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Passive immunotherapy for Middle East Respiratory Syndrome coronavirus infection with equine immunoglobulin or immunoglobulin fragments in a mouse model

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ABSTRACT

Middle East Respiratory Syndrome (MERS) is a highly lethal pulmonary infection caused by a coronavirus (CoV), MERS-CoV. With the continuing spread of MERS-CoV, prophylactic and therapeutic treatments are urgently needed. In this study, we prepared purified equine $F(ab')_2$ from horses immunized with MERS-CoV virus-like particles (VLPs) expressing MERS-CoV S, M and E proteins. Both IgG and $F(ab')_2$ efficiently neutralized MERS-CoV replication in tissue culture. Passive transfer of equine immune antibodies significantly reduced virus titers and accelerated virus clearance from the lungs of MERS-CoV infected mice. Our data show that horses immunized with MERS-CoV VLPs can serve as a primary source of protective $F(ab')_2$ for potential use in the prophylactic or therapeutic treatment of exposed or infected patients.

1. Introduction

Middle East Respiratory Syndrome (MERS)-CoV is an emerging pathogen that causes severe pneumonia in humans in the Arabian Peninsula and in travelers from this region (Assiri et al., 2013a; Zaki et al., 2012b; Zumla et al., 2015). Human-to-human spread has been documented (Assiri et al., 2013b). While infections of immunocompetent patients generally present with only mild symptoms, the elderly and patients with pre-existing illnesses such as diabetes or renal failure are likely to develop more severe disease (Assiri et al., 2013a). As of September 21, 2016, 1806 cases with 643 deaths (35.6% mortality) had been reported to the World Health

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Organization, although the actual number of infections could be much larger since mild, asymptomatic or undiagnosed cases are likely to be common (Drosten et al., 2014).

As yet there are neither licensed vaccines nor any prophylactic or therapeutic treatments effective against MERS-CoV. Given the ability of coronaviruses to rapidly adapt to new hosts, a major public health concern is that MERS-CoV will further adapt to replication in humans, triggering a global severe acute respiratory syndrome (SARS)-like pandemic (Peiris et al., 2004; Zaki et al., 2012a).

As of now, the most promising treatment is the passive administration of anti-MERS-CoV neutralizing antibodies. Several research groups have developed and produced anti-MERS patient-derived or humanized monoclonal neutralizing antibodies *in vitro* that were able to protect MERS-CoV infected mice (Corti et al., 2015; Li et al., 2015; Zhao et al., 2014). However, since these antibodies react with a single epitope on the MERS-CoV spike (S) protein and since coronaviruses are prone to mutate, this approach has raised concerns about possible antibody escape (Corti et al., 2015; Sabir et al., 2016).

Recently, we showed that sera from Middle East dromedary camels contained high levels of anti-MERS-CoV neutralizing antibodies. Passive immunotherapy with sera from these animals significantly reduced virus loads and accelerated virus clearance from the lungs of MERS-CoV infected mice (Zhao et al., 2015). This provides proof of concept that immune animal sera are potentially useful in the treatment of patients with MERS (Hayden et al., 2014). Passive immunotherapy with animal sera or antibodies has been successfully used to prevent rabies and to neutralize snake venom (Both et al., 2012; Gutierrez et al., 2014). Convalescent plasma used to treat patients with SARS has been found safe and has demonstrated some efficacy in a study with a small number of patients (Mair-Jenkins et al., 2015). However, neutralizing antibody titers in MERS patients are generally low and the limited number of MERS survivors makes this approach impractical (Drosten et al., 2013).

Here, we show that immunization of healthy horses with MERS-CoV virus-like particles (VLPs) expressing MERS-CoV S, M and E proteins induces strong polyclonal neutralizing antibodies against MERS-CoV. Since administration of whole antibodies can induce allergic responses in some humans, we further tested $F(ab')_2$ fragments prepared by digestion of antibody with pepsin. Prophylactic or therapeutic treatment of MERS-CoV infected mice with either IgG or $F(ab')_2$ significantly decreased the virus load in their lungs.

