Research in Veterinary Science 117 (2018) 196–199

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Short communication: Immune responses in sows induced by porcine sapovirus virus-like particles reduce viral shedding in suckled piglets

Bo Yang[a,b], Bin Yang[a], Xingna Shan[a], Baoyu Li[a], Xiaojun Ma[b], Xiangping Yin[a], Yun Zhang[a], Yongsheng Liu[a], Xi Lan[a,*]

[a] State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China
[b] College of Veterinary Medicine, Gansu Agricultural University, Lanzhou 730070, China

ARTICLE INFO

Keywords:
Porcine sapovirus
Virus-like particles
Viral shedding
Piglets

ABSTRACT

Porcine sapovirus (PoSaV) is a potential threat to public health owing to its capacity for reassortment with human sapovirus strains. However, there is still no vaccine available for the prevention and control of this infectious disease. In this study, we developed PoSaV virus-like particles (VLPs) using a baculovirus expression system. Immunization with PoSaV VLPs induced high titers of serum antibody specific for VP1 in sows. The results of our challenge study demonstrated that maternally-derived antibodies (MDA) induced by VLP immunization dramatically reduced viral shedding of PoSaV in the feces of next generation piglets. Therefore, the results of this study indicate that the immune responses of sows elicited by PoSaV VLPs can inhibit in vivo viral replication in their offspring and represent a promising strategy for developing vaccines against PoSaV.

Sapovirus (SaV) is one of the primary causes of serious viral gastroenteritis in humans, particularly in younger individuals (Liu et al., 2014). In recent years, SaVs were detected in many countries, such as Canada, Japan, the Netherlands, England, North Korea, and China (Kim et al., 2006; L'Homme et al., 2009; Martella et al., 2008; Reuter et al., 2010; Wang et al., 2006). Thus far, SaV has been detected in many additional species, including swine (Guo et al., 2001b), mink (Guo et al., 2001a), cows (Mijovski et al., 2010), dogs (Li et al., 2011) and bats (Tse et al., 2012). Therefore, it is possible that SaV has the capability to disseminate across species, posing a potential threat to public health.

Like other viruses in the Caliciviridae family, SaV is a small (27–40 nm in diameter) positive sense single-stranded, non-enveloped RNA virus with a 7.3–8.3 kb nucleotide genome and two or three main open reading frames (ORFs). For SaV, ORF1 encodes a polyprotein that is cleaved into multiple viral proteins, including 2C-like NTPase, 3C-like protease, VPg, 3D-like RNA-dependent RNA polymerase and the major capsid protein (VP1). ORF2 encodes the minor and poorly characterized structural protein (VP2) (Bank-Wolf et al., 2010). ORF3 overlaps with ORF1, encoding a protein of unknown function (Hansman et al., 2007). The capsid protein VP1 is the main structural protein of PoSaV to protect the viral nucleic acid and possesses multiple immunogenic epitopes that induce both humoral and cellular immunity. Moreover, VP1 was closely correlated with the process of virus assembly and infection (Chen et al., 2006). PoSaV was first identified in fecal specimens in the United States by electron microscopy in 1980 (Saif et al., 1980), but there is still no effective vaccine thus far for prevention of the disease.

Virus-like particles (VLPs) are analogs of the natural and original structure of the virus, assembled from viral structural proteins but lacking any genetic material. The significant advantage of VLPs over inactivated or attenuated virus vaccination is the combination of outstanding safety with strong immunogenicity. Importantly, VLPs contain functional VPs responsible for cell penetration by the virus, which ensures efficient cell entry (Chroboczek et al., 2014). Furthermore, baculovirus expression systems have been widely used for the production of recombinant proteins and the construction of VLPs (Vicente et al., 2011). In the past decade, SaV VLPs have already been developed (Hansman et al., 2005; Hansman et al., 2006; Oka et al., 2009), but even though PoSaV VLPs have been generated and reported (Guo et al., 2001c), the protective efficacy of PoSaV VLPs has not yet been investigated, despite this being of great importance for control of swine diarrhea. Therefore, the aim of this work was to investigate the PoSaV VLPs developed in this study can be used as a vaccine candidate for control of PoSaV.

