PV re-emergence and the existence of long-term PV excreters.

OPV must be eliminated in the polio-free world because of two reasons: First, attenuated PV particles in OPVs can revert into virulent, transmissible viruses (circulating vaccine derived PVs, cVDPV). Second, immune-deficient patients exposed to OPVs can excrete immune deficient vaccine derived PVs (iVDPVs) over a long period [2]. In addition, the production of OPVs and IPVs would be impossible in a polio-free world because it requires the amplification of PVs in large quantities. Therefore, an alternative virus-free approach for the production of polio vaccines is urgently needed.

PV possesses a single-stranded RNA genome that is approximately 7.5 kb in length [3]. The PV genome encodes one polyprotein that is cleaved by virus-encoded proteinases to generate P1, P2, and P3. P1 is subsequently cleaved by the 3CD proteinase into the viral structural proteins VP1, VP3, and VP0. VP0 is further cleaved into VP2 and VP4 [4]. These viral structural proteins form virus capsids. Most protective epitopes are located on the virus capsid and mainly localize on VP1, VP2, and VP3 [5,6].

Numerous viral structural proteins can self-assemble into VLPs. These VLPs structurally resemble natural viral particles and retain comprehensive antigenic epitopes. VLP vaccines against HPV, HBV, and influenza viruses have been used in clinical practice for years and have shown superb efficiency and safety [7–9].

Early endeavors to generate PV-VLPs vaccines were hampered by the low production levels of VLPs and the poor immunogenicity of PV-VLPs. The structural instability of PV-VLPs may account for their poor immunogenicity. Fox et al. [10] recently identified mutations that are capable of enhancing the thermostability of PV. PV-VLPs that carry stabilizing mutations (PV-sVLPs) are as immunogenic as IPV. This characteristic suggests that PV-sVLPs have the potential to replace currently used PV vaccines.

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In this study, recombinant baculovirus vectors were constructed to express the capsid structural proteins of PV. This work demonstrated that individually expressed VPs (VP1, VP0, and VP3-2A) form VLPs. PV-sVLPs were produced in large quantities by using a baculovirus expression vector system (BEVS). The humoral and cellular immune responses induced by the produced VLPs were evaluated in immunized mice. Results suggest that PV-sVLPs could serve as safe PV vaccines in the polio-free world.

2. Materials and methods

2.1. Cells, viruses, plasmids, and strains

The insect cell line Sf9 was obtained from ATCC. pFastBac™ Dual and DH10Bac were acquired from Thermo Fisher Scientific (Invitrogen). SF-α cells were isolated by the Southern China United Vaccine Institute. PVs Sabin 1, Sabin 2, and Sabin 3 (attenuated PV strains) were provided by the Center of Disease Control and Prevention of Guangzhou, Guangdong Province, PRC.

2.2. Main reagents and antibodies

Restriction enzymes, polymerases, DNA markers, protein markers, and BCA Protein Assay Kits were procured from Thermo Fisher Scientific. Grace's cell culture medium and FBS were acquired from GIBCO. EXCELL-420 was purchased from Sigma. Cellfectin® II Reagent was obtained from Thermo Fisher Scientific. Bio-Plex Pro™ Mouse Cytokine Th1/Th2 Assay (Bio-Rad) was performed in accordance with the manufacturer's instructions. MAB 234 for PV1, MAB 1050 for PV2, MAB 520 for PV3, and D antigen International Standard for IPV 12/104 were purchased from NIBSC. A Sf9 Insect Cell HCP ELISA kit was obtained from Cygnus Technologies. IPV was procured from Sanofi Pasteur (40 D antigen unit of PV1, 8 D antigen unit of PV2, and 32 D antigen unit of PV3). Anti-IPV polyclonal antibodies were generated by immunizing rabbits with IPV vaccines. Anti-guinea pig GP64 antibodies and horseradish peroxidase (HRP)-conjugated anti-rabbit GP64 antibodies were produced through the immunization of guinea pigs and rabbits with the GP64 protein, respectively.

2.3. Construction of the transfer vector

The 3CD and P1 coding sequences of PV1 (Mahoney strain), PV2 (MEF-1 strain), and PV 3 (Saukett strain) were optimized for insect codon usage and synthesized (cloned in a pUC57 plasmid) by GenScript Incorporation. Transfer plasmids were generated by using pFastBac™ Dual.

2.4. Generation of recombinant baculoviruses

Recombinant baculoviruses were generated by using the Bac-to-Bac system (Invitrogen). In brief, recombinant plasmids were transformed into DH10Bac, wherein whole expression cassettes between Tn7R and Tn7L were transferred from the transfer vector to the bacmid through site-specific transposition. Recombinant baculoviruses were isolated, amplified, stored, and titrated in accordance with standard methods for the Bac-to-Bac system.

PV VLPs (PV-VLPs and PV-sVLPs) were produced by infecting SF-α cells cultured in EXCELL-420 medium with the recombinant baculoviruses. Cells were cultured in spinner flasks for small-scale production. The supernatant and the cells were harvested and subjected to Western blot analysis at 6 days post infection (dpi).

