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RESEARCH PAPER

Study of Fusion Protein and Attachment Glycoprotein of Nipah Virus Expressed in Recombinant Baculovirus

WANG Xi-Jun[1], HU Sen[1], GE Jin-Ying[2], WANG Qing-Hua[3], QIN Li-Ting[4], BU Zhi-Gao[1,*]

1 National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, China
2 College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China
3 National Diagnostic Center for Exotic Animal Disease, National Animal Quarantine Institute of Ministry of Agriculture, Qingdao 266032, China
4 College of Animal Technique and Science, Shandong Agricultural University, Tai'an 271018, China

Abstract: The envelope attachment glycoprotein (G) and fusion protein (F′) of Nipah virus (NiV) play a key role in viral entry and induction of neutralization antibody. In this study, recombinant baculoviruses, rBac-NF and rBac-NG, were generated to express F and G proteins of NiV. The expressions of recombinant G (rNG) and F (rNF) proteins in rBac-NF and rBac-NG-infected cells were confirmed by Western blot. Both rNG and rNF showed sensitive and specific antigenic reaction to rabbit serum anti-Nipah virus in indirect immunofluorescence detection and indirect ELISA. Immunization with rBac-NF and rBac-NG-infected insect cells elicited G and F protein-specific antibody responses in mice. Furthermore, the G and F protein-specific antibodies could neutralize the infectivity of the VSVΔG*F/G, the NiV F and G envelope glycoproteins of pseudotype recombinant Vesicular Stomatitis Virus expressing green fluorescence protein. The results demonstrated that the F and G proteins expressed by the recombinant baculoviruses could be safe diagnostic antigens for the surveillance and monitoring of NiV and could also be promising subunit vaccines for the prevention of NiV.

Key Words: Nipah virus; fusion protein; attachment glycoprotein; recombinant baculovirus

In late September 1998, a group of patients associated with pig farming in the suburbs of Ipoh city within the Kinta district of Perak state in Peninsular Malaysia were infected with acute febrile encephalitis that was associated with high mortality[1]. Initially, the illness in pigs was attributed to Classical Swine Fever. The mortality in humans was attributed to Japanese encephalitis (JE), a mosquito-borne RNA virus. However, the vaccination for Swine Fever and JE and efforts to control mosquitoes failed to arrest the epidemic. By December 1998, the outbreak had spread to Sungai Nipah village and Bukit Pelandok (the biggest pig-farming region) in the state of Negeri Sembilan. A month later, a group of 11 human cases of febrile encephalitis illness along with one case of death was reported among abattoir workers in Singapore who had handled pigs from the outbreak regions in Malaysia[2]. In March 1999, a novel paramyxovirus, Nipah virus (NiV), was isolated from the cerebro-spinal fluid of a patient from Sungai Nipah village, who was subsequently identified with encephalitis; this virus was the aetiological agent that was responsible for the outbreak. By December 1999, a total of 283 human cases of acute febrile encephalitis including 109 cases of death associated with the outbreak were reported, with a mortality rate of 38.5 %[3].

The NiV and the related Australian Hendra virus (HeV) form the Henipavirus genus within the paramyxovirus family. Among the paramyxoviruses, these viruses had remarkable

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* Corresponding author. E-mail: zgb@bhvri.ac.cn

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abilities to infect and cause potentially fatal diseases in a number of host species, including humans. The natural reservoir of HeV was fruit bats of the *Pteropid* genus[4,5]. Therefore, fruit bats were suspected to be the natural reservoir of NiV[6]. In 2002, NiV was isolated from the urine samples and swabs from partially eaten fruits by flying foxes of *Pteropus* genus. The world distribution of flying foxes extends from the western Indian Ocean islands through Southeast Asia, including southwest Pacific Islands, and Australia excluding Tasmania[7]. In 2001 and 2002, serological evidences indicated that the neutralizing antibody to NiV was found in fruit bats from Bangladesh, the northern India, and Cambodia. In 2004, there was an outbreak of NiV in Bangladesh, in which 30 people were infected and 18 were killed.