The recombinant baculovirus, containing the coding sequences of the PoSaV CH430 VP1 (GenBank accession no.: KF204570) were generated by using the Bac-to-Bac™ system (Invitrogen) (Luckow et al., 1993). Propagation of the recombinant virus was performed according to standard procedures as per the manufacturer's instructions

* Corresponding author at: Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 1 Xujiaping, Chengguan District, Lanzhou 730046, China.
E-mail addresses: yangbin02@caas.cn (B. Yang), yinxiangping@caas.cn (X. Yin), zhangyun03@caas.cn (Y. Zhang), liuyongsheng@caas.cn (Y. Liu), lanxi@caas.cn (X. Lan).

https://doi.org/10.1016/j.rvsc.2017.12.016
Received 25 May 2017; Received in revised form 14 December 2017; Accepted 26 December 2017

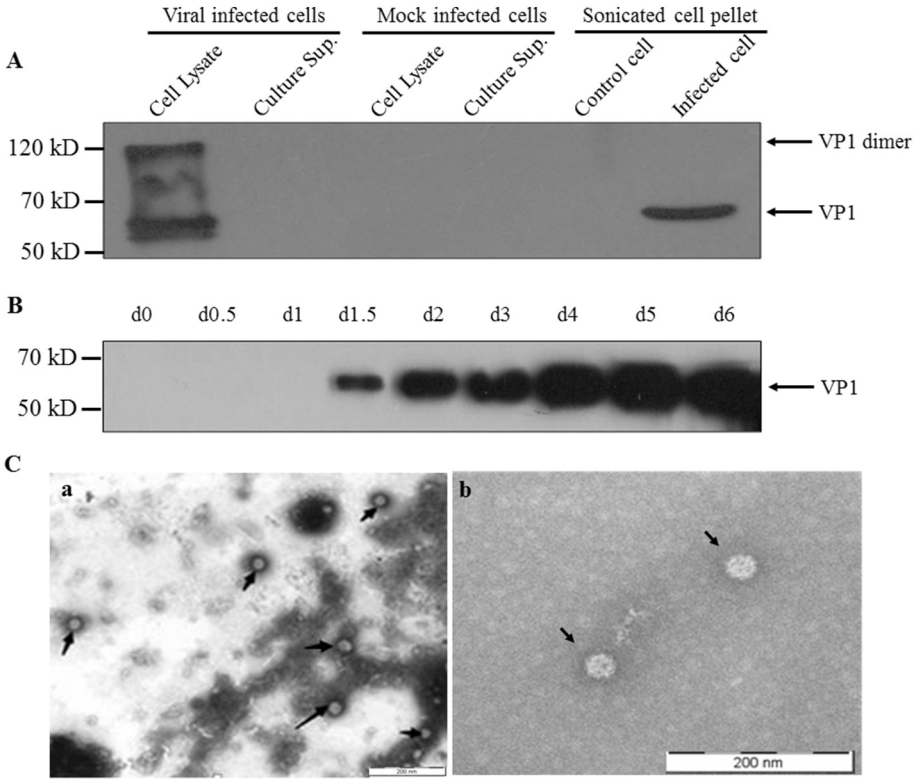

Fig. 1. Identification of PoSaV VP1 expression and VLPs formation. (A) Sf9 cells were inoculated with P2 recombinant baculovirus at 48 h post-infection and subjected to western blot analysis with anti-VP1 monoclonal antibody, showing the expected band at 60 kDa (monomer) or 120 kDa (dimer). (B) The time course of VP1 expression in sf9 cells was also analyzed by western blot. (C) The PoSaV VP1 protein assembled into spherical VLPs. (a) Purified VLPs were negatively stained and visualized by TEM. (b) Magnified views of VLPs.

