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Receptor-binding domain-based subunit vaccines against MERS-CoV

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ABSTRACT

Development of effective vaccines, in particular, subunit-based vaccines, against emerging Middle East respiratory syndrome (MERS) caused by the MERS coronavirus (MERS-CoV) will provide the safest means of preventing the continuous spread of MERS in humans and camels. This review briefly describes the structure of the MERS-CoV spike (S) protein and its receptor-binding domain (RBD), discusses the current status of MERS vaccine development and illustrates the strategies used to develop RBD-based subunit vaccines against MERS. It also summarizes currently available animal models for MERS-CoV and proposes a future direction for MERS vaccines. Taken together, this review will assist researchers working to develop effective and safe subunit vaccines against MERS-CoV and any other emerging coronaviruses that might cause future pandemics.

1. Introduction

Middle East respiratory syndrome (MERS) is a newly emerged infectious disease caused by a novel β-coronavirus (β-CoV), MERS coronavirus (MERS-CoV). MERS-CoV was first identified in humans in Saudi Arabia on June 12, 2012 (Zaki et al., 2012). Although the spread of MERS-CoV among humans has been limited, MERS-CoV infection has been linked to several family clusters and healthcare workers (Assiri et al., 2013; Memish et al., 2013b, 2013c), providing evidence for human-to-human transmissibility of the virus. MERS-CoV has demonstrated an increasing trend of infection since its first identification. Sporadic MERS cases have been reported in at least 21 countries in the Middle East, Africa, Europe, Asia and North America. As of November 7, 2014, a total of 909 laboratory-confirmed cases including 331 deaths (mortality rate ~36%) were reported (http://www.who.int/csr/don/07-november-2014-mers/en/). The geographic spread and rapid increase of MERS cases during the past several months have raised concerns of pandemic potential, even though the consequences of such pandemic might be less severe than those caused by severe acute respiratory syndrome coronavirus (SARS-CoV), another β-CoV which led to a worldwide outbreak in 2003 (Peiris et al., 2003). As a practical control strategy against the potential outbreak of MERS-CoV-caused emerging infectious disease, development of effective vaccines has become a high priority.

A number of studies have pointed out the transmission hosts for MERS-CoV. It is reported that bat-derived coronaviruses, particularly bat coronavirus HKU4, have close phylogenetic relationship with MERS-CoV and that dipeptidyl peptidase 4 (DPP4), the receptor for MERS-CoV, is also the receptor for HKU4. While HKU4 prefers bat DPP4 over human DPP4, MERS-CoV has adapted to use human DPP4, in addition to bind bat DPP4, suggesting that (1) bats are potential natural reservoirs for MERS-CoV; and (2) bat coronaviruses remain a threat to human health because of their potential for cross-species transmission (Annan et al., 2013; Cui et al., 2013; Ithete et al., 2013; Memish et al., 2013a; Yang et al., 2014). In addition to bats, camels have recently become a focus for the study of MERS-CoV transmission since MERS-CoV neutralizing antibodies and MERS-CoV gene fragments have been identified in dromedary camels, and infectious MERS-CoV has been recovered from infected camels (Briese et al., 2014; Chu et al., 2014; Drosten et al., 2014; Haagmans et al., 2014; Meyer et al., 2014; Nowotny and Kolodziejek, 2014; Reusken et al., 2013). The fact that humans were infected with MERS-CoV after exposure to infected camels suggests that camels are the most likely intermediate transmission hosts of MERS-CoV (Memish et al., 2014). Most recently, MERS-CoV RNA fragments were

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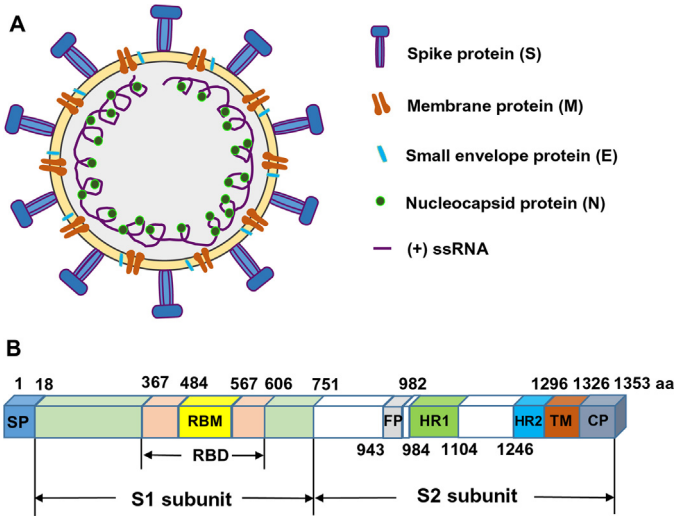

Fig. 1. Schematic structures of MERS-CoV and its spike protein RBD. (A) Schematic structure of MERS-CoV. MERS-CoV contains a positive, single-stranded RNA and four structural proteins, including S, M, E and N. (B) Spike protein of MERS-CoV and its RBD. MERS-CoV S protein contains S1 and S2 subunits, and their functional regions with specific amino acid residues are shown. SP, signal peptide. RBD, receptor-binding domain. RBM, receptor-binding motif within RBD. FP, fusion peptide. HR1 and HR2, heptad repeats 1 and 2. TM, transmembrane domain. CP, cytoplasmic tail.

detected in an air sample collected from the barn that sheltered MERS-CoV-infected camels (Azhar et al., 2014), indicating possible airborne transmission of MERS-CoV between animals and humans. Although the spread of MERS-CoV among humans is limited and inefficient (Drosten et al., 2014), the increased infection rate among healthcare workers during the month of April 2014 (http://www.who.int/csr/disease/coronavirus_infections/archive_updates/en/) has raised concerns of future epidemic potential, which call for the development of effective and safe vaccines to prevent and control MERS (Hotez et al., 2014).