The envelope attachment glycoprotein (G) and fusion protein (F) of NiV are major structural proteins that mediate membrane fusion between the virion and the host cell and elicite neutralization antibody. In this study, the recombinant baculoviruses, rBac-NF and rBac-NG, were generated to express F and G proteins of NiV. Recombinant G (rNG) and F (rNF) proteins expressed by rBac-NF and rBac-NG showed sensitive and specific antigenic reaction to rabbit serum anti-NiV. Immunization with rBac-NF and rBac-NG-infected insect cells elicited G and F protein-specific antibody responses in mice. Furthermore, the G and F protein-specific antibodies could neutralize the infectivity of the VSVΔG*F/G, the NiV F and G-envelope glycoproteins of pseudotype recombinant Vesicular Stomatitis Virus expressing green fluorescence protein. The results demonstrated that the F and G proteins expressed by the recombinant baculoviruses could be safe diagnostic antigens for the surveillance and monitoring of NiV and could also be promising subunit vaccines for the prevention of NiV.

1 Materials and methods

1.1 Plasmids and antisera

Plasmids pUC 18-NiV-F (full-length ORF of NiV F gene was inserted into *Sam* I site of vector pUC18) and pMD18-T-NiV-G (full-length ORF of NiV G gene was inserted into *Eco*R V site vector pMD18-T) were stored in our laboratory. Polyclone serum from rabbits immunized with inactivated NiV was kindly provided by Dr. L. Wang, CSIRO, Australia.

1.2 Virus and cells

sf9 insect cells and vero E6 cells were stored in our laboratory. The recombinant Vesicular Stomatitis Virus pseudotype, VSVΔG*G, in which the VSV envelope protein G gene was replaced with the green fluorescent protein gene and complemented with VSV G glycoprotein expressed in trans, was kindly provided by Dr. Whitt MA. VSVΔG*F/G pseudotype was prepared by cotransfecting 293T cells with pCAGG-G and pCAAGG-F following infection with VSVΔG*G, as described previously. The infection unit (IU) of VSVΔG* F/G in filtered supernatant of 293T cell culture was determined on BHK-21 cells by account of cells expressing GFP under a fluorescence microscope, typically about 10^7/mL without concentration[8,9]. The VSVΔG*F/G stocks were stored at − 80 °C until use.

1.3 Construction of recombinant baculoviruses

pFastBac1-NiV-F was generated by cloning *Eco*R I-*Pst* I fragment from pUC 18-NiV-F into the *Eco*R I-*Pst* I site of pFastBac1. To generate pFastBac1-NiV-G, a fragment encoding NiV G protein was excised from pMD18-T-NiV-G by digestion with *Sal* I and *Xba* I and then cloned into the *Sal* I-*Xba* I site of pFastBac1. Briefly, pFastBac1-NF and pFastBac1-NG were transformed into DH10BAC competent cells containing Bacmid (baculovirus shuttle vector plasmid) and helper plasmid and were plated on LB solid medium plates containing 50 μg/mL kanamycin (TaKaRa Dalian, China), 7 μg/mL gentamicin (TaKaRa Dalian, China), 10 μg/mL tetracycline (TaKaRa Dalian, China), 100 μg/mL X-gal (TaKaRa Dalian, China), and 40 μg/mL IPTG (TaKaRa Dalian, China). After 24–48 h of incubation at 37 °C, white colonies were selected and grown overnight in LB medium with the antibiotics. Plasmid recombinant Bacmids were extracted from the overnight cultures as described in the manual, and were identified by PCR with primers M13-48f andM13-47r. sf9 insect cells were transfected with 1 μg of the recombinant Bacmids DNA using the Cellfectin® Reagent (Invitrogen). Recombinant viruses were identified by PCR with primers M13-48f and M13-47r. A single recombinant virus plaque was isolated. The virus was further amplified using Sf9 cells. The IU of the recombinant baculoviruses, rBac-NiV-F and rBac-NiV-G, were measured by account of plaque. The recombinant baculoviruses were stored at −80 °C until use.