2.5. Production and purification of PV VLPs

Cells for PV VLPs production and purification were cultured in a ReadyToProcess™ WAVE 25 (single-use bioreactors, GE Healthcare Life Sciences). The supernatant was harvested through centrifugation at 10,000*g* (Beckman, USA) and concentrated via ultrafiltration with a tangential flow unit (Millipore). Pellets were dissolved in PBS after separation with a 25% (w/w) sucrose cushion and the purified through gel filtration. Then, the purified PV VLPs were collected and subjected to SDS-PAGE and Western blot analyses.

2.6. Electron microscopy

PV VLPs were adsorbed for 5 min at room temperature onto copper grids coated with carbon Formvar, briefly washed with water (1 min), and then stained with 1% uranyl acetate for 1 min. The grids were examined under a JEM-2010 transmission electron microscope (JEOL) operated at 80 V. The PV VLPs were also analyzed with a cryo-electron microscope (Titan Krios) operated at 300 kV.

2.7. Analysis of host cell protein and residual DNA contents

The host cell protein contents of PV VLPs were quantified by using a Sf9 Insect Cell HCP ELISA kit (Cygnus Technologies). Residual baculovirus was detected through ELISA. In this assay, anti-guinea pig GP64 antibody was used as a coating and the HRP-conjugated anti-rabbit GP64 antibody was applied for detection.

Residual DNA was first denatured, fixed on a cellulose nitrate membrane, prehybridized in the hybridization solution for 30 min, and then hybridized overnight with the digoxigenin-labeled DNA probes of the Sf9 host and the baculovirus.

2.8. Protein concentration and D antigen ELISA

Protein concentration was quantified by using a BCA Protein Assay Kit (Thermo Fisher Scientific).

The wells of 96-well microtiter plates were coated with 100 μl of monoclonal antibodies (MAB 234 for PV1, MAB 1050 for PV2, and MAB 520 for PV3) and incubated at 4 overnight for the measurement of D antigen content via ELISA. The plates were blocked with 5% FBS in PBST (PBS containing 0.05% Tween 20) at 37 for 2 h. The International Standard for IPV 12/104 (D antigen) and PV VLPs were added to the appropriate wells and incubated at 37 for 1 h. D antigen was detected through incubation with the anti-IPV antibody (rabbit serum) at 37 °C for 1 h and then with HRP-conjugated Goat Anti-Rabbit IgG antibody at 37 °C for 1 h. TMB mixture (Millipore) was added to the wells, which were then incubated for 15–20 min for color development. Then, 0.2 M of H_2SO4 was added to stop the reaction, and the absorbance was measured at 450 nm in a 96-well plate reader.

2.9. Thermostability

The thermostability of PV VLPs was investigated through incubation at 37 for 24 days. The PV VLPs were divided into multiple components, and one component was incubated at 37 every 2 days. Then, the D antigen content was measured through ELISA, and the remaining D antigen of PV VLPs after incubation at 37 for 6, 12, 18, 24 days relative to no incubation were calculated.

2.10. Immunization

Female BALB/c mice aged 6–8 weeks were randomly divided into 20 groups (n = 8 for each group). PV-VLPs and PV-sVLPs were diluted to concentrations of 40, 10, and 2 μg/ml. Thus, 18 treat-

ment groups were obtained. Mice in another group were immunized with IPV (3-fold dilution of IPV, 13.33 D antigen unit of PV1, 2.67 D antigen unit of PV2, and 10.67 D antigen unit of PV3) produced by Sanofi Pasteur. Mice in the negative control group were immunized with PBS. All the above sample vaccines (including PV-VLPs, PV-sVLPs, IPV, and PBS) contained 200 μg/ml of aluminum adjuvant. Each mouse was injected intraperitoneally with 0.5 ml of the sample vaccine and received a booster injection at weeks 2 and 4. Blood samples were collected from the BALB/c mice at week 5 for serological analysis.

2.11. Serum neutralization assay

Vero cells (2×10^5 cells/well) were cultured in 6-well plates overnight until 80% confluent for the measurement of neutralization titers. Serum samples from immunized mice were serially diluted and mixed with an equal volume of virus (100 PFU). Serum samples from mice immunized with PV-VLPs, PV-sVLPs, IPV, or PBS were mixed with Sabin 1, Sabin 2, or Sabin 3. The mixtures were incubated in 6-well plates for 2 h and then mixed with 4 ml of 1% methylcellulose. Neutralization titers were determined as the highest dilutions that could result in a 50% reduction in CPE.

2.12. Luminex cytokine analyses

The splenocytes of the immunized mice were isolated at week 5. Splenocyte suspensions were seeded in 24-well plates at a density of 2×10^6 cells per well. Subsequently, the splenocytes of mice immunized with PV-VLPs or PV-sVLPs were stimulated with 5 μg of the corresponding VLPs, and the splenocytes of the mice immunized with IPV or PBS were stimulated with 5 μg of PV-sVLPs. The negative and positive controls for these assays were stimulated with RPMI 1640 Medium and 5 μg of ConA, respectively. The supernatants were collected, and cytokine (IL-2, IL-4, IL-5, IL-10, IL-12, GM-CSF, IFN-γ, and TNF-α) concentrations were determined by using Bio-Plex Pro™ Mouse Cytokine Th1/Th2 Assay by Bio-Plex® 200 Systems.

