ORIGINAL ARTICLE

Chimeric influenza-virus-like particles containing the porcine reproductive and respiratory syndrome virus GP5 protein and the influenza virus HA and M1 proteins

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Abstract Both porcine reproductive and respiratory syndrome and swine influenza are acute, highly contagious swine diseases. These diseases pose severe threats for the swine industry and cause heavy economic losses worldwide. In this study, we have developed a chimeric virus-like particle (VLP) vaccine candidate for porcine reproductive and respiratory syndrome virus (PRRSV) and H3N2 influenza virus and investigated its immunogenicity in mice. The HA and M1 proteins from the H3N2 influenza virus and the PRRSV GP5 protein fused to the cytoplasmic and transmembrane domains of the NA protein were both incorporated into the chimeric VLPs. Analysis of the immune responses showed that the chimeric VLPs elicited serum antibodies specific for both PRRSV GP5 and the H3N2 HA protein, and they stimulated cellular immune responses compared to the responses to equivalent amounts of inactivated viruses. Taken together, the results suggested that the chimeric VLP vaccine represents a potential strategy for the development of a safe and effective vaccine to control PRRSV and H3N2 influenza virus.

Introduction

Influenza A virus is a segmented, negative-stranded RNA virus belonging to the family *Orthomyxoviridae*. Among the large variety of species that influenza A viruses infect naturally, swine influenza virus (SIV) causes an acute, highly contagious respiratory disease in swine. Epithelial cells in the swine respiratory tract have receptors for both avian and mammalian influenza viruses [13]. Therefore, pigs might serve as "mixing vessels" for the generation of new reassortant strains with pandemic capacity. Three predominant subtypes are prevalent in different countries: H1N1, H3N2, and H1N2.

Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of porcine reproductive and respiratory syndrome (PRRS), is a member of the family *Arteriviridae* in the order *Nidovirales* [5]. This virus causes one of the most economically important infectious diseases for the swine industry worldwide [24]. PRRS is predominantly characterized by reproductive failure in breeding swine, pre-weaning mortality, and respiratory disorders in pigs of all ages [1, 27, 36].

Vaccination has been an effective way to reduce the incidence of diseases resulting from influenza virus and PRRSV infections. Compared to conventional vaccines such as killed vaccine and attenuated vaccine, virus-like particles (VLPs) have been demonstrated to be a promising alternative candidate [33, 38, 39],. As a new form of vaccine candidate, the non-infectious nature of VLPs and their lack of viral genomic material are attractive safety features

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that may be suitable for a variety of viruses [9, 11, 16, 19, 33, 41]. Both B cell-mediated antibody and specific T-cell-mediated cellular responses were elicited by VLPs. VLPs not only mimic the overall structure of the virion, but they also present conformational epitopes of surface proteins, which can be readily recognized and processed by antigen-presenting cells [2, 12, 14, 15, 25]. The protective effects of VLPs have been demonstrated in clinical and preclinical trials [3, 14, 15, 28, 32]. An important advance would be the development of new VLPs with an enhanced breadth of immunity, which could potentially be used to prevent infection by PRRSV and SIV.

In a previous study, we expressed the GP5 proteins of PRRSV on the surface of chimeric VLPs, which elicited a humoral and cellular immune response and a neutralization antibody response to PRRSV [38, 39]. Based on this platform, we hope to generate chimeric VLPs for protection against both PRRS and influenza. In this study, we chose the H3N2 influenza virus as the basis for VLP production. As expected, the fusion protein NA/GP5 in combination with HA and M1 effectively formed chimeric VLPs. Next, we demonstrated that the chimeric VLPs induced a potent immune response to both PRRSV and H3N2 SIV in a BALB/c mice model. Hence, the results suggested that the chimeric VLP vaccine is a new vaccine candidate for protection against both PRRS and H3N2 influenza.