1.4 Indirect immunofluorescence assay

According to reference 10, the monolayer of sf9 insect cells were infected with 1:10 dilutions of rBac-NiV-F, rBac-NiV-G, and wild-type baculovirus. The infected cells were harvested until 90 % of the cells showed CPE, and were then spread on glass slides for air drying. Following this, the cells were fixed with 95 % ethanol and were allowed to interact with 20-fold dilution of serum from rabbits immunized with inactivated NiV and with nonimmunized rabbit serum, respectively. The glass slides were washed with PBST and then interacted with 1:50 dilution of the fluorescein isothiocyanate-conjugated anti-rabbit IgG antibodies. The glass slides were then washed again with PBST and observed under afluorescence microscope (Leica DMIRES2).

1.5 Production of antiserum

pET-NiV-F was generated by PCR-amplifying NiV F gene fragment (976–1 479 nt) with the primers NiV-F-f: 5′-GTG TTCGAATTCATCGAGATCGGGTTCTG-3′ and NiV-F-r: 5′-GATGATGTCGACGGAGAGCATGGAG-3′ and cloned into

the *Eco*R I-*Sal* I site of prokaryotic expression vector pET-30a(+). pET-NiV-G was generated by PCR amplification of NiV G gene fragment (319–1 002 bp) with the primers, NiV-G-f: 5′-ACCGACGAATTCCCCAAGG TGTCCCTGAT -3′ and NiV-G-r: 5′-GAGGACGTCGACCTGGTGCTGGT TGTA-3′ and cloned into the *Eco*R I-*Sal* I site of prokaryotic expression vector pET-30a(+). pET-NiV-F and pET-NiV-F were transformed into BL21 competent cells and were expressed by IPTG inducing, and the proteins that fused to His-tag at its both termini were purified using Ni-NTA (Pierce). Ten-to eight-week-old female BALB/c mice (Weitong Lihua Experimental Animal Inc., Beijing) were immunized by subcutaneous injection with 10 μg of the proteins, respectively, by boosting with the same dose of proteins at 21 days and 42 days after priming. The proteins were emulsified with Freund's complete adjuvant (CFA) in initial immunization and then emulsified with Freund's incomplete adjuvant (FIA) in second and third immunizations. Mouse blood was collected by retro-orbital bleeding at 14 days after final proteins immunization and stored at −20 °C until use.

1.6 Production of recombinant antigens

According to reference 11, sf9 cells were infected with 1:10 dilutions of rBac-NiV-F, rBac-NiV-G, and wild type baculovirus and harvested 72 h postinfection. The cells were then collected by 1 000 r/min for 10 min. The cell pellet was resuspended in 10 % volume PBS of primal culture fluid and frozen and thawed twice, and then shattered by supersonic waves. Cell debris was removed by centrifuging at 1 000 r/min for 10 min and the supernatant was stored at −80 °C as antigens.

1.7 Western blot

The lysates of rBac-NiV-F, rBac-NiV-G, and wild-type baculovirus-infected sf9 cells were separated on 12 % SDS-PAGE. For Western-blot analysis, the proteins were transferred from the gels to nitrocellulose filter. Following transfer, the membranes were blocked with 10 % nonfat dry milk and incubated overnight with a 1:100 dilution of polyclone serum from mice immunized with purified recombinant partial NiV F or NiV G, which were generated from *E. coli* bacteria, suspended in buffer (1× PBS, 0.05 % Tween 20, and 5 % nonfat dry milk). A 1:2 500 dilution of HRP-conjugated goat anti-mouse IgG (Sigma) was used to detect protein-antibody complexes, which were subsequently visualized with DAB.