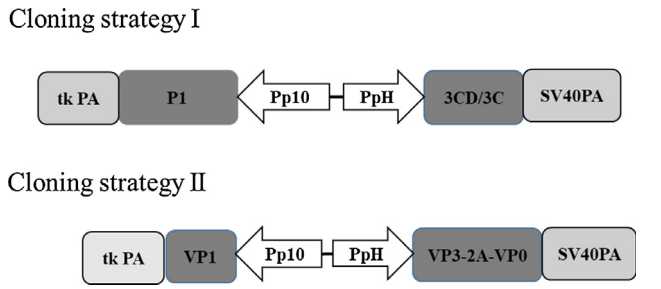


Fig. 1. Schematic illustration of the construction of PV transfer vector. (The 2A sequence was from Porcine Teschovirus-1 and was GSGATNFSLLKQAGDVEENPGP).

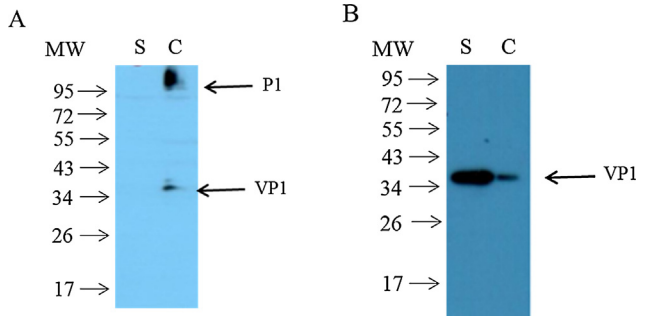


Fig. 2. Analysis of the levels of PV structural proteins of Cloning strategy I and Cloning strategy II by Western-blot. The anti-IPV polyclonal which generated by immunizing rabbits with IPV vaccines was used as an primary antibody. S: Supernatant, C: Cells. (A) Cloning strategy I, (B) Cloning strategy II.

3. Results

3.1. Production of PV VLPs

PV structural proteins are encoded by the viral P1 gene and cleaved by the viral 3CD proteinase during translation. To express all these proteins using one recombinant baculovirus vector, we compared two construction strategies (Fig. 1). In the first construct, in the same bacmid, the P1 coding sequence was cloned under the p10 promoter, while the 3CD coding sequence was cloned under the pH promoter. For the second construct, the 3CD coding

Table 1
Recombinant baculoviruses with thermostable amino acid mutations.

Baculoviruses	VP0	VP1	VP3
Bac-PV1-s	R18G, T94A, D125E,	V196L, H248P	L119M, Q178L
Bac-PV2-s	/	T41I, Y159F	Q178L
Bac-PV3-s	T67A, L86I, L293M, D309E	T105M, F132L	H19Y, L85F

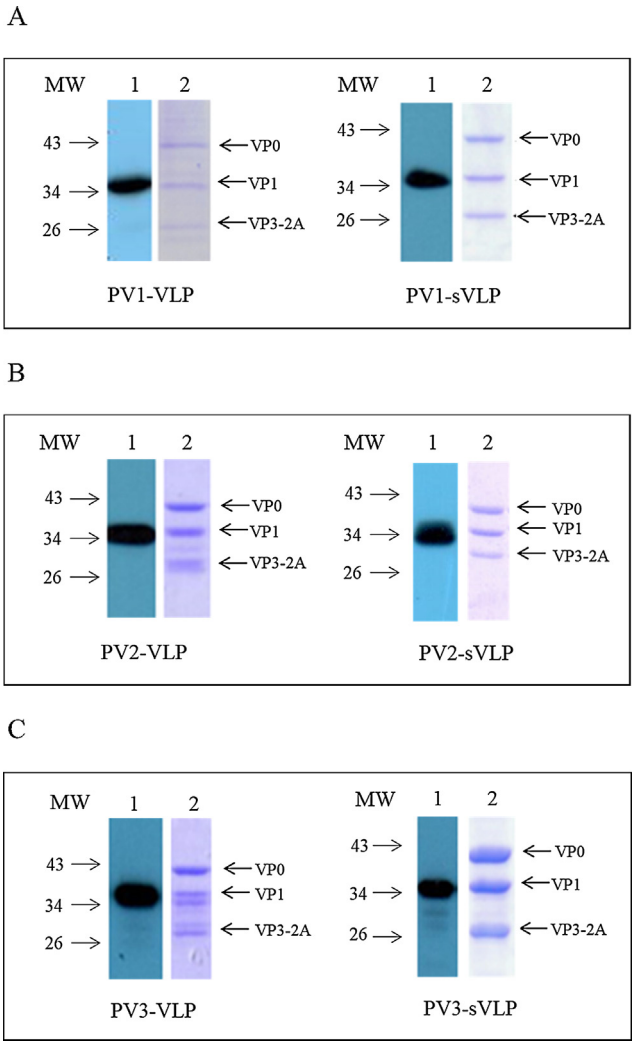


Fig. 3. Analysis of the purified PV VLPs. The anti-IPV polyclonal which generated by immunizing rabbits with IPV vaccines was used as an primary antibody. 1: Western-blot, 2: SDS-PAGE. (A) PV1-VLP and PV1-sVLP, (B) PV2-VLP and PV2-sVLP, (C) PV3-VLP and PV3-sVLP.

sequence was excluded. Instead, the VP1 coding sequence was cloned under the p10 promoter, whereas the VP3-2A–VP0 fusion protein was placed under the pH promoter. The 2A peptide of porcine teschovirus-1 is an auto cleavable peptide [11,12] that will cleave VP3–2A–VP0 into VP3–2A and VP0. In this construct, VP1, VP0, and VP3-2A will be generated in the absence of the 3CD proteinase. We compared the expression levels of VLP through Western blot analysis by using the anti-IPV polyclonal antibody. As indicated in Fig. 2, we selected construction strategy II for the subsequent production of PV-VLPs because it produced more PV structural proteins than construction strategy I.