Materials and methods

Cells, viruses, and plasmids

Spodoptera frugiperda Sf9 cells were maintained in serum-free SF900II medium (Gibco) at 28 °C in spinner flasks at a speed of 100 rpm. The PRRSV strain GDKP/3/08 [18] was propagated on MARC-145 cells that were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with penicillin-streptomycin and 10 % fetal calf serum at 37 °C and 5 % CO_2. The H3N2 strain of the influenza virus (A/swine/Guangdong/01/1998(H3N2)) was propagated in MDCK cells under the same conditions. 293 T cells were maintained in DMEM supplemented with penicillin-streptomycin and 10 % fetal calf serum at 37 °C and 5 % CO_2.

The HA, NA and M1 genes of the H3N2 influenza virus (accession numbers FJ830855.1, FJ830857.1 and FJ830858.1), the GP5 gene of PRRSV (accession number GQ374441), and the NA/GP5 fusion gene were inserted into the pFast-Bac-Dual vector as described previously [38, 39]. The NA/GP5 and M1 genes were cloned into the same vector (pFast-Bac-Dual) under the control of different promoters. All of the plasmids were confirmed by DNA

sequencing to ensure that no additional changes were introduced during the PCR.

Generation of recombinant baculoviruses

The recombinant baculoviruses (rBVs) were derived from the transfer plasmids pFast-Bac-Dual-HA, pFast-Bac-Dual-GP5 and pFast-Bac-Dual-NA/GP5-M1 using the Bac-to-Bac baculovirus expression system. The viruses harvested from the supernatant were subjected to three rounds of plaque purification.

Protein expression

Sf9 cells were co-infected with rBVs expressing NA/GP5-M1 and HA at different ratios (0.5, 1, 2, 3, 5, and 6) and then incubated for 72 h at 28 °C. The Sf9 cells showed a high degree of cytopathology. The culture supernatants were collected and centrifuged at 2000×g for 30 min at 4 °C and analyzed by western blot. The expressed influenza virus proteins HA and M1 were detected with mouse polyclonal sera against the H3N2 influenza virus. The fusion protein NA/GP5 was detected with mouse polyclonal sera against PRRSV. Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG polyclonal antibodies were used as the secondary antibody (PTGLAB, USA).

Preparation of chimeric VLPs

To produce VLPs containing HA, M1 and NA/GP5, Sf9 cells were co-infected with rBVs expressing the HA and NA/GP5-M1 proteins at ratios of 5 and 2. After incubation for 72 h at 28 °C, the VLPs were harvested and purified using 20 %-30 %- 60 % (w/v) discontinuous sucrose step density gradient ultracentrifugation at 100,000×g for 60 min at 4 °C. The fractions were collected and analyzed for the presence of the HA, M1, and NA/GP5 proteins by western blot. The NA/GP5 content of chimeric VLPs was quantified by grayscale scanning of an SDS-PAGE gel. The hemagglutination activity of the VLPs was determined using chicken red blood cells. For electron microscopy, the sucrose-gradient-purified VLPs were applied to a carbon-coated Formvar grid for 2 min. Then, the grid was immediately stained with 1 % phosphotungstic acid (pH 6.5) for 60 s. The stained VLPs were observed by transmission electron microscopy (JEM-100CX-II, JEOLLTD, Japan).

Animal immunization

Four groups of six-week-old, female, inbred SPF BALB/c mice (n = 8) were housed in microisolator units and allowed free access to food and water. The mice were immunized intramuscularly (i.m.) with 10 µg of the

chimeric VLPs containing 2 µg NA/GP5 proteins with complete Freund's adjuvant (CFA) for the primary immunization (weeks 0) or incomplete Freund's adjuvant (IFA) for subsequent boosts (weeks 2 and 4). The positive control groups were immunized i.m. with 1 ‰ formalin-inactivated PRRSV in which the amount of GP5 was equal to that of the chimeric VLPs or 1 ‰ formalin-inactivated H3N2 influenza virus in which the amount of HA was equal to that of the chimeric VLPs. PBS was injected as the negative control. Blood samples were collected before immunization on the 14th, 28th, and 42nd day after primary immunization.

The experiments were carried out in accordance with the ethical guidelines for animal protection in China and approved by the Sun Yat-sen University Animal Ethics Committee. All procedures were performed under anesthesia, and all efforts were made to minimize suffering.