1.8 Antigenicity of the recombinant proteins expressed by recombinant baculoviruses in indirect ELISA

ELISA plates were coated with the above-mentioned lysates of sf9 cells infected with rBac-NiV-F, rBac-NiV-G, and wild-type baculovirus at 1:100 dilution in carbonate buffer (pH 9.6) overnight at 4 °C and then blocked for 2 h at 37 °C with 5 % nonfat dry milk in PBST.Serum sample (serum from rabbits immunized with inactivated NiV and serum from nonimmunized rabbits) were tested in 1: 200 dilutions in 5 % nonfat milk in PBST. HRP-conjugated goat anti-rabbit IgG was diluted 1: 4 000 in PBST. The substrate solution used was OPD. After 25 min of incubation in the dark, the reaction was stopped with the addition of 50 μL of 2 mol/L H_2SO_4 and the optical density at 490 nm was measured in an ELISA microplate reader (Bio-Rad, Benchmark plus). Sera were run in triplicate. Negative and positive control sera were included in each assay.

1.9 Immunization of mice

sf9 cells were infected by rBac-NiV-F, rBac-NiV-G, and wild-type baculovirus. When 90 % of the cells showed CPE, cells and the supernatant were harvested as immunizing antigens. Ten- to eight-week-old female BALB/c mice (Weitong Lihua Experimental Animal Inc., Beijing, China) were immunized by an intraperitoneal injection with 250 μL of the immunizing antigen and intramuscular injection with 100 μL of the immunizing antigen, respectively, by boosting with the same dose of proteins at 21 days after priming. Mouse blood was collected by retroorbital bleeding at 21 days after final immunization and was stored at −20 °C until use.

1.10 Immunogenicity of the recombinant proteins expressed by recombinant baculovirus

ELISA plates were coated with recombinant partial NiV F or NiV G, which were generated from *E. coli* bacteria, at 5 μg/mL in carbonate buffer (pH 9.6) overnight at 4 °C, and then blocked for 2 h at 37 °C with 5 % nonfat dry milk in PBST. Serum samples (serum from mice immunized with recombinant NiV F and G expressed by the recombinant baculoviruses and serum from nonimmunized mice) were tested in 1:50 dilutions in 5 % nonfat milk in PBST. HRP-conjugated goat anti-rabbit IgG was diluted 1: 2 500 in PBST. The substrate solution used was OPD. After 25 min of incubation in the dark, the reaction was stopped by the addition of 50 μL of 2 mol/L H_2SO_4 and the optical density at 490 nm was measured in an ELISA microplate reader (Bio-Rad, Benchmark plus). Sera were run in triplicate. Negative and positive control sera were included in each assay.

1.11 Neutralization test

It has been reported that hamsters passively administered with neutralization antibody were protected from lethal challenge of NiV. Because of the unique biological and genetic features, NiV are categorized as biological safety level-4 (BSL-4) pathogens, which severely limits the laboratory facilities that are required for study with live viruses. In this study, we developed a highly safe and sensitive, single-round infectivity system was developed for the neutralization antibody assay without the need of live NiV. The VSVΔG*F/G mimicked the infection of real NiV and entering NiV susceptible target cells BHK-21 by F and G-mediated fusion, and was used to replace live NiV for the neutralization antibody assay.

To perform the neutralizing antibody assay, 1×10^5 IU VSVΔG*F/G was added at each step of a serial two-fold dilution of heat-inactivated immunized mouse sera (serum from mice immunized with recombinant baculovirus expressed recombinant NiV F protein (rNF), serum from mice immunized with recombinant NiV G protein (rNG), and serum from mice immunizied with wild type baculovirus, 30 min at 56 °C) in triplicate wells. After incubation for 1 h at 37 °C, the mixtures containing 5×10^4 IU VSVΔG*F/G were added to the rinsed Vero E6 monolayers. Sera (serum from rabbit immunized with inactivated NiV and serum from non-immunized rabbits) were included in each test as controls. The GFP-expressing cells were counted at 16 h postinfection (hpi) under a fluorescence microscope. The neutralizing titers were expressed as the reciprocal of the highest serum dilution that gave 80 % reduction in the number of GFP-expressing cells.