A group of amino acid mutations that can enhance the thermostability of PV particles has been previously identified [10]. We generated Bac–PV1-s, Bac–PV2-s, and Bac–PV3-s to express the mutated forms of PV structural proteins for the comparison of the immunogenicity of the PV-VLPs mutants (Table 1). As shown in Fig. 3, the presence of stabilizing mutations drastically increased the production of VP1, VP3-2A, and VP0 from PV1, PV2, and PV3. This effect may be attributed to the enhanced structural stability of the PV proteins.

3.2. Analysis of the structure, thermostability, and purity of PV-sVLPs

To verify that the expressed PV structural proteins indeed assembled into VLPs, the purified proteins were observed by transmission electron microscopy (TEM). A high concentration of uniform PV-like particles with diameters of 27–30 nm was readily observed (Fig. 4).

A sandwich ELISA was applied to investigate the amounts of D antigen in PV-VLPs and PV-sVLPs. In contrast to that in previously reported assays, in our assay, we coated plates with mAbs specific for D antigens to enrich and capture VLPs that contain D antigen epitopes and employed polyclonal antibodies to detect captured VLPs. As shown in Table 2, the amount of D antigen units presented by PV-sVLPs was more than 3.9 times that presented by the same amount of PV-VLPs.

We also utilized our developed ELISA procedure to investigate the thermostability of PV-sVLPs and PV-VLPs. We measured the amount of remaining D antigen after incubation at 37 for 6, 12, 18, and 24 days. As shown in Fig. 5, the D antigen content of PV-sVLPs remained constant but that of PV-VLPs decreased drastically.