Determination of antibody titers by ELISA

Briefly, 96-well plates were coated with 100 µl of antigens at a concentration of 3 µg/ml in coating buffer (0.1 M sodium carbonate, pH 9.6) at 4 °C overnight. The antigens used as targets were extracts prepared from 293 T cells transfected with pFast-Bac-Dual-HA (to express HA protein) or pFast-Bac-Dual-GP5 (GP5 protein) [38, 39]. The plates were then blocked with PBS containing 0.05 % Tween-20 and 1 % BSA at 37 °C for 1 h and incubated with serial dilutions of each sample at 37 °C for 1 h. Following thorough washing in PBS containing 0.05 % Tween-20, all of the samples were incubated with horseradish peroxidase (HRP)-labeled goat anti-mouse IgG with a stock concentration of 1 mg/ml, 1:10,000 diluted in PBS containing 0.05 % Tween-20 and 0.1 % BSA at 37 °C for 1 h. The unbound antibodies were removed, and the wells were thoroughly washed. The substrate 3,3,5,5-tetramethylbenzidine (TMB, Sangon, China) in citrate-phosphate buffer (pH 5.0) containing 0.01 % H_2O_2 was used for color development. The reaction was terminated with 0.2 M H2S04, and the absorbance was determined at 450 nm using a spectrophotometer (Bio-Tek ELx800UV, USA).

Cytokine assays

Lymphocytes were isolated from the spleens for cytokine ELISPOT assays. Briefly, spleens were carefully rinsed with sterile PBS and depleted of erythrocytes by treatment with ammonium chloride (0.1 M, pH 7.4). Following thorough washing with PBS, cells were isolated from spleens using Mouse 1 × Lymphocyte Separation Medium (DAKEWE, China). The collected cells were centrifuged at 800×g for 30 min at room temperature and then resuspended in Lympho-Spot Serum-Free Medium Rodent

(DAKEWE). Cell viability was determined by staining with 0.4 % trypan blue (Sigma). Pre-coated anti-mIFN-γ or anti-mIL-4 (3 µg/ml in coating buffer, BD/PharMingen) 96-well plates (Millipore) were incubated with 200 ml Lympho-Spot Serum-Free Medium Rodent at 25 °C for 10 min and then were incubated with splenocytes isolated from vaccinated mice at 5×10^6/well. Splenocytes were stimulated with GP5 protein or HA protein at a concentration of 10 µg/ml. Additional wells of cells were stimulated with PMA (10 ng/ml), and ionomycin (500 ng/ml) or were mock stimulated. The plates were incubated for 20 h at 37 °C with 5 % CO_2. Plates were thoroughly washed with PBS containing 0.05 % Tween-20 and incubated with biotinylated anti-mIFN-γ or anti-mIL-4 at 37 °C for 1.5 h. Then, the plates were washed and incubated with streptavidin conjugated to alkaline phosphatase at 37 °C for 1.5 h. Following extensive washing, antibody-cytokine-antibody complexes were incubated with stable BCIP/NBT chromagen at 25 °C for 45 min. The plates were rinsed with ddH2O and air dried at 25 °C for 2 h. The spots were counted using an ImmunoSpot ELISPOT reader (Bio-reader4000, BIO-Sys, Germany).

Virus neutralization assay

The sera were complement-inactivated at 56 °C for 30 min before testing, serially diluted twofold in DMEM without serum, and then mixed with PRRSV ($100TCID_{50}$). The mixtures were added to prewashed Marc-145 cells growing in 96-well tissue culture dishes and incubated at 37 °C with 5 % CO_2 for 96 h. The cells were examined to observe the appearance of the cytopathic effects (CPE). The neutralization titers were expressed as the reciprocal of the highest serum dilution that neutralized 100 $TCID_{50}$ of PRRSV in 50 % of the wells.