2 Results

2.1 Construction and identification of recombinant baculoviruses

NiV F gene and G gene, encoding full-length F and G proteins, were cloned into pFastBac1, pFastBac1-NiV-F and pFastBac1-NiV-G were constructed, which were transformed into DH10BAC competent cells. The recombinant Bacmids were extracted and PCR amplification of rBacmid-NiV-F and rBacmid-NiV-G with primers M13-48f and M13-47r produced 3.9 kb and 4.1 kb fragments, respectively. sf9 insect cells were transfected with the recombinant Bacmids to generate the recombinant viruses, rBac-NiV-F and rBac-NiV-G. PCR amplification of the recombinant virus with primers M13-48f andM13-47r produced also 3.9 kb and 4.1 kb fragments. The results demonstrated that NiV F gene and G gene had inserted into the recombinant baculoviruses rBacmid-NiV-F and rBacmid-NiV-G, respectively.

Lysates of rBac-NiV-F, rBac-NiV-G, and wild-type baculovirus-infected sf9 cells were separated by 12 % SDS-PAGE and Western blot. The NiV F0 (61 kD), F1 (49 kD), and G (66 kD) specific band were probed with polyclone serum from mice that were immunized with purified recombinant partial NiV F or NiV G which was generated from *E. coli* bacteria (Fig. 1 and Fig. 2).

2.2 rNF and rNG as antigen-detecting specific antibodies in serum from rabbits immunized with inactivated NiV in indirect ELISA

For detecting reactionogenicity of rNF and rNG expressed by the recombinant baculoviruses, ELISA plates were coated with the lysate of sf9 cells that were infected by rBac-NiV-F and rBac-NiV-G and detected with 1:200 dilution of polyclone serum from rabbits immunized with inactivated NiV (kindly provided by Dr. L. Wang, CSIRO, Australia). The results showed that the average OD_{490nm} for rNF and rNG were 0.181 and 0281, respectively, in indirect ELISA, in which rNF and rNG were detected using serum from rabbits immunized with inactivated NiV; the average OD_{490nm} for rNF and rNG were 0.063 and 0.055, respectively, in detecting serum from non-immunized rabbits in indirect ELISA; the P/N values were 2.9 and 5.1 (Fig. 3).

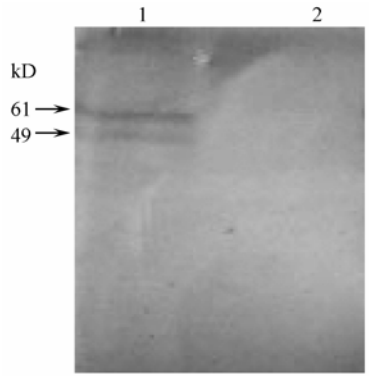

Fig. 1 Western-blot detecting expression of rNF in insect cells infected by rBac-NiV-F with polyclone serum from mice immunized with purified recombinant partial NiV F which was generated from *E. coli* bacteria

1: lyses of insect cells infected with rBac-NiV-F; 2: lyses of insect cells infected with wild-type baculovirus.

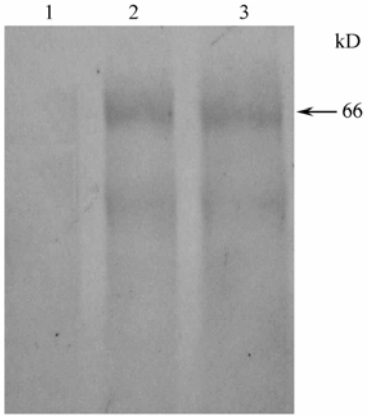

Fig. 2 Western-blot detecting expression of rNG in insect cells infected by rBac-NiV-G with polyclone serum from mice immunized with purified recombinant partial NiV G which was generated from *E. coli* bacteria

1: lyses of insect cells infected with wild-type baculovirus; 2,3: lyses of insect cells infected with rBac-NiV-G.