2. MERS-CoV and its spike protein receptor-binding domain

MERS-CoV belongs to lineage C of β-CoV with a close relationship to the bat coronaviruses HKU4 and HKU5 and is the first known lineage C β-CoV associated with human infections (Chan et al., 2013b; Woo et al., 2012). MERS-CoV is a positive-sense, single-stranded RNA virus whose genome encodes four major structural proteins, including spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N), each with unique functions (Fig. 1A). The E protein is a transmembrane protein which forms an ion channel on the viral surface, while the N protein interactively functions with the M protein and other N molecules encapsulating genomic RNA (Hurst et al., 2010).

Among the four structural proteins of MERS-CoV, the S protein plays the most important roles in virus infection and pathogenesis. It displays as a trimer on the viral membrane surface. The precursor S protein is cleaved into two noncovalently associated subunits: the distal subunit S1 and the membrane-anchored subunit S2. The MERS-CoV S1 subunit contains the receptor-binding domain (RBD), including a core structure and an accessory subdomain receptor-binding motif (RBM), while the S2 subunit consists of a putative fusion peptide, transmembrane domain and two heptad repeat regions, termed heptad repeats 1 and 2 (HR1 and HR2) (Fig. 1B) (Chen et al., 2013b; Lu et al., 2013a; Wang et al., 2013).

Although MERS-CoV and SARS-CoV share similar core structures to maintain conformational integrity, their RBMs are different (Chen et al., 2013b; Li et al., 2005). As such, the receptors recognized by both coronaviruses are distinctively different. Angiotensin-converting enzyme 2 (ACE2) is a functional receptor for SARS-CoV, while DPP4 (also known as CD26) is an identified receptor for MERS-CoV (Li et al., 2003; Raj et al., 2013). The crystal structures of SARS-CoV RBD complexed with its receptor ACE2 have identified the RBD of SARS-CoV as residues 306–527 and the RBM as residues 424–494 (Li et al., 2005). Crystal structure analysis of MERS-CoV RBD alone or RBD/DPP4 complex has mapped the RBD to residues 367–588 and 367–606, respectively, in the S protein of MERS-CoV, with the RBM spanning residues 484–567 (Chen et al., 2013b; Lu et al., 2013a; Wang et al., 2013). Fig. 2 lists the crystal structures of SARS-CoV and MERS-CoV RBDs and their complexes with respective receptors.

MERS-CoV undergoes two major processes to infect target cells. First, the virus binds to the cellular receptor DPP4 via the RBD in the S1 subunit. Second, the HR1/HR2 complex in the S2 subunit forms a fusion core, leading to cell-virus membrane fusion, thereby mediating MERS-CoV entry into the target cells (Gao et al., 2013; Lu et al., 2014). Therefore, like SARS-CoV S protein, the S protein of MERS-CoV plays an essential role in receptor binding, membrane fusion and cell entry. We have previously demonstrated that the S protein, or, more specifically, the RBD, of SARS-CoV played an essential role in developing SARS vaccines (Du et al., 2009a; Jiang et al., 2012b). It is thus expected that the RBD of MERS-CoV S protein will be an important target for developing vaccines against MERS (Lu et al., 2013b; Ma et al., 2014b).

3. Current status in developing MERS vaccines

Antibodies induced by SARS-CoV RBD do not cross-neutralize MERS-CoV infection since different receptors are recognized by the two coronaviruses, suggesting that developing a safe and effective MERS vaccine is a high priority (Du et al., 2013b, 2013c). Since MERS is a newly-emerged viral disease, no vaccines have been developed for clinical use. However, a number of vaccine candidates have been tested and/or proven effective in *in vitro* preclinical studies. Current updates on MERS vaccine development, including the possibility for developing certain vaccine types as candidates against MERS, are discussed below.

It was revealed that a recombinant modified vaccinia virus Ankara (MVA) expressing the full-length MERS-CoV S protein, MVA-MERS-S, produced neutralizing antibodies in immunized mice against infections from MERS-CoV in cell cultures *in vitro* (Song et al., 2013), providing a basis for developing viral vector-based MERS vaccines. In addition, full-length infectious cDNA clones of MERS-CoV have been constructed using reverse genetics systems, and relevant infectious viruses could be rescued and propagated in Vero A66 and Huh-7 (human liver) cells (Almazán et al., 2013; Scobey et al., 2013). Reports have also shown that a full-genome sequence of MERS-CoV (Jordan-N3/2012 strain) exhibited stability after sequential passages in two mammalian cell lines: Vero (African green monkey kidney) and MRC5 (human lung) (Frey et al., 2014). The above studies indicate the potential for developing live-attenuated viruses as MERS vaccine candidates. Moreover, it was reported that high titers of specific antibodies with neutralizing activity can be generated in mice through vaccination with nanoparticles expressing the full-length MERS-CoV S protein, suggesting the possibility of developing nanoparticle-based MERS vaccines (Coleman et al., 2014a).

In addition to the aforementioned vaccine types, epitope-based and subunit vaccines also show promise against MERS-CoV infection or are under investigation for their efficacy. For example, recent studies in sequence analysis and computational prediction have identified an immunogenic and conserved epitope, WDYPKCDRA, in the RNA-directed RNA polymerase protein of human coronaviruses, supporting the concept of designing and