Hemagglutination inhibition assay

The hemagglutination inhibition (HI) assay was used to assess the ability of functional HA-specific antibodies to inhibit agglutination of chicken erythrocytes. Briefly, sera were treated with receptor-destroying enzyme and serially diluted twofold in v-bottom 96-well microtiter plates. An equal volume (50 µl) of four adjusted hemagglutination units of the inactivated H3N2 strain of influenza virus was added to each well. The plates were covered and incubated at room temperature for 20 min followed by the addition of 50 µl of 1 % red blood cells (RBCs) in PBS. The HI titer was determined as the reciprocal dilution of the last row that contained non-agglutinated RBCs. Negative serum controls were included for each plate. The geometric mean HI titers and standard error were calculated within each group.
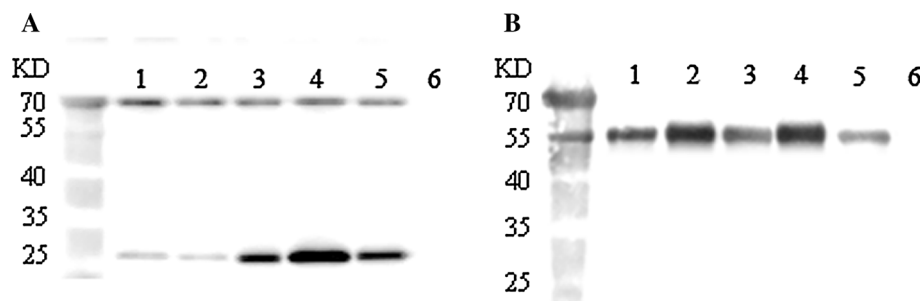
Fig. 1 Western blot analysis of protein expression in the culture supernatant from infected Sf9 cells. (A) Expression of influenza virus HA and M1 proteins in the culture supernatant of Sf9 cells infected with rBVs at different ratios. rBV-HA: rBV-NA/GP5-M1 = 0.5:2 (lane 1), 1:2 (lane 2), 3:2 (lane 3), 5:2 (lane 4), 6:2 (lane 5), and negative control (lane 6, culture supernatant from Sf9 cells infected with wild-type BVs). (B) Expression of the NA/GP5 fusion protein in the culture supernatant of Sf9 cells infected with rBVs at different ratios. rBV-HA: rBV-NA/GP5-M1 = 0.5:2 (lane 1), 1:2 (lane 2), 3:2 (lane 3), 5:2 (lane 4), 6:2 (lane 5), and negative control (lane 6, culture supernatant from Sf9 cells infected with wild-type BVs)

Statistical analysis

All parameters were recorded for individual mice within all groups. Statistical comparisons of the data between groups were carried out using an analysis of variance test (ANOVA). Statistical analyses were performed using Student's two-tailed test with equal variance. P-values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

Protein expression

Cells were coinfected with recombinant baculoviruses expressing NA/GP5-M1 and HA proteins at different ratios. The culture supernatant was harvested and analyzed by western blot (Fig. 1A and B). Different ratios led to different levels of protein expression. The results suggested that infection with rBVs expressing HA and NA/GP5-M1 proteins at a ratio of 5:2 was optimal for chimeric VLP production.

Production and analysis of VLPs

Chimeric VLPs were produced and released into the culture supernatant of insect cells coinfected with rBVs expressing HA, M1, and NA/GP5 as described. The VLPs were purified by sucrose gradient ultracentrifugation and characterized by western blot. The fractions containing VLPs could be observed at a sucrose density between 30-60 %, and the incorporation of HA, M1, and NA/GP5 protein into the VLPs was confirmed (Fig. 2A and B). Electron microscopic examination of negatively stained samples revealed the presence of VLPs with a diameter of approximately 100 nm (Fig. 2C). The HA titer was 64.

Antibody responses to immunization in mice

The immunogenicity of the chimeric VLPs was evaluated in a mouse immunization trial in the presence of Freund's adjuvants. HA protein was used as an antigen for H3N2 influenza virus antibody detection. Mice vaccinated with the VLPs and inactivated virus vaccine formulations elicited serum anti-HA protein antibody responses following the first vaccination that were boosted by the second vaccination (Fig. 3B). The group in which VLPs were infected intramuscularly and the positive control group showed considerable IgG titers that were significantly higher than those of the negative control group ($p < 0.01$, Fig. 3B). The differences in IgG titers between the VLP group and the positive control was not statistically significant ($p > 0.05$).