2.3 rNF and rNG as antigens detecting specific antibodies in serum from rabbits immunized with inactivated NiV in IFA

For detecting reactionogenicity of rNF and rNG expressed by the recombinant baculoviruses, sf9 cells infected by

rBac-NiV-F or rBac-NiV-G or wild-type baculoviruses were detected using serum from rabbits immunized with inactivated NiV in IFA. The sf9 cells infected by rBac-NiV-F and rBac-NiV-G showed strong positive fluorescence signal in detecting serum from rabbits immunized with inactivated NiV (Fig. 4A, 4B) and negative fluorescence signal in detecting serum from non-immunized rabbits (Fig. 4C, 4D). The sf9 cells infected by wild-type baculoviruses showed negative fluorescence signal in detecting using serum from rabbits immunized with inactivated NiV (Fig. 4E). The results showed that rNF and rNG expressed by the recombinant baculoviruses possess good sensitivity and specificity in IFA.

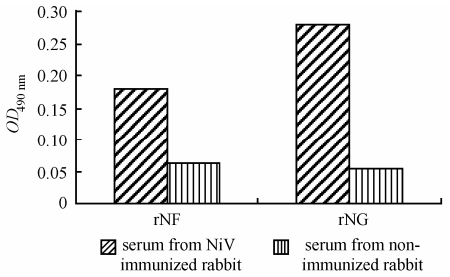

Fig. 3 Indirect ELISA detecting serum antibodies of NiV fusion and attachment glycoprotein in serum from rabbits immunized with inactivated NiV based on rNF and rNG as antigens

Fig. 4 IFA assay detecting serum antibodies of NiV fusion and attachment glycoprotein in serum from rabbit immunized with inactivated NiV based on rNF and rNG as antigens

2.4 The recombinant partial NiV F or NiV G generated from *E. coli* bacteria as antigen detecting specific antibodies in serum from rabbits immunized with rNF and rNG in indirect ELISA

For detecting immunogenicity of rNF and rNG expressed by the recombinant baculoviruses, ELISA plates were coated with purified recombinant partial NiV F or NiV G which were generated from *E. coli* bacteria and detected using 1:50 dilution of polyclone serum from mice immunized with rNF or rNG. The results showed that the average OD_{490nm} for recombinant partial NiV F and NiV G were 0.236 and 0.208, respectively, in indirect ELISA, in which purified recombinant partial NiV F or NiV G were detected using serum from mice immunized with rNF or rNG expressed by the recombinant baculoviruses; the average OD_{490nm} for recombinant partial NiV F or NiV G were 0.059 and 0.052 in detecting serum from nonimmunized mice in indirect ELISA; both P/N values were 4 and 4, respectively (Fig. 5 and Fig. 6).

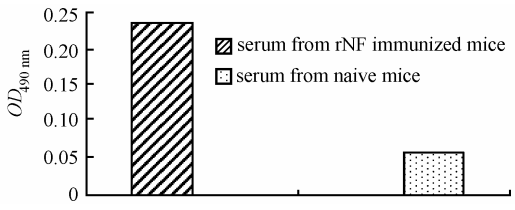

Fig. 5 Indirect ELISA detecting serum antibodies of NiV attachment glycoprotein in serum from mice immunized with recombinant F protein based on F protein fragment F′ as antigen

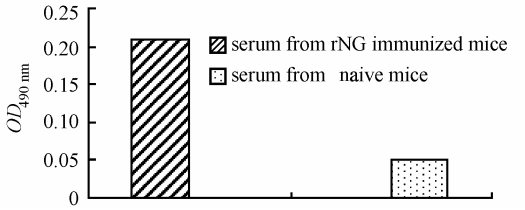

Fig. 6 Indirect ELISA detecting serum antibodies of NiV attachment glycoprotein in serum from mice immunized with recombinant G protein based on G protein fragment G′ as antigen

2.5 Neutralization test

The VSVΔG*F/G mimicked the infection of real NiV and entering NiV susceptible target cells BHK-21 by F and G-mediated fusion, and was used to replace live NiV for neutralization antibody assay. The result showed that immunization with insect cells infected with rBac-NF and rBac-NG elicited G and F protein-specific antibody responses in mice. Furthermore, the G and F protein-specific antibodies neutralized the infectivity of the VSVΔG*F/G. The neutralizing titers were expressed as the highest serum dilution that gave 80 % reduction in the number of GFP-expressing cells. The titers of serum from mice immunzed with rNG, serum from mice immunized with rNF, and serum from rabbits immunized with inactivated NiV were 128, 64, and 32. However, serum from nonimmunized rabbits and serum from mice immunizied with wild type baculovirus

could not neutralize the infectivity of the VSVΔG*F/G (Fig. 7).

