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RESEARCH ARTICLE

Newcastle disease virus-based MERS-CoV candidate vaccine elicits high-level and lasting neutralizing antibodies in Bactrian camels

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Abstract

Middle East respiratory syndrome coronavirus (MERS-CoV), a member of the *Coronaviridae* family, is the causative pathogen for MERS that is characterized by high fever, pneumonia, acute respiratory distress syndrome (ARDS), as well as extrapulmonary manifestations. Currently, there are no approved treatment regimens or vaccines for MERS. Here, we generated recombinant nonvirulent Newcastle disease virus (NDV) LaSota strain expressing MERS-CoV S protein (designated as rLa-MERS-S), and evaluated its immunogenicity in mice and Bactrian camels. The results revealed that rLa-MERS-S showed similar growth properties to those of LaSota in embryonated chicken eggs, while animal immunization studies showed that rLa-MERS-S induced MERS-CoV neutralizing antibodies in mice and camels. Our findings suggest that recombinant rLa-MERS-S may be a potential MERS-CoV veterinary vaccine candidate for camels and other animals affected by MERS.

Keywords: Newcastle disease virus, MERS-CoV, neutralizing antibodies, camels

1. Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV), a member of the c lineage in the genus *Beta coronavirus*, causes high fever, pneumonia, acute respiratory distress syndrome (ARDS), as well as extrapulmonary manifestations including gastrointestinal symptoms, lymphopenia, acute kidney injury (Yeung *et al*. 2016), hepatic inflammation, and pericarditis (Wong *et al*. 2015). So far, MERS-CoV is responsible for 1 800 laboratory-confirmed cases of human infection, including at least 640 deaths (WHO, http://www.who.int/emergencies/mers-cov/en).

The majority of human cases have been reported in the Middle East, likely due to the existence of dromedary camels, which have been confirmed to carry live MERS-CoV and may be the potential source of human infections (Azhar *et al*. 2014; Memish *et al*. 2014). Furthermore, due to globalization, geographical barriers are more easily bypassed than in the past. Since bats are the potential natural hosts of MERS-CoV (Annan *et al*. 2013; Memish *et al*. 2013) and are in limited contact with humans, the most effective strategy would be to suppress circulation of the virus in camels before MERS-CoV escalates into a global pandemic in humans. With the increasing number of

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MERS cases, there is an urgent need to develop vaccines or specific drugs targeted at epidemic MERS-CoV (Modjarrad *et al*. 2016; Zumla *et al*. 2016).

Newcastle disease virus (NDV) belongs to the genus *Avulavirus* in the *Paramyxoviridae* family. NDV is classified as lentogenic (nonvirulent), mesogenic (moderately virulent), or velogenic (highly virulent) according to their pathogenicity in poultry. Lentogenic strains, such as the LaSota, have also been applied as a vaccine vector targeting human and other animal diseases (Ge *et al*. 2007, 2010; Di Napoli *et al*. 2010a, b). NDV is innately advantageous as a potential vaccine vector for the following reasons: Firstly, NDV is antigenically distinct from the mammalian paramyxoviruses, it does not typically cause an productive infection in mammals. Secondly, the pre-existing immunity against mammalian paramyxoviruses does not interfere with the replication capacity of NDV. In addition, the safety profile of NDV has been confirmed in many non-human primates as well as humans (Bukreyev *et al*. 2006; Bukreyev and Collins 2008; Khattar *et al*. 2010; Kortekaas *et al*. 2010).

As a membrane-anchored structural protein of MERS-CoV, spike (S) protein mediates viral receptor binding and entry (Belouzard *et al*. 2012; Millet and Whittaker 2014). S protein is the primary target for anti-coronavirus vaccine design (Zhao *et al*. 2014), and studies have demonstrated that S protein is immunogenic and can induce neutralizing antibodies which plays crucial role in anti-CoV infection (Hofmann *et al*. 2004; Enjuanes *et al*. 2008; Du *et al*. 2009; Pascal *et al*. 2015). Currently, several MERS-CoV candidate vaccines, such as DNA vaccines (Muthumani *et al*. 2015), virus like particles (VLPs) (Wang *et al*. 2016) as well as recombinant viral vectored vaccines. Of note, the recombinant viral vectored MERS vaccines, such as modified vaccinia Ankara (Song *et al*. 2013) or adenovirus (Kim *et al*. 2014) demostrated good immunogenicity and provided protection for mice, nonhuman primates (NHPs) and camels against MERS-CoV challenge. Herein, we generated a recombinant NDV LaSota virus expressing MERS-CoV S protein and evaluated its immunogenicity in mice and camels.