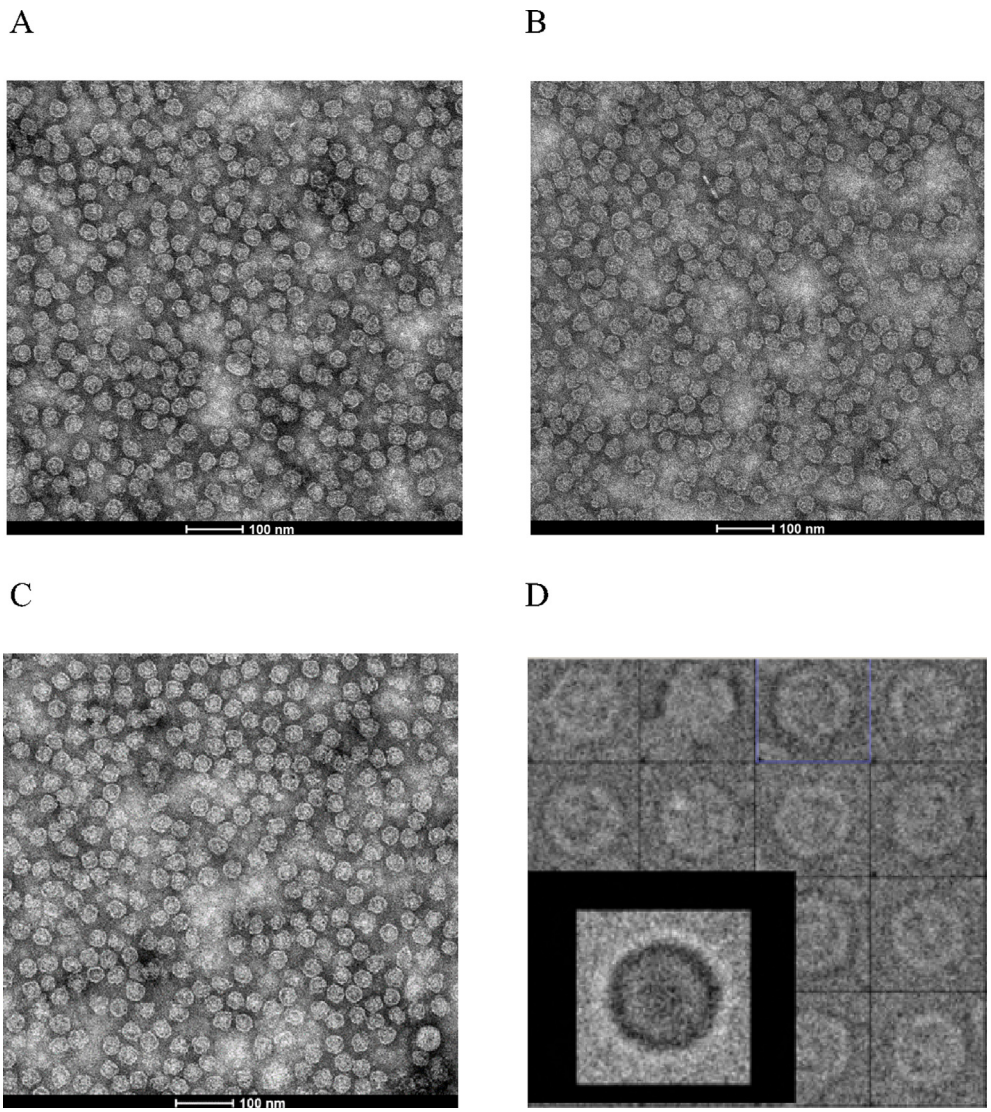


Fig. 4. Electron microscope and Cryo-electron analysis of purified PV-sVLPs. PV-sVLPs were analyzed by transmission electron microscopy (TEM) and the diameter was approx. 27–30 nm. The bar is 100 nm. (A) electron microphotographs of PV1-sVLP, (B) electron microphotographs of PV3-sVLP, (C) electron microphotographs of PV2-sVLP, (D) Cryo-electron microphotographs of PV2-sVLP.

Table 2
D antigen unit (DU) of 1 μg PV-VLPs and PV-sVLPs.

Types	PV-VLPs (DU/μg)	PV-sVLPs (DU/μg)	Fold
1	0.065	0.32	4.92
2	0.050	0.197	3.94
3	0.42	2.4	5.71

These results confirm that the introduction of mutations enhanced the thermostability of VLPs.

Given that we aimed to develop PV-sVLP-based human vaccines, we applied an ELISA method to determine the purity of the purified VLPs. Our ELISA results suggest that the purity of the purified PV-sVLPs exceeded 98.5%, whereas the amount of residual DNA from Sf9 and the baculovirus was less than 50 pg/mg PV-sVLPs.

3.3. Immunization with PV-sVLPs elicited strong immune response against PV

To compare the immunogenicity of PV-VLPs and PV-sVLPs, we immunized mice with different amounts of corresponding VLPs and examined the titers of PV-neutralizing antibodies through neutralization assays. A group of mice was immunized with trivalent IPV vaccine (equivalent to 13.33 D antigen unit of PV1, 2.67 D antigen unit of PV2, and 10.67 D antigen unit of PV3) from Sanofi Pasteur for comparison. As shown in Fig. 6, PV1-sVLP, PV2-sVLP, and PV3-sVLP induced significantly high titers of neutralizing antibodies in a dose-dependent manner. Compared with the same amount of PV-VLPs, PV-sVLPs presented more D antigens and induced higher levels of neutralizing antibody titers (Fig. 6).

To determine PV-specific cellular immune responses, we measured the levels of IFN-γ that were excreted by the splenocytes of BALB/c mice upon PV-VLPs or PV-sVLPs stimulation. As shown in Fig. 7A, although PV-VLPs and PV-sVLPs induced IFN-γ in a dose-dependent manner, the level of IFN-γ expression induced by PV-sVLPs was higher than that induced by PV-VLPs.

We also evaluated the expression of cytokines that were correlated with the Th1 and Th2 types of immune responses (Fig. 7A to 7E). The levels of Th1-polarizing cytokines (IFN-γ and IL-2), Th2-polarizing cytokines (IL-5 and IL-10), and Th1 and Th2-polarizing cytokines (GM-CSF) all significantly increased in the PV-sVLP immunization group relative to those in the PBS control group.

These results suggest that the specific humoral and cells immune response of mice could be effectively stimulated through immunization with PV-sVLPs.

4. Discussion

PV remains prevalent only in Pakistan, Afghanistan, and Nigeria given the successful application of combined IPV and OPV vaccines. Worldwide PV eradication is imminent. Nevertheless, the systematic strategy of polio vaccination should be continued for the following decades to maintain the necessary community immunity at a safe level in the post-polio era. However, the current methods for IPV and OPV vaccine production require the amplification of PVs in tissue culture. This approach will no longer be feasible in