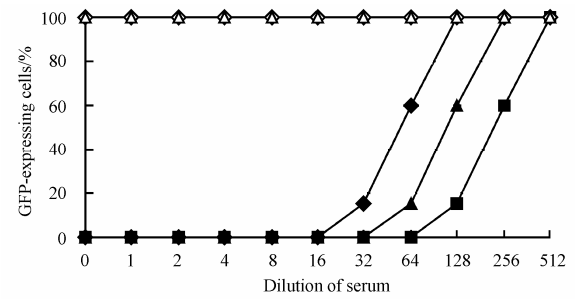

Fig. 7 Neutralization curves of serum from mice immunized with rNF and rNG

1×10^4 IU VSVΔG*F/G was added at each step of a serial two-fold dilution of heat-inactivated immunized mouse serum (serum from rNF immunized mice (▲) and serum from rNG immunized mice (■) and serum from wild type baculovirus immunized mice (△), 30 min at 56 °C) in triplicate wells. After incubation for 1 h at 37 °C, these mixtures containing 1×10^4 IU VSVΔG*F/G were added to the rinsed Vero E6 monolayers. Serum from inactivated NiV immunized rabbits (◆) and non-immunized rabbits (◇) were included in each test as controls. The GFP-expressing cells were counted at 16 h post-infection (hpi) under a fluorescence microscope. The neutralizing titers were expressed as the reciprocal of the highest serum dilution that gave 80 % reduction of the number of GFP-expressing cells.

3 Discussion

In this study, recombinant baculoviruses, rBac-NiV-F and rBac-NiV-G, were generated for expressing F and G proteins. The expressions of recombinant G (rNG) and F (rNF) protein in rBac-NF and rBac-NG-infected cells were confirmed by Western blot. Both rNG and rNF showed sensitive and specific antigenic reaction to rabbit serum anti-NiV in indirect immunofluorescence detection and indirect ELISA. Furthermore, the results of the neutralization tests and indirect ELISA demonstrated that cell suspension of sf9 insect cells infected by the recombinant baculoviruses, rBac-NiV-F and rBac-NiV-G, elicited G ad F protein-specific antibody responses in mice. Moreover, the G and F protein-specific antibodies neutralized the infectivity of the VSVΔG*F/G entering NiV susceptible target cells. Therefore, the recombinant NiV F and G proteins expressed by the recombinant baculovirus could be to play a significant role in the study of epidemiology of NiV encephalitis, the serological monitoring of wildlife, and the appraisal to specific immune response of protective immunogen in the development process of a new generation vaccine.