Importantly, the chimeric VLPs induced significant IgG titers against the PRRSV GP5 protein compared with the negative control group ($p < 0.01$, Fig. 3A), and the difference in IgG titers between the VLP group and the positive control group was not statistically significant ($p > 0.05$) which was the same as the immune response to the HA protein. These data show that the fusion of the ectodomain of NA did not hinder the function of GP5 as an antigen. Meanwhile, the production of an immune response to the HA protein indicated that these chimeric VLPs administered via the intramuscular route could effectively induce a response to two different antigens that was comparable to that induced by the positive control vaccine.

Cell-mediated immunity promoted by chimeric VLPs in mice

The numbers of IFN-γ and IL-4 cytokine-secreting cells were determined using specific and sensitive ELISPOT assays. Spleens were harvested from vaccinated mice, and lymphocytes were isolated after the last immunization. The
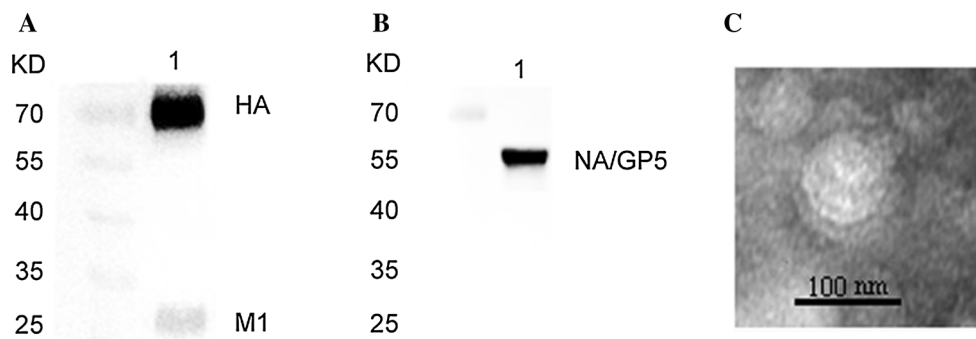
Fig. 2 Production and analysis of chimeric VLPs. (A) Western blot analysis of the influenza virus HA and M1 proteins. VLPs in the supernatant were purified by sucrose gradient centrifugation. Then, the bands from the 30-60 % sucrose gradient were detected by western blot. The expected positions of HA and M1 are indicated on the right. The molecular weight of each protein was determined using a wide-range molecular weight marker (170 to 10 kDa, Fermentas, MBI, USA). (B) Western blot analysis of the NA/GP5 fusion protein. The bands from the 30-60 % sucrose gradient were detected. The expected position of the NA/GP5 fusion is indicated on the right. (C) Negative staining electron miscropy of chimeric VLPs composed of HA, M1, and NA/GP5 fusion proteins. Bars represent 100 nm
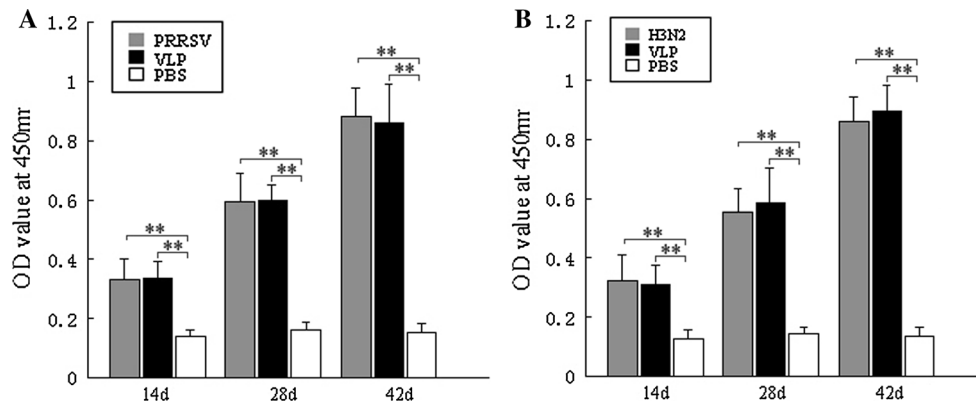