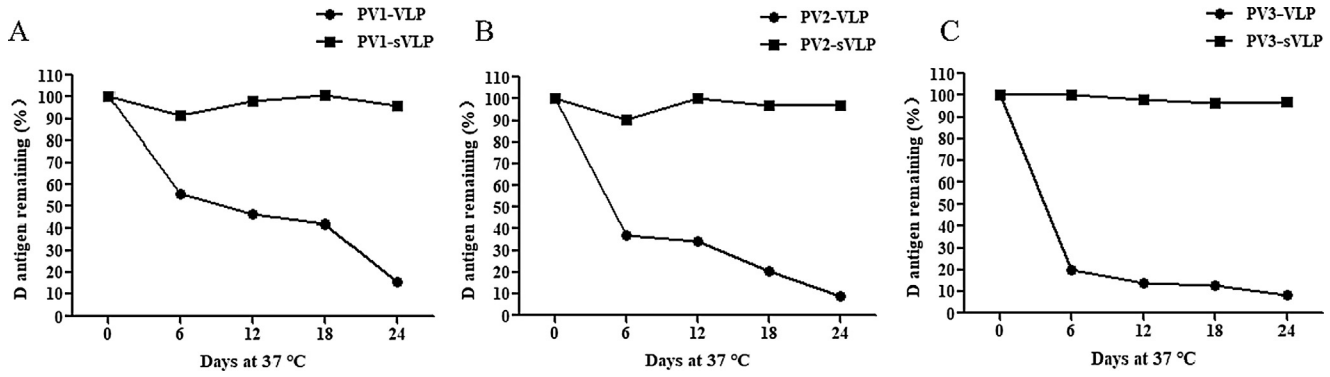


Fig. 5. Thermostability of PV-VLPs and PV-sVLPs. D antigen remaining of PV-VLPs and PV-sVLPs after incubation at 37 for 6, 12, 18, 24 days relative to no incubation. (A) PV1-VLP and PV1-sVLP, (B) PV2-VLP and PV2-sVLP, (C) PV3-VLP and PV3-sVLP.

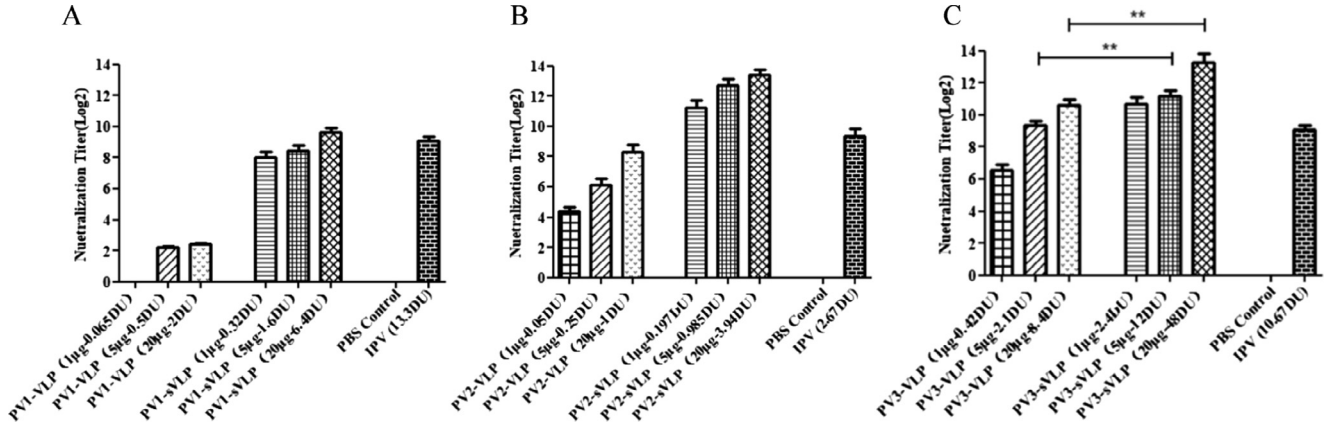


Fig. 6. PV neutralizing antibody in BALB/c mice immunized with PV-VLPs and PV-sVLPs. (A) PV1-VLP and PV1-sVLP, (B) PV2-VLP and PV2-sVLP, (C) PV3-VLP and PV3-sVLP. All results were expressed as mean ± standard deviation (S.D.) for eight mice in each group (*P < 0.05, **P < 0.01 and ***P < 0.001).

a polio-free world. This deficiency may be addressed through the production of noninfectious VLP vaccines.

VLP vaccine technology has become the mainstream method for recombinant vaccine production in recent years. VLPs are nanoparticles that are free from infectious viral genomic sequences and are generated by self-assembled viral structural proteins that present important protective epitopes. VLP vaccines are associated with numerous advantages. First, VLPs can be produced in large quantities in a heterologous host and can be easily purified because of their particle structure and high molecular weight. Second, VLPs can efficiently stimulate a protective immune response by themselves or in combination with the appropriate adjuvants. Third, VLPs can display chimeric epitopes that function as multivalent VLPs when used as scaffold cores. Fourth, VLPs are safer than conventional viral vaccines because they lack viral genomic sequences. VLP vaccines for Hepatitis B [13], human papillomavirus [7], and hepatitis E [14] have been approved, and other VLP candidate vaccines are currently under clinical evaluation.

The expression of stable VLPs in large quantities has been the major challenge encountered by VLP vaccine technology. Efforts to generate PV VLPs by using different expression systems were initiated decades ago [15–18] because of the importance of PVs