Paramyxoviruses have two glycoproteins at the surface, namely, attachment glycoprotein and fusion glycoprotein. The attachment glycoprotein is associated with virion attachment to the cellular receptor and has been designated as the hemagglutinin-neuraminidase protein (HN), the hemagglutinin protein (H), or the G protein, which has neither hemagglutinating nor neuraminidase activities, whereas, the F glycoprotein induces fusion between the viral and cellular membranes. G and F acted in concert to bring about fusion. The existing studies showed that only coexpression of the attachment glycoprotein and fusion glycoprotein of Newcastle disease virus[12], Human parainfluenza virus[13], and Measles virus[14] induced fusion. In addition, only coexpression of the G protein and F protein of NiV also induced fusion. The binding of G protein and the cellular receptor induced a series of configuration changes in the F protein, which further induced the fusion of viral envelope and cellular membrane or the fusion of cellular membranes, resulting in the entry of the nucleocapsid into the cytoplasm and the formation of typical syncytia. For most paramyxoviruses, the heterotypic fusion activity that has been measured is considerably lower than a virus's homotypic fusion activity. However, the NiV and HeV envelope glycoproteins could efficiently mediate fusion in heterologous envelope combinations with each other. The existing studies confirmed that the G and F proteins of NiV could mediate high-level fusion of BSC-1 cells, U373 cells, BHK 21 cells, and cat embryonic cells[15]. This study demonstrated that the F protein precursor F0 expressed by the recombinant baculovirus could be cleaved to produce F1 subunit and F2 subunit similar to natural F proteins. In this study, the recombinant baculoviruses rBac-NiV-F and rBac-NiV-G coinfected sf9 insect cells that induced quiet syncytia, which suggested that the recombinant F and G proteins possess partial biological activity.

The envelope glycoprotein of RNA virus plays key role in binding the virus to the host cells, viral entry into host cells, and in eliciting the neutralization antibody. The recombinant Vesicular Stomatitis Virus (VSV) possesses the same competence of producing pseudotype virus to bear foreign viral envelope proteins as the retrovirus. The VSV envelope protein G gene was replaced with the foreign viral envelope protein gene by the reverse genetic technique of negative strand RNA virus. The foreign envelope proteins were assembled on the envelope of VSV to produce the VSV-pseudotype, which resemble the donor virus of the foreign envelope protein in the function of binding acceptor, the mechanism of viral entry and the sensibility and specificity of the neutralization antibody. Therefore, the envelope protein G gene of VSV and retrovirus were replaced with the report gene and complemented with VSV or retrovirus G glycoprotein expressed in trans to produce VSV pseudotype, which was used to replace the biosafety high-level virus or the virus that the observation of its cytopathic effect (CPE) is difficult in the study of the detecting of neutralization antibody, the characteristics of receptor binding, antigenicity

and epitope[16,17]. Presently, NiV does not exist in China and NiV was not assented to initiate live virus. Therefore, VSV envelope protein G gene, in this study, was replaced with the green fluorescent protein gene, and complemented with NiV G and F glycoprotein expressed in trans, which produced a safe, stable, and high titer VSV pseudotype that possessed the competency of infection and duplication. The VSV pseudotype replaced live NiV. The neutralizing antibody assay obtained satisfactory effectiveness when the VSV pseudotype replaced live NiV.

The passive immunization protection test, in small animal model, demonstrated that the neutralization antibody formed the effective protection to lethal challenge of NiV[18]. G and F of NiV were major viral structural proteins, which induced neutralization antibody responses. In this study, immunization with insect cells was infected by the recombinant baculoviruses, thereby expressing NiV G and F proteins, eliciting G and F protein-specific antibody responses in mice. Furthermore, the G and F protein-specific antibodies neutralized the infectivity of the VSVΔG*F/G. rNG that induced higher titer of neutralization antibody (>128) than rNF (>64). Both rNG and rNF induced higher titer of neutralization antibody than the titer of neutralization antibody of serum from rabbits immunzied with inactivated NiV (>32).The study of the Measles virus showed that HN protein induced higher titer of neutralization antibody than F protein, *in vivo*[19]. In addition, the comparative study of DNA immunization of NiV G and F protein showed that G protein induced higher titer of neutralization antibody and ELISA antibody than F protein (the result is reported in other article). Baculovirus possess the competence of transient infection to mammalian cell. Therefore, the recombinant baculoviruses that expressed specific immunogen induced effective specific immune responses[20]. In this study, the cell suspension of sf9 insect cells infected by the recombinant baculovirus immunized mice, in which rNF, rNG and the recombinant baculovirus were the immunogen to form immunostimulation. At present, the vaccine against NiV has not been successfully developed. Consequently, F and G protein expressed by the recombinant baculoviruses could be safe diagnostic antigens for the surveillance and monitoring of NiV and promising subunit vaccines for the prevention of NiV.

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