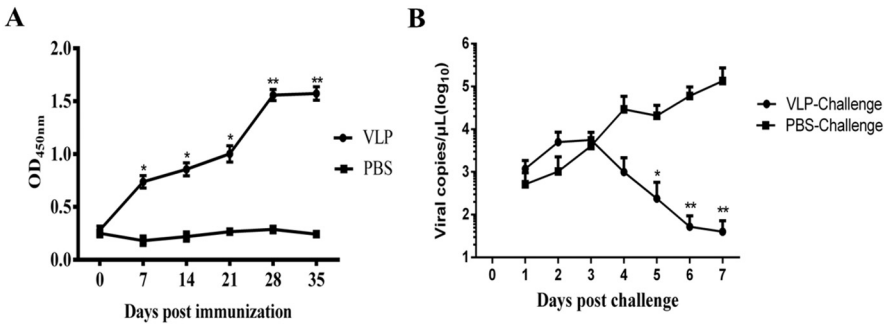

Fig. 2. Porcine SaV specific antibody induced by VLP immunization and protected against viral challenge in neonates. (A) Two groups of sows were immunized with PoSav VLPs or mock-immunized, and boost-immunized with the same immunogen at 14 days post-primary immunization. Serum was collected on a weekly basis following immunization. VP1-specific antibodies in the serum were measured by indirect ELISA. The data are expressed as mean ± SD of each group among the 5 sows. Data are the mean ± SD of five sows per group. (B) For monitoring of daily viral shedding after challenge with PoSaV, quantitative real-time RT-PCR was employed for measuring viral loads in fecal samples. Data are the mean ± SD of five piglets per group. *$p < 0.05$ vs. PBS, **$p < 0.01$ vs. PBS.

(Invitrogen). The second generation of recombinant virus was inoculated onto sf9 cells and VP1 protein expression was detected by western blot. The results showed that VP1 was expressed in the cell lysate rather than the culture supernatant (Fig. 1A). Interestingly, besides the VP1 band observed at around 60 kDa, our data consistently showed a strong signal at 120 kDa, indicating that VP1 dimers were also formed in this expression system (Fig. 1A). However, only VP1 monomers could be detected in the cell pellet after sonication treatment (Fig. 1A). We next measured the time course of VP1 expression in sf9 cells, and our results demonstrated that VP1 expression was observed as early as 36 h post-infection and increased with progressing infection (Fig. 1B). Furthermore, because the level of VP1 expression at 5 day post infection (dpi) was higher than other time post infection (Fig. 1B), the cells were harvested at 5 dpi and then subjected to three freeze/thaw cycles and ultrasonication by centrifugation at 5000 rpm for 30 min at 4 °C. The treated cell lysate samples were filtered through a 0.45 μm filter and layered onto a 2 ml 20%, 2 ml 30%, 3 ml 45% and 3 ml 60% sucrose cushion in TNE buffer (50 mM Tris-HCL, 1 mM EDTA and 1 M NaCl) from top to bottom in 14 × 89 mm Ultra-Clear™ centrifuge tubes (Beckman, USA). The samples were centrifuged at 35000 rpm for 3 h using a Beckman SW41Ti rotor and divided into 10 fractions for each tube after centrifugation. All fractions were examined for PoSaV VLPs using a transmission electron microscopy (TEM) JEM-1010 (JEOL, Tokyo, Japan). TEM showed that the VP1 protein assembled into spherical VLPs with diameters ranging from 38 to 43 nm, as indicated by the arrows (Fig. 1C-a). The size and structure of the magnified VLPs were clearer (Fig. 1C-b) and similar to PoSaV wild-type virus particles. These data suggest that the PoSaV VLPs could be successfully assembled and recovered from an insect/baculovirus expression system.

Large White sows were eight month at first farrowing and purchased from Jingning Boyutong Breeding Co., Ltd. (Gansu Province, China) and housed in a comfortable, disinfected animal room. Forty days before farrowing, five healthy pregnant sows were first intramuscularly immunized with 120 μg VLPs mixed with an equal volume of ISA 206 adjuvant (SEPPIC, France) and boosted at 14 days post-primary immunization. A negative control group of five sows was intramuscularly injected with sterile PBS mixed with an equal volume of ISA 206 adjuvant. Sera were collected on days 0, 7, 14, 21, 28, and 35 for detection of PoSaV specific antibodies. As shown in Fig. 2A, the serum antibody against PoSaV VP1 was generated around 1 week after primary immunization with VLPs. The level of serum antibody was increased following boost immunization, and reached a peak at 2 weeks post-booster (Fig. 2A). Meanwhile, no VP1 antibody was detected in the mock-immunized sows (Fig. 2A). This result demonstrated that humoral immune responses were specifically induced by PoSaV VLP