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Recombinant Newcastle disease viral vector expressing hemagglutinin or fusion of canine distemper virus is safe and immunogenic in minks

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ABSTRACT

Canine Distemper Virus (CDV) infects many carnivores and cause several high-mortality disease outbreaks. The current CDV live vaccine cannot be safely used in some exotic species, such as mink and ferret. Here, we generated recombinant lentogenic Newcastle disease virus (NDV) LaSota expressing either envelope glycoproyein, heamagglutinine (H) or fusion protein (F), named as rLa-CDVH and rLa-CDVF, respectively. The feasibility of these recombinant NDVs to serve as live virus-vectored CD vaccine was evaluated in minks. rLa-CDVH induced significant neutralization antibodies (NA) to CDV and provided solid protection against virulent CDV challenge. On the contrast, rLa-CDVF induced much lower NA to CDV and fail to protected mink from virulent CDV challenge. Results suggest that recombinant NDV expressing CDV H is safe and efficient candidate vaccine against CDV in mink, and maybe other host species.

1. Introduction

Canine distemper is a widespread infectious disease affecting carnivores. It is caused by the canine distemper virus (CDV), which is an enveloped nonsegmented negative-strand RNA virus belonging to the *Morbillivirus* genus in the Paramyxoviridae family [1–4]. CDV is highly infectious and causes systemic and often fatal disease in dogs and other terrestrial carnivores [2]. The lethality of CDV infection varies among susceptible species, ranging from 0% for domestic cats to 100% for ferrets [4]. For domestic dogs, mortality rates can reach 50% [5]. Currently, attenuated CDV strains are routinely used as live vaccines against CDV infection in dogs, minks, and other carnivores. The extensive use of modified live CDV vaccines has greatly reduced the number of outbreaks of CD. Current modified live vaccine strains are safe and effective in dogs, but some cases of canine distemper infection in immunized dogs are still reported [6–9]. One well-known problem for conventional live CDV vaccines is that the effects of primary vaccination are affected by residual maternally derived antibodies [10]. Moreover, conventional live attenuated vaccines are not safe for minks, ferrets, and some forms of wildlife [11–13]. A safer and more efficient vaccine without these limitations is still unavailable.

Newcastle disease virus (NDV), one member of the Paramyxoviridae family, is classified as nonvirulent (lentogenic), moderately virulent (mesogenic) or highly virulent (velogenic) for poultry [14]. Lentogenic strains contain fewer basic amino acids at the protease cleavage site of the fusion (F) protein precursor than other strains and can only be cleaved by trypsin-like extracellular proteases, which are largely confined to the respiratory tract. However, highly virulent strains are cleaved by ubiquitous intracellular proteases, and this can cause systemic infection [15,16]. Currently, lentogenic strains, such as the La Sota strain, are used as live attenuated vaccine vectors against NDV and AIV in poultry [17–19]. NDV has been actively developed and evaluated for use as a vaccine vector for the control of disease in humans and other animals [20–24]. NDV does not usually cause sustained infection in mammals because of host-range restriction and it is antigenically distinct from mammalian paramyxoviruses, suggesting that mammals would not be susceptible to NDV. Host-range restriction

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and antigenic distinction indicate that NDV is a promising vaccine vector for humans and other mammals. Here, we generated a recombinant NDV expressing hemagglutinin, or fusion protein, which are the major antigens for protective immune responses against CDV. The feasibility of these recombinant NDVs as live virus-vectored CD vaccines was here evaluated in minks.

2. Materials and methods

2.1. Cells and viruses

HEp-2 cells (American Type Culture Collection, ATCC No. *CCL-10*), BHK-21 cells (ATCC No. CCL-10), and Vero cells (ATCC No. CCL-81) used for rescue, titration or propagation of virus were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Carlsbad, CA, U.S.) containing 10% fetal bovine serum (FBS) (Gibco). MVA-T7 was propagated and titrated in chicken embryo fiber cells as described previously [25]. The CDV vaccine strain Rockborn-20/8(CDV/R-20/8) and the recombinant CDV expressing enhanced green fluorescent protein (rCDV-EGFP) was propagated and titrated in Vero cells [26]. The CDV virulent strain TM/JL2008 was isolated from a naturally infected Tibetan mastiff in 2008 and passaged for five generations in a transfected Vero cell line that stably expressed dog SLAM receptor (Vero-SLAM). It was then titrated and used to challenge Vero-SLAM cells. NDV rLaSota strain were grown and titrated in 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs by inoculation of the allantoic cavity [17]. Viral titration results were calculated using the method described by Reed and Muench [27]. All viruses were stored at −70 °C before use.

