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RESEARCH PAPER

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

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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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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