Fig. 3 Serum IgG in mice. Six-week-old BALB/c mice were vaccinated via intramuscular injection at weeks 0, 2, and 4 with chimeric VLPs, PRRSV, H3N2, or PBS. The serum for each group was tested for the presence of IgG antibody by indirect ELISA using the GP5 or HA protein as an antigen. The optical densities were read at 450 nm. The bars represent the arithmetic means of the antibody titers ± standard errors. A. PRRSV-specific IgG antibodies in mice. B. H3N2-specific IgG antibodies in mice. The difference in IgG antibody titers between the experimental groups were statistically significant (**p < 0.01)

cells were stimulated with either GP5 protein or HA protein. Our data showed that significant numbers of IFN-γ- and IL-4-secreting cells were induced in the chimeric VLP group or the positive control group, but not in the negative control mice (P < 0.05), whether GP5 protein (Fig. 4A and B) or HA protein (Fig. 4C and D) was used as the stimulus. It is notable that the mice immunized with the chimeric VLPs elicited both Th1- and Th2-type cellular immune responses. This might be helpful to suppress viral replication and reduce viral infection for both the PRRSV and H3N2 influenza virus.

PRRSV-specific neutralizing antibodies elicited by VLPs

Because neutralizing activities play an important role in the first line of defense against PRRSV infection and the clearance of PRRSV, most likely conferring protective immunity, we performed neutralization assays. As shown in Fig. 5, PRRSV-specific neutralizing antibodies were detected on the 14th day after primary immunization, they were elevated on the 28th day, and they increased to the highest level in the 42nd day. Furthermore, no neutralizing antibodies against PRRSV were detectable in the PBS group.

Hemagglutination-inhibition activity in sera

The HI results demonstrated that a single intramuscular immunization with the VLP vaccine in the presence of adjuvant elicited a relatively modest HI titer against the inactivated H3N2 influenza virus strain, and the two booster immunizations induced a high HI titer, indicating that the VLPs were strongly immunogenic (Fig. 6).
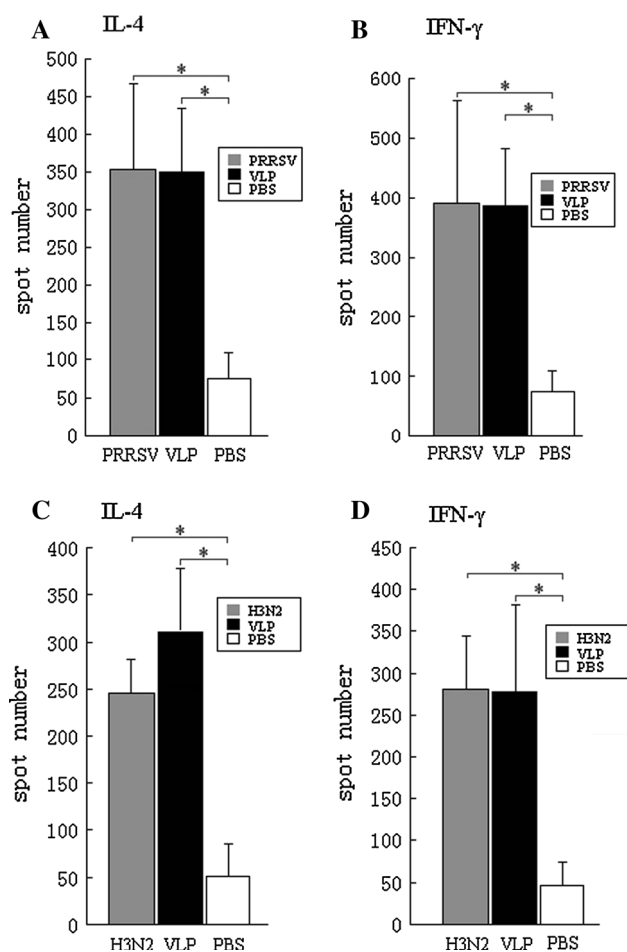
Fig. 4 ELISPOT assays of IFN-γ and IL-4 cytokine-secreting splenocytes in mice. Splenocytes were isolated from immunized mice 3 weeks after the final immunization, and cytokine-secreting cells were examined by ELISPOT assays. The spots for cytokine-producing cells from the spleen were counted and expressed based on 5×10^6 cells per well. A. The IL-4 level stimulated by the GP5 protein. B. The IFN-γ level stimulated by the GP5 protein. C. The IL-4 level stimulated by the HA protein. D. The IFN-γ level stimulated by the HA protein. The differences in number of cytokine-secreting cells between the experimental groups were statistically significant (*p < 0.05)
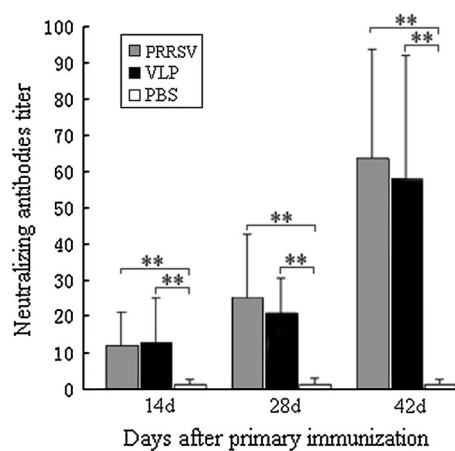