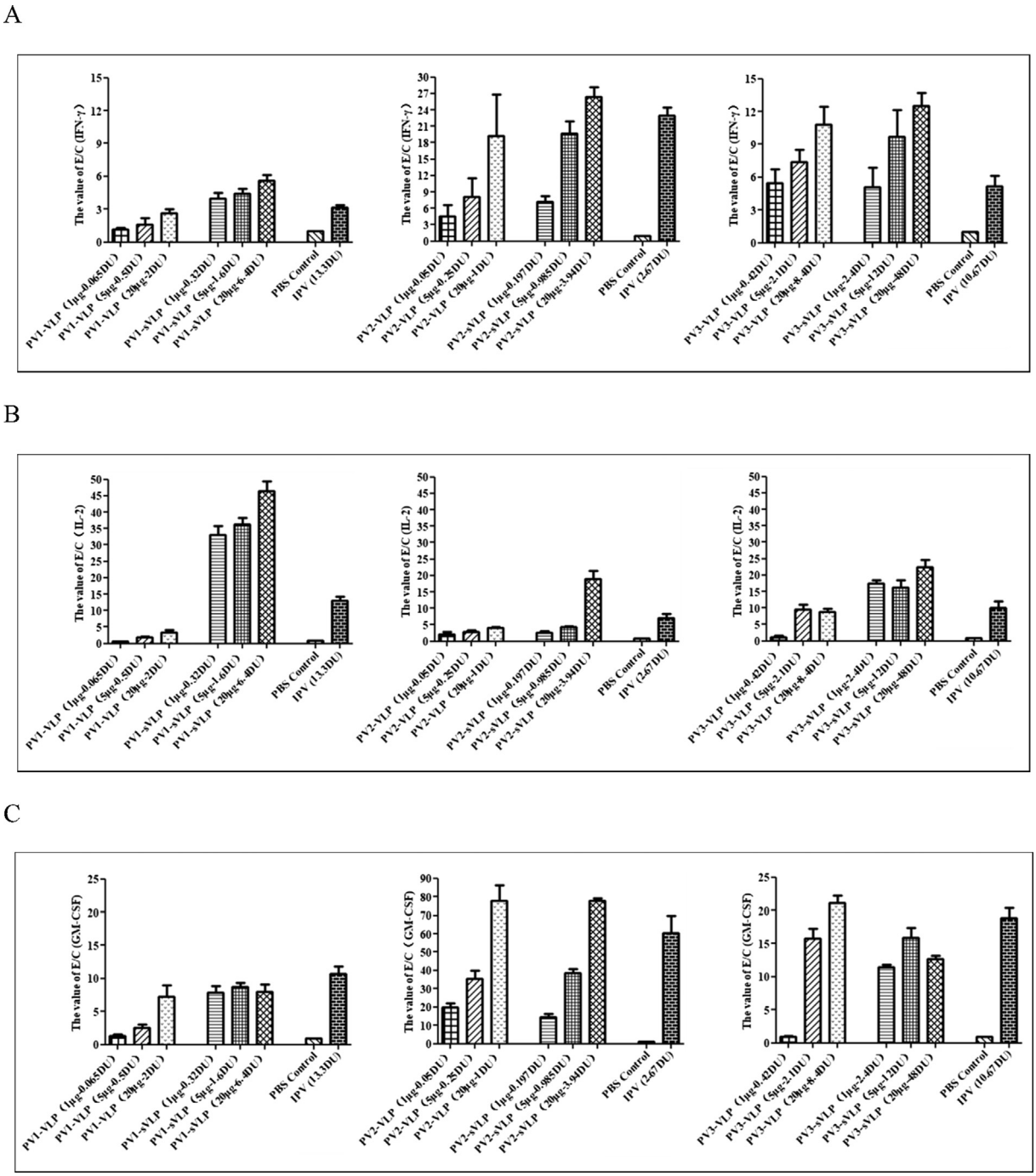
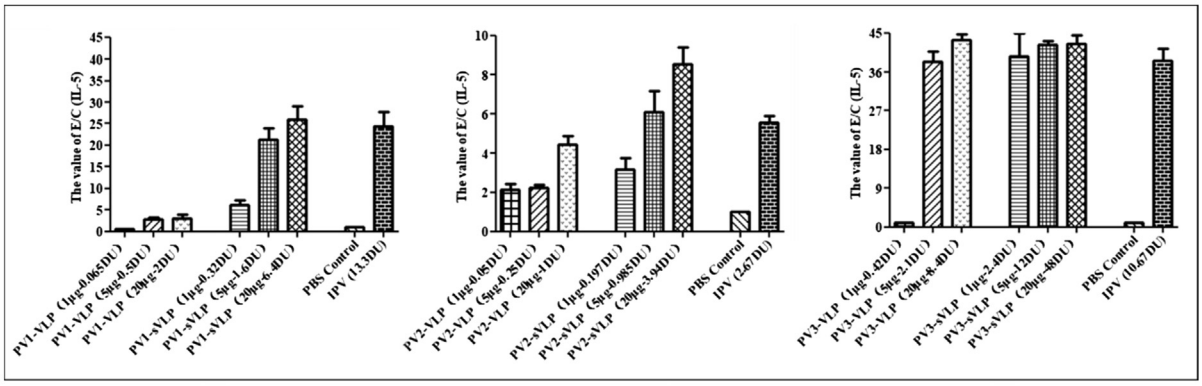


Fig. 7. The value of E/C (E: Concentration of cytokines secreted by PV VLPs stimulated splenocytes of vaccine groups, C: Concentration of cytokines by PV VLPs stimulated splenocytes of PBS) by PV VLPs (PV-VLPs or PV-sVLPs) stimulated splenocytes. (A) was IFN-γ, (B) was IL-2, (C) was GM-CSF, (D) was IL-5, (E) was IL-10. The splenocytes were derived at one week post vaccination and then stimulated by 5 μg/well purified PV VLPs for 48 h. The culture supernatant was collected and measured by Luminex.

