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Short communication

Peste des petits ruminants virus-like particles induce both complete virus-specific antibodies and virus neutralizing antibodies in mice

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A B S T R A C T

Peste des petits ruminants virus (PPRV), an etiological agent of peste des petits ruminants (PPR), is classified into the genus *Morbillivirus* in the family *Paramyxoviridae*. In a previous study, a recombinant baculovirus has been constructed to co-express the PPRV matrix (M), haemagglutinin (H) and nucleocapsid (N) proteins in insect cells, causing budding of PPR virus-like particles (VLPs) from insect cell membranes by viewing of ultrathin section with a transmission electron microscope. In this follow-up study, these PPR VLPs were purified by sucrose density gradient centrifugation for immunizing mice twice. Three weeks post-primary immunization and 2 weeks post-secondary immunization, all serum samples were obtained and subsequently subjected to indirect ELISA detection on complete virus-specific antibodies. In addition, all serum samples, which were collected 2 weeks post-secondary immunization, were used for virus neutralization test on PPRV neutralizing antibodies. The results showed that the purified PPR VLPs induced both types of antibodies mentioned above in mice, indicating a given potential of VLP-based vaccine candidate against PPR.

1. Introduction

Peste des petits ruminants (PPR) is an acute or subacute, highly contagious and economically important disease of small ruminants. Peste des petits ruminants virus (PPRV), an etiological agent of PPR, is classified into the genus *Morbillivirus* in the family *Paramyxoviridae*. The PPRV genome encodes six structural proteins, namely, nucleocapsid (N) protein, phosphoprotein (P), matrix (M) protein, fusion (F) protein, hemagglutinin (H) protein and large (L) protein. During the past three decades, different PPRV isolates, like Nigeria 75/1 and Sungri/96, were successfully attenuated by serial passages in Vero cells (Saravanan et al., 2010). As demonstrated to be very efficient in the protection of sheep and goats against virulent challenges, these avirulent PPRVs as efficacious immunogens are now widely used to produce commercially available vaccines against PPR.

Several studies were involved in development of a new generation of PPR vaccines characterized by DIVA (differentiating infected from vaccinated animals), such as viral vector vaccines (Diallo et al., 2002; Qin et al., 2012; Herbert et al., 2014; Rojas et al., 2014a,b), all of which however have not been commercialized as yet. Another type of vaccine candidate is the subunit vaccine. Recombinant H protein of rinderpest virus (sharing the highest homology with PPRV) expressed in insect cells can elicit not only humoral but also cell-mediated immune responses in cattle (Sinnathamby et al., 2001). Furthermore, the transiently expressed PPRV H protein was found to be biologically active in possessing hemadsorption and neuraminidase activities (Seth and Shaila, 2001). Therefore, the PPRV H protein is a potential candidate for incorporation in the subunit vaccine.

Owing to their safety and efficacy at relatively high levels *in vivo* (Jeoung et al., 2011; Bucarey et al., 2014; Deng et al., 2014; Kim et al., 2014), virus-like particles (VLPs) are increasingly being considered as potential subunit vaccines against viral diseases. VLPs, composed of one or more structural proteins but no genomes of native viruses, mimic the organization and conformation of authentic virions but have no capability of self-replication in cells. To date, animal virus VLPs have been generated from a broad spectrum: VLPs of structurally simple viruses, *e.g.*, porcine circovirus VLPs (Yin et al., 2010); VLPs with lipid envelope, *e.g.*, avian influenza virus VLPs (Lee et al., 2011); VLPs with multiple protein layers, *e.g.*, bluetongue virus VLPs (Belyaev and Roy, 1993). Due to their immunogenic properties and high safety profile, VLPs represent one of the most appealing strategies for immunization to prevent viral diseases, as highlighted systematically in recent reviews (Liu et al., 2012, 2013a,b).

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In principle, owing to high-fidelity simulation of parental viruses to display densely repetitive epitopes, VLPs are commonly more immunogenic than a single recombinant protein (Bright et al., 2007), therefore potentially yielding safer vaccine candidates even without the need for any adjuvant (Zhang et al., 2000; McGinnes et al., 2011). A variety of VLPs have proven to be efficient for the induction of neutralizing antibodies and to be protective in animal models (Pushko et al., 2005; Stewart et al., 2012; Tretyakova et al., 2013). More importantly, since VLPs containing either monovalent or multivalent antigen can be produced in compliance with the requirements for serological surveillance, VLP-based vaccines play a promising role in DIVA strategies for vaccination against animal diseases.

In a previous study, PPR VLPs comprising the PPRV M, H and N proteins have been produced by a baculovirus expression system (Liu et al., 2014b). In this follow-up study, these PPR VLPs were purified by sucrose density gradient centrifugation for further immunization of mice twice. Serum samples were collected after immunization, and then were subjected either to indirect ELISA detection on complete virus-specific antibodies or to virus neutralization test on PPRV neutralizing antibodies.

2. Materials and methods

2.1. Cells and viruses

Spodoptera frugiperda (Sf9) cells were grown in Sf-900™ III serum-free medium (Life technologies, Carlsbad, USA) at 27 °C in suspension culture for infection with recombinant baculoviruses, which were successfully constructed to co-express intracellularly the PPRV M, H and N proteins in the previous work (Liu et al., 2014b). The number of Sf9 cells was counted microscopically with a hemocytometer to determine cell density, while cell viability was judged by trypan blue dye exclusion (Yamaji et al., 1999). PPRV Nigeria 75/1 was obtained from the China Institute of Veterinary Drug Control. Vero cells were cultured in Dulbecco's modified eagle's medium (Life technologies, Carlsbad, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 25 mM HEPES (Sigma, St. Louis, USA) at 37 °C in 5% CO_2 (Wang et al., 2013).