2. Materials and methods

2.1. Antigen preparation

MERS-CoV VLPs were produced and purified as previously described (Wang et al., 2016). In brief, army worm Sf9 cells were infected with a single recombinant baculoviruses co-expressing MERS-CoV structural protein genes S, M, and E, at a multiplicity of infection (MOI) of 0.5. Culture supernatants were harvested at 96 h post-infection and centrifuged at 2000 g for 30 min to remove cell debris. Following centrifugation of the clarified supernatants at 100,000 g for 1 h at 4 °C the resulting VLP pellets were resuspended in PBS and loaded onto a 30–40–50% discontinuous sucrose gradient. After an additional centrifugation at 100,000 g for 1.5 h at 4 °C, bands between 30 and 40% sucrose containing MERS-CoV VLP were collected.

2.2. Animal immunization

Four 4-year-old healthy horses received multi-point intramuscular injections of 0.5, 1.5, 2, 3, and 5 mg MERS-CoV VLPs in 4 ml PBS at weeks 0, 2, 4, 6, and 8, respectively. Freund's complete adjuvant (Sigma) was included in the first dose, and incomplete adjuvant in the remaining ones. Sera were collected from the jugular vein 2 weeks after each injection, and stored at −20 °C before further analysis.

2.3. MERS-CoV specific antibody measurement

MERS-CoV specific antibodies in the sera were measured by an indirect enzyme-linked immunosorbent assay (ELISA) using purified MERS-CoV receptor-binding domain (RBD) protein (i.e., S protein residues 358–662 cloned into the pET-30a expression vector and purified by Ni-NTA affinity chromatograph column). Briefly, 96-well microtitration plates (Corning Costar, USA) were pre-coated with 100 μL purified RBD antigen diluted in 0.05 mol/L carbonate sodium buffer (pH 9.6) to a final concentration of 1 μg/mL and incubated at 4 °C overnight. After blocking with skimmed milk for 2 h at 37 °C, 100 μL twofold serially diluted serum samples were added to the wells, and incubated at 37 °C for 1 h. The plates were washed three times with PBS containing 0.05% Tween-20 (PBST), before addition of 100 μL HRP-labeled rabbit antibody against horse IgG (Bioss, China; 1:20,000) and incubation at 37 °C for 1 h. After washing with PBST, 100 μL 3, 3′, 3, 5'-tetramethylbenzidine (TMB) (Sigma, USA) as substrate was added to each well and incubated for 30 min. The reaction was stopped with 50 μL 2 M H_2SO_4. Optical densities at 450 nm were measured in an ELISA plate reader (Bio-Rad, USA).

2.4. Immunoglobulin purification

Horse antiserum was diluted with 2 vol of normal saline (0.9% NaCl) and a half volume of saturated ammonium sulfate was then added and mixed gently at room temperature for 30 min before centrifugation at 5000 g for 20 min. The resulting sediment was redissolved in saline and mixed with a one-third volume of saturated ammonium sulfate. After incubation at ambient temperature for 30 min and centrifugation at 5000 g for 20 min, the second sediments were dissolved in normal saline and dialyzed against normal saline to remove any remaining ammonium salt.

2.5. Immunoaffinity chromatography

Immunoaffinity resins were prepared by coupling 10 mg RBD protein to 0.02 M sodium periodate-activated Sepharose 4B (4 g), and then incubating with 150 μL sodium borohydride for 30 min. After reaction with 1 M Tris (pH 7.5) for 30 min, a purified IgG sample was diluted 9-fold with PBS and incubated with the RBD resin overnight at 4 °C with constant rotation. The flowthroughs (anti-RBD depleted) were collected, and then the flowthroughs were tested against the RBD protein by ELISA to ensure RBD-specific IgG all bound with the RBD Sepharose 4B. After washing with PBS, the bound antibodies (anti-RBD) were eluted in 0.2 M glycine-HCl buffer (pH 2.7). The eluates were neutralized with 1 M Tris buffer (pH 9.0), and then dialyzed against PBS. All samples were adjusted to the same protein concentration and sterilized by passage through microspin filters (0.2 μm pore size; Millipore). Neutralizing activity of the IgG, RBD-specific IgG, and flowthroughs were tested.

2.6. $F(ab')_2$ preparation

The pH of the horse antiserum was adjusted to 3.3 with 1 mol/L HCl. Following incubation with pepsin (10000 IU/mL) at 30 °C for 2.5 h, the reaction was stopped by adjusting the pH to 7.2 with 1 mol/L NaOH. The solution was then applied to Protein-A and