Fig. 5 PRRSV-specific neutralization antibodies in mice. Mice were immunized as in Fig. 3. Serum samples were collected on the 14th, 28th, and 42nd day after primary immunization to determine the neutralization antibody titers. The data represent the mean S.D. for eight mice. The differences in the neutralization antibody titers between the experimental groups were statistically significant (**p < 0.01)
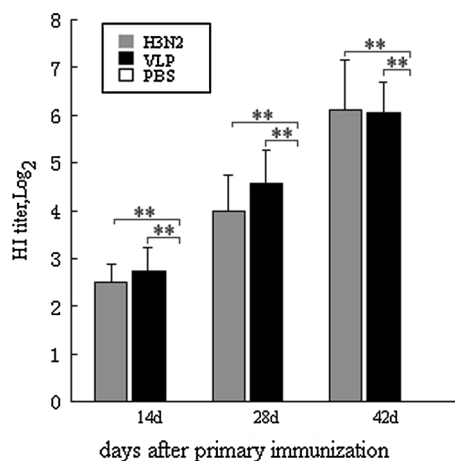

Fig. 6 Hemagglutination-inhibition (HI) titers. Serum samples were collected on the 14th, 28th, and 42nd day after primary immunization of mice (8 per group) vaccinated with chimeric VLPs, inactivated H3N2 virus, or PBS. The HI responses were assessed compared with the H3N2 viruses. The differences in HI titers between the experimental groups were statistically significant (**p < 0.01)

Discussion

PRRSV and SIV coinfect pigs under certain conditions. These two agents associated with *Mycoplasm hyopneumoniae* (MHYO), *Pasturella multocida,* and porcine circovirus type 2 (PCV2) have been reported to constitute the porcine respiratory disease complex (PRDC) [37]. A previous study indicated that SIV and PRRSV are the primary etiological agents associated with respiratory disease in pigs [7]. The presence of PRRSV between vaccinations reduced the efficacy of the vaccine, but it did not negatively impact either the systemic or local antibody response to either SIV vaccination or challenge [29]. Previously, we prepared chimeric VLPs composed of the M1 protein from H1N1 influenza virus and a fusion protein, denoted as NA/GP5, containing the cytoplasmic and transmembrane domains of the H1N1 virus NA protein and the PRRSV GP5 protein. We demonstrated that immunization of BALB/c mice with these chimeric VLPs could stimulate a robust immune response [38, 39]. Based on this result, an important advance would be the development of new vaccines, which could be used to prevent coinfection by PRRSV and SIV.