2.2. Purification of PPR VLPs

The PPR VLPs were purified by sucrose density gradient centrifugation as described previously (Liu et al., 2014b). In short, healthy Sf9 cells (1.5×10^6 cells/mL) were infected with recombinant baculoviruses at an MOI of 5 at 27 °C in suspension culture at a shaking speed of 120 rpm. At 96 h post-infection, the supernatant was obtained from the culture by centrifugation at 7000 rpm for 20 min in a JA-10 rotor (Beckman J-20XP; Beckman Coulter, Fullerton, USA) to remove any cells and debris. The clear supernatant was spun in an ultracentrifuge (Beckman LE-80K; Beckman Coulter, Fullerton, USA) at 25,000 rpm at 4 °C for 90 min using SW 32 Ti swinging bucket rotor to pellet the VLPs. The pellet was resuspended in 8 mL PBS. This was overlaid on an 8 mL step sucrose gradient of 20–40–60% (w/v) prepared in PBS. Gradients were spun in the ultracentrifuge (Beckman LE-80K; Beckman Coulter, Fullerton, USA) at 28,000 rpm at 4 °C for 90 min using SW 32 Ti swinging bucket rotor. Finally, two white protein layers were visible in the tube, and the upper one containing VLPs was collected with a syringe followed by resuspension with PBS. The protein concentration of purified product was analyzed by the NanoDrop® ND-1000 Spectrophotometer (Thermo, Waltham, USA).

2.3. Immunization of mice

A total of 15 6-week-old female Balb/c mice from the Vital River Laboratories, China, were housed under pathogen-free conditions in micro-isolator cages in the animal quarters at the National Research Center for Exotic Animal Diseases, China. The mice were distributed randomly into three groups (five mice/group), namely the group A, B and C. All protocols requiring open cages were accomplished in a biosafety cabinet. All animal experiments involved in this study were performed under a guideline of the *Administration Rule of Laboratory Animal*, China.

On the first day, the group A mice were immunized subcutaneously using purified VLPs (0.3 mg total protein/mouse) mixed with complete Freund's adjuvant (Sigma, St. Louis, USA) (v/v: 1/1); the group B mice were immunized subcutaneously using purified VLPs (0.3 mg total protein/mouse) without adjuvant; the group C mice were immunized subcutaneously using sterile PBS (0.8 mL/mouse). After 3 weeks, a small amount of blood samples from all groups were obtained from tail vein nicks and subjected to centrifugation to separate sera.

Subsequently, the group A mice were boosted subcutaneously using purified VLPs (0.2 mg total protein/mouse) mixed with incomplete Freund's adjuvant (Sigma, St. Louis, USA) (v/v: 1/1); the group B mice were boosted subcutaneously using purified VLPs (0.2 mg total protein/mouse) without adjuvant; the group C mice were boosted subcutaneously using sterile PBS (0.5 mL/mouse). Two weeks post-secondary immunization, a large amount of blood samples from all groups were obtained from the orbital sinuses after anesthetization, and then were subjected to centrifugation to separate sera.

2.4. Indirect ELISA detection

All serum samples, collected both 3 weeks post-primary immunization and 2 weeks post-secondary immunization, were used for indirect ELISA detection on titer of antibodies against complete PPRVs. Briefly, the wells of polystyrene microtiter plates were coated with cell culture-derived and bromoethleneimine-inactivated PPRVs, obtained from a commercially available PPRV competitive ELISA kit (BDSL, North Ayrshire, UK). After incubation for 1 h at 37 °C, the wells were washed three times with PBS-Tween 20 (PBST) buffer. The coated wells were blocked with 100 μL of 2% BSA at 37 °C for 1 h, washed three times with PBST buffer and then incubated with 100 μL PBST buffer containing sera with different consecutive deliquations (from 1:500 to 1:512,000) at 37 °C for 1 h. After incubation, the coated wells were washed 3 times with PBST buffer followed by incubation with 50 μL horseradish peroxidase-conjugated rabbit anti-mouse IgG solution, obtained from the PPRV competitive ELISA kit (BDSL, North Ayrshire, UK) as mentioned above, at 37 °C for 1 h. After incubation of secondary antibodies, the coated wells were washed 3 times with PBST buffer, and then reacted with 50 μL ortho-phenylenediamine (OPD; Sigma, St. Louis, USA) solution with H_2O_2 at room temperature in the dark for at least 10 min. The reaction was stopped by 50 μL H_2SO_4 (1 M) and the absorbance was measured at 492 nm using a Tecan Sunrise™ microplate reader (TECAN, Männedorf, Switzerland). The antibody titer was defined as the highest dilution of serum, at which a P/N value (A_{492} of post-immunization serum/A_{492} of negative control serum) is greater than 2.1. All the P/N values under the condition of sequential dilutions were analyzed using the GraphPad Prism version 5.0 software.

2.5. Virus neutralization test

All serum samples, which were collected 2 weeks post-secondary immunization, were used for virus neutralization test.