2. Materials and methods

2.1. Viruses and cells

BHK-21 and Vero-E6 cells were grown in Dulbecco's minimum essential medium (DMEM) containing 10% fetal bovine serum (FBS). The NDV vector virus rLa was rescued from the genomic cDNA of the NDV LaSota vaccine strain as previously described (Ge *et al*. 2007). Recombinant NDV was grown and titrated in 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs by allantoic cavity inoculation. A recombinant Vesicular stomatitis virus (VSV) vectored virus expressing MERS-CoV S protein and enhanced green fluorescence protein (eGFP), designated as VSVΔG-eGFP-MERS, was used to determine the induction of neutralizing antibodies by MERS-CoV. The recombinant VSV-vectored virus (VSVΔG-eGFP-MERS) was generated by replacing the *G* gene of the recombinant VSV expressing eGFP with the MERS-CoV *S* gene as described previously (Li *et al*. 2006; Liu *et al*. 2015). VSVΔG-eGFP-MERS was grown and titrated in Vero E6 cells. Modified vaccinia Ankara expressing the T7 RNA polymerase (kindly provided by Dr. Bernard Moss, the National Institutes of Health, Bethesda, MD, USA) was grown and titrated in primary chicken embryo fibroblasts (Wyatt *et al*. 1995). All viruses were stored at −70°C before use.

2.2. Plasmid construction and virus rescue

To construct the full-length recombinant NDV genomic cDNA, MERS-CoV (GenBank accession no. KF186567.1) *S* gene was amplified by PCR from synthesized cDNA (Invitrogen, Shanghai, China) by using the following primers: 5′-GACT**GTTTAAAC**TTAGAAAAAATACGGGTAGAAGT*GCCACC*ATGATACACTCAGTGTTTCTACTG-3′, and 5′-GACT**GTTTAAAC**TCATTAGTGAACATGAACCTTATG-CGGCTCGAG-3′, in which the NDV gene end and gene start sequences (underlined), the optimal Kozak sequence (italic) and the *Pme*I restriction sites (bold) were introduced. *S* gene was introduced into NDV genomic cDNA through a unique *Pme*I site in the P-M intergenic region. The resultant plasmid was designated as prLa-MERS-S and used for virus rescue following established protocol as described previously (Ge *et al*. 2007). The rescued virus was designated as rLa-MERS-S. The presence of *S* gene in the NDV genome was confirmed by sequencing of the entire viral genome. S protein expression in rLa-MERS-S infected cells was confirmed by indirect immunofluorescence and Western blot assay.

2.3. Assessment of virus pathogenicity

The pathogenicity of rLa-MERS-S in poultry was determined by mean death time (MDT), intracerebral pathogenicity index (ICPI), and intravenous pathogenicity index (IVPI) in embryonated SPF chicken eggs or in SPF chickens according to the OIE Manual (OIE 2004).

To assess the pathogenicity of the recombinant viruses in mice, 2 groups of 10 six-week-old female Balb/c mice (Vital River, Beijing, China) were intramuscularly (*i.m.*) injected with 1×10^8 EID_{50} (50% embryo infectious dose) rLa-MERS-S or rLa in 0.1 mL diluted allantoic fluid and intranasally (*i.n.*) inoculated with 3×10^7 EID_{50} rLa-MERS-S or rLa in 0.03 mL diluted allantoic fluid. The third group of 10 mice was *i.m.* injected with 0.1 mL and *i.n.* inoculated with 0.03 mL PBS