2.2. Plasmid construction

The cDNA of the CDV H and F genes was amplified from genome RNA of CDV/R-20/8 by RT-PCR. The primers for amplification of H gene cDNA were 5′-GACTGTTTAAACTTAGAAAA AATACGGGTAGAAGT*GCCGCCACC*ATGCTCTCCTACCAAGACAAGGT-3′ and 5′-GACTGTTTAAACTCAGGGATTTGAACG GTTACATG-3′. These primers included the gene start and gene termination sequences of the NDV genome (underlined) and the Kozak sequence (italics). The primers for F gene cDNA were 5′-GACTGTTTAAACTTAGAAAAAATACGGGTAGAAGTGCCACCA TGCA-CAAGGAAATCCCCAA-3′ and 5′-GACTGTTTAAACTCAGAGTGATCTCA CATA GGA-3′. The H and F gene cDNA was inserted into the NDV genome cDNA by *Pme* I site in the P-M intergenic region, as described previously [17,22]. The resultant plasmids were designated pLa-CDVH and pLa-CDVF, respectively.

2.3. Rescue of recombinant viruses from cloned cDNA

The rescue of recombinant NDV viruses from cloned cDNA was described previously, as followings: HEp-2 cells grown in a six-well plate were infected with MVA-T7 at a multiplicity of infection (MOI) of 1 and then transfected with pL-CDVH (1 μg) or pL-CDVF together with 3 helper plasmids [pT7-NP (0.4 μg), pT7-P (0.2 μg), and pT7-L (0.2 μg)]. After 16 h of incubation at 37 °C, the medium was replaced with 2 ml Opti-MEM (Invitrogen Corp., Carlsbad, CA, U.S.) containing 0.5 μg of TPCK trypsin and the cells were incubated for another 3 d at 37 °C. The supernatant was then inoculated into the allantoic cavities of 10-day-old embryonated SPF eggs. After 72 h of incubation at 37 °C, the allantoic fluid was harvested and the virus was identified by hemagglutination assay using 0.5% chicken red blood cells. The resultant recombinant viruses were designated rL-CDVH and rL-CDVF.

2.4. Immunofluorescence (IFA)

BHK-21 cells were plated on cover slips in dishes 35 mm in diameter and infected with rLaSota, rL-CDVH or rL-CDVF for 24 h. Then cells were fixed in pre-chilled 3% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min at room temperature and stained with mice polyclonal antibodies against CDV or chicken serum against NDV followed by TRITC-conjugated goat anti-mouse IgG (Sigma) or a FITC-conjugated rabbit anti-chicken IgG (Sigma) [17,26]. Cells were incubated with DAPI for 5 min to stain the nuclei. Images were taken by sequential scanning at each wavelength on a Leica confocal laser microscope. Mock-infected cells were used as controls.

2.5. Assessment of pathogenicity

To determine the pathogenicity of rL-CDVH and rL-CDVF in poultry, the mean death time (MDT), intracerebral pathogenicity index (ICPI), and intravenous pathogenicity index (IVPI) were determined in embryonated SPF chicken eggs or in SPF chickens as described before according to the OIE Manual [17,28].

To assess the pathogenicity of the recombinant viruses in mice, three groups of 19 six-week-old female Balb/c mice (Vital River, Beijing, China) were intramuscularly (*i.m.*) injected with 0.1 ml of diluted allantoic fluid containing 10^8 EID_{50} (50% embryo infectious dose) rL-CDVH, rL-CDVF or rLaSota and intranasally (*i.n.*) inoculated with 0.03 ml of diluted allantoic fluid containing 3×10^7 EID_{50} rL-CDVH, rL-CDVF or rLaSota. Another 19 mice were *i.m.* injected with 0.1 ml and *i.n.* inoculated with 0.03 ml PBS as mock infection control. Every 3 mice were anesthetized at 3, 5, and 7 days after infection and lung, heart, spleen, kidney, and brain tissues were collected for isolation if virus by inoculation of eggs. The remaining 10 mice were monitored daily for changes in body weight.

2.6. Vaccination

Six-week-old minks were arranged in five groups of ten animals each and each were given two doses of different viruses by intramuscular routine (*i.m.*) in 2 ml of PBS-diluted at 3-week intervals. Three groups of minks were inoculated with rL-CDVH, rL-CDVF, and NDV LaSota at a dosage of 2×10^9 EID_{50}. One group was inoculated with a mixture of rL-CDVH and rL-CDV-F containing 1×10^9 EID_{50} (rL-CDVH/F). Another group was inoculated with $10^{5.8}$ $TCID_{50}$ of CDV/R-20/8. The sera were collected at specific points and time before and after vaccination for CDV neutralization antibody and NDV NA assays. None of the minks had been inoculated with CD vaccine previously.

2.7. Serologic assays

The titers of neutralization antibodies (NA) to CDV and NDV in mink serum were each determined as described previously [26].

2.8. Challenge study

Two weeks after the second vaccination, four minks in each group were anesthetized and challenged with virulent CDV strain TM/JL2008 at a dosage of 2×10^4 $TCID_{50}$ by intranasal administration. The clinical symptoms were observed and body temperatures were measured daily. To detect viral shedding, swab samples were collected from the mucosa of the nose and eyes at 3, 5, 7, 14, and 21 days. These samples were soaked in 0.5 ml of MEM supplement. The samples were centrifuged and the supernatant was diluted 10-fold in 96-well plates in triplicate (0.1 ml per well). Subsequently, 2×10^5 Vero-SLAM cells in 0.1 ml of 2 × MEM supplemented with 20% FCS, 400 unit/ml penicillin were added to each well. Then the