The influenza virus virion is surrounded by a lipid membrane containing two major glycoproteins, HA and NA. The HA protein is the most abundant glycoprotein and is responsible for the attachment of the virus to terminal sialic acid residues on host cell receptors and mediating fusion between viral and cellular membranes [10]. Influenza VLPs could be obtained by the expression of four viral proteins (HA, NA, M1, M2) [17], three viral proteins (HA, M1, NA) [4, 8, 28, 32, 35], two viral proteins (HA, M1) [30, 33, 34], or even HA alone [6]. Recently, it has been demonstrated that influenza VLPs can be made from M2 fusion proteins [40]. Therefore, influenza VLPs were chosen as a platform on which PRRSV proteins (GP5) can be expressed. A previous report showed that, based on the assembly of Newcastle disease (ND) VLPs [22], it was possible to develop chimeric VLPs containing respiratory syncytial virus (RSV) G proteins using ND VLPs as a platform [23], as well as ND VLPs containing RSV G and F proteins [21]. In our study, we showed that the HA protein could assemble with NA/GP5 and M1 to form chimeric VLPs. Compared to our previous results, HA had no influence on the formation of chimeric VLPs, but the size and morphology of the chimeric VLPs were more similar to those of the natural influenza virus. The HA titer of the chimeric VLPs indicated that HA was structurally and functionally incorporated into the particles. The results suggested that it might be feasible to generate chimeric influenza VLPs in this way.

The immunogenicity of chimeric VLPs in mice demonstrated that they were effective for inducing immunity. Our data showed that both the inactivated virus and the chimeric VLP vaccine generated comparable amounts of PRRSV-specific or H3N2-influenza-virus-specific antibodies, as measured by ELISA. Splenocytes proliferated vigorously and produced both IFN-γ and IL-4 in response to stimulation with GP5 or HA antigen. Following three immunizations, the VLP vaccine induced HI antibodies against the homologous influenza strain in mice. Our findings provide evidence that chimeric VLPs can induce protective immunity against H3N2 influenza virus similarly to the killed virus. Meanwhile, when we measured the neutralizing antibody titers, significantly elevated neutralizing antibodies to PRRSV were achieved in the VLP group as well as with inactivated PRRSV ($P < 0.05$). This result demonstrated that the PRRSV GP5 protein co-incorporated with influenza H3N2 NA and formed chimeric VLPs with HA and M1, which could be identified and transferred efficiently as a PRRSV antigen. It was reported recently that VLPs of the hepatitis B virus core protein containing five mimotopes of infectious bursal disease virus (IBDV) protected chickens against IBDV [38, 39]. The use of both genetically engineered norovirus VLPs incorporating relevant epitopes from multiple strains and multivalent vaccine formulations increases the breadth of the immune response to diverse variants within a genotype [26]. In summary, we found that the chimeric VLPs induced antibodies against two diseases at the same time, in contrast to the chimeric VLPs in our previous study.

The data presented in this study showed that intramuscular immunization of mice with these chimeric VLPs induced systemic immune responses, including both humoral and cellular immune components. Similar to our research, chimeric SARS-CoV S glycoprotein and influenza M1 efficiently form VLPs that protect mice against challenge with SARS-CoV [20]. Experimental triple-HA VLPs containing HA proteins derived from the H5N1, H7N2, and H2N3 viruses were immunogenic and protected ferrets from challenge with all three potentially pandemic viruses. Additionally, VLPs containing HA subtypes derived from the seasonal H1N1, H3N2, and type B influenza viruses protected ferrets from three seasonal influenza viruses [31]. Therefore, it is clear that the chimeric VLPs were as effective as the killed viruses. The results showed that the immune responses to these chimeric VLPs were similar to those to the VLPs composed of NA/GP5 and M1 proteins. The less an animal needs to be handled to deliver vaccines, the better. The presence of multiple virus targets in a single vaccine will be a new advantage of the chimeric VLP vaccine. Such vaccines could also reduce the burden of vaccine production and administration in comparison with regular vaccination. After this basic step, studies of the protective efficacy of chimeric VLPs in a swine model need to be carried out.

In summary, in this study, we provide a proof of concept for a chimeric VLP vaccine generating an anti-PRRSV and anti-H3N2-influenza-virus immune response. These chimeric VLPs could serve as a promising vaccination strategy to control PRRS and H3N2 influenza in swine. Although the response to immunization with the chimeric VLPs was not more effective than the response to inactivated viruses, the fact that chimeric VLPs are not infectious and lack viral genomic material means that they could provide a safer method to prevent viral infection. Furthermore, the production of the chimeric VLPs is faster and presents a lower risk relative to the traditional method. Thus, this approach has greater potential for vaccine development.

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