D



E

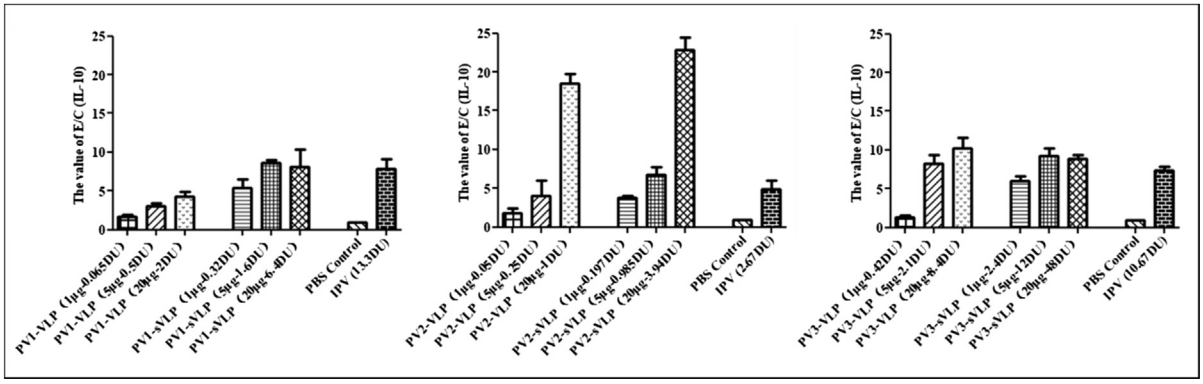


Fig. 7 (*continued*)

eradication. Urakawa et al. (1989) produced PV3 VLP with a BEVS platform [15] by cloning the complete viral ORF into a baculovirus vector. Nevertheless, they obtained low VLP yields (60 μg/L). Their results suggested that uncertain viral proteinase activity might have contributed to low VLP production. Subsequently, Rombaut et al. (1997) obtained PV1 VLP by co-express P1 and 3CD in yeast [18]. They reported a production yield of less than 100 μg/L, which is similar to that reported by Urakawa et al. In the present study, we first cloned 3CD/3C and P1 coding sequences separately but in the same bacmid. Similar to that observed in the yeast system, the production of recombinant PV capsid proteins (represented by VP1) in our system was low. Given the possibility of insufficient P1 cleavage by the 3CD/3C proteinase in insect cells and to eliminate the potential cytotoxicity exerted by 3CD/3C [19], we opted to forego 3CD/3C in the expression system in our second construction strategy. Instead, we placed the sequence of the autocleavage peptide 2A between VP3 and VP0. We placed the VP1 coding sequence directly under the P10 promoter, as was previously done to develop a vaccine against the O-type foot-and-mouth disease virus, to further enhance the expression of the VP1 protein [20]. Our strategy produced high levels of VP3-2A, VP0, and VP1 that assembled into PV-VLPs efficiently and provided VLP yields of 2 mg/L. The production yield of our strategy is anticipated to reach the requirement for industrial production after further process optimization.

In this study, we showed for the first time that PV-VLPs could assemble independently of the viral proteinase 3CD. Furthermore, our results indicate that even wild-type PV-VLPs have considerable D antigen contents. Whether this property is unique to our unconventional PV-VLP formation strategy and/or to our BEVS platform remain to be determined. The generation of a high-resolution three-dimensional structure would help answer these questions.

Early endeavors to generate PV-VLPs were hampered mainly by their extremely low protective immunogenicity [15–18]. Later studies indicated that PV particles are thermally unstable. Mutations capable of enhancing the thermostability of PV have been recently identified [10].All of these mutations are located in the VP0, VP1, and VP3 regions. A PV3 sVLP carrying thermostability-enhancing mutations was generated in a plant. This sVLP showed considerable protective effects against PV infection in a mouse model [21]. In the present study, we introduced thermostability-enhancing mutants and produced the respective PV-sVLPs of all three types of viruses. Our results suggest that PV-sVLPs are highly stable (Fig. 5) at 37 and present high amounts of D antigen units (Table 2). We found that PV-sVLPs induced neutralization antibody titers at levels higher than those induced by their respective PV-VLPs and at levels similar to those induced by IPVs (Fig. 6). In addition, the high levels of IFN-γ (Fig. 7A) and IL-2 (Fig. 7B) production observed in mice immunized with PV-sVLPs mice is suggestive of an enhanced Th1 type of immune response. Meanwhile, the increased production of Th2-polarizing cytokines, IL-5, IL-10 (Fig. 7D and 7E), and the Th1/Th2-polarizing cytokine GM-CSF (Fig. 7C) indicates that PV-sVLPs could induce a Th1/Th2 integrated immune response that enhances T-cell activation and antibody production.

In summary, we successfully established a BEVS platform for the production of PV-sVLPs in large quantities. Our results show that the produced VLPs can induce strong humoral and cellular immune responses in mice. Our data imply that our BEVS platform is a efficient expression system for PV-sVLP production. The pro-

duction and administration of highly immunogenic PV-sVLPs are safer than those of IPV and OPV. Thus, the production of PV-sVLPs through the BEVS platform represents a promising approach for the development of vaccines against PV infection. Our approach for PV vaccine development should be included in the current global effort to end polio safely.

Conflict of interest

The authors declared that there is no conflict of interest.

Acknowledgments

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