Taylor & Francis
Taylor & Francis Group

REVIEW

Vaccines for the prevention against the threat of MERS-CoV

Lanying Du[a], Wanbo Tai[a,b], Yusen Zhou[b] and Shibo Jiang[a,c]

[a]Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY, USA; [b]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, China; [c]Key Laboratory of Medical Molecular Virology of Ministries of Education and Health, School of Basic Medical Sciences, Fudan University, Shanghai, China

ABSTRACT

First identified in 2012, Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) is listed as a new Category C Priority Pathogen. While the high mortality of MERS-CoV infection is further intensified by potential human-to-human transmissibility, no MERS vaccines are available for human use. This review explains immune responses resulting from MERS-CoV infection, describes MERS vaccine criteria, and presents available small animal models to evaluate the efficacy of MERS vaccines. Current advances in vaccine development are summarized, focusing on specific applications and limitations of each vaccine category. Taken together, this review provides valuable guidelines toward the development of an effective and safe MERS vaccine. This article is written for a Special Focus Issue of *Expert Review of Vaccines* on 'Vaccines for Biodefence'.

Introduction

Since first emerging in Saudi Arabia in June 2012, cases of Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) infection have been reported from 26 countries. Saudi Arabia has the largest number of MERS cases, followed by South Korea. As of 2 February 2016, 1638 MERS cases, including 587 deaths (case fatality rate: ~36%), have been reported to the WHO [1–3]. Cases of MERS kept increasing in Saudi Arabia and reached at 1297 as of 24 February 2016 [4].

Similar to other coronaviruses (CoVs), including severe acute respiratory syndrome coronavirus (SARS-CoV) [5] and porcine epidemic diarrhea virus [6], MERS-CoV is a zoonotic virus and originates from bats, suggesting that bats are the most likely natural reservoir of MERS-CoV [7–10]. However, unlike SARS-CoV, which utilizes small animals, such as palm civets and raccoon dogs, as its intermediate hosts [11,12], MERS-CoV depends on dromedary camels [13–17]. Nevertheless, human-to-human transmission of MERS-CoV does occur, and unprepared health-care facilities could become a major source for human infection and transmission (Figure 1), as shown by the incidence of MERS-CoV cases in South Korea in 2015 [1,18–21]. Several family clusters infected with MERS-CoV have also been revealed [22–25]. Recently, MERS-CoV has been added to the NIAID list as a Category C Priority Pathogen [26]. This places it in the same category as SARS-CoV and, as such, it has the potential to be used as a biological weapon. Therefore, steps toward prevention strategies need to be taken, particularly the development of effective and safe vaccines.

CoV genera are classified as α, β, γ, and δ (Figure 2) [2,27–31]. The δ CoV, a new genus in the Coronaviridae family, usually infects birds, including HKU16, HKU17, and HKU21, or mammals, such as porcine deltacoronavirus HKU15 and new strains recently identified in United States, which causes porcine diarrhea [28,31–34]. Several α and β CoVs can infect humans, but only MERS-CoV and SARS-CoV have led to regional or global human outbreaks [1,2,20,35–40]. Although MERS-CoV and SARS-CoV are β CoVs, it is interesting to note that MERS-CoV is phylogenetically related to bat-CoVs HKU4 and HKU5 (Figure 2) [2,41–43]. Unlike HKU5, HKU4 can bind to bat and human dipeptidyl peptidase 4 (DPP4), the receptor of MERS-CoV, to directly infect bat cells, or indirectly infect a number of human cells by exogenous stimulation [8,44,45]. Two mutations, S746R and N762A, in the surface spike (S) protein may be responsible for transmitting MERS-CoV from bats to humans (Figure 1), thus explaining the origins of MERS-CoV in bats [7,9,44].

Similar to other CoVs, the MERS-CoV genome is a single positive-stranded RNA. It contains two large replicase open reading frames (ORFs), ORF1a and ORF1b, encoding two proteases, a papain-like protease and 3C-like protease, which are conserved in all other CoVs [2,43,46]. The downstream ORF2, ORF6, ORF7, and ORF8 of MERS-CoV genome are believed to encode S, envelope (E), membrane (M), and nucleocapsid (N) structural proteins, respectively, all having different functions (Figure 3A,B) [2,43]. MERS-CoV E protein, for example, promotes virulence [46,47]. The surface S protein is composed of S1 and S2 subunits, respectively, responsible for cellular receptor DPP4 binding via the receptor-binding domain (RBD), and fusion of virus and cell membranes, thereby mediating the entry of MERS-CoV into target cells (Figure 3C) [45,48–50]. The MERS-CoV RBD consists of a core structure, which is homologous to that of the SARS-CoV S protein RBD, and a receptor-binding motif, which is unique to MERS-CoV, thus determining viral pathogenesis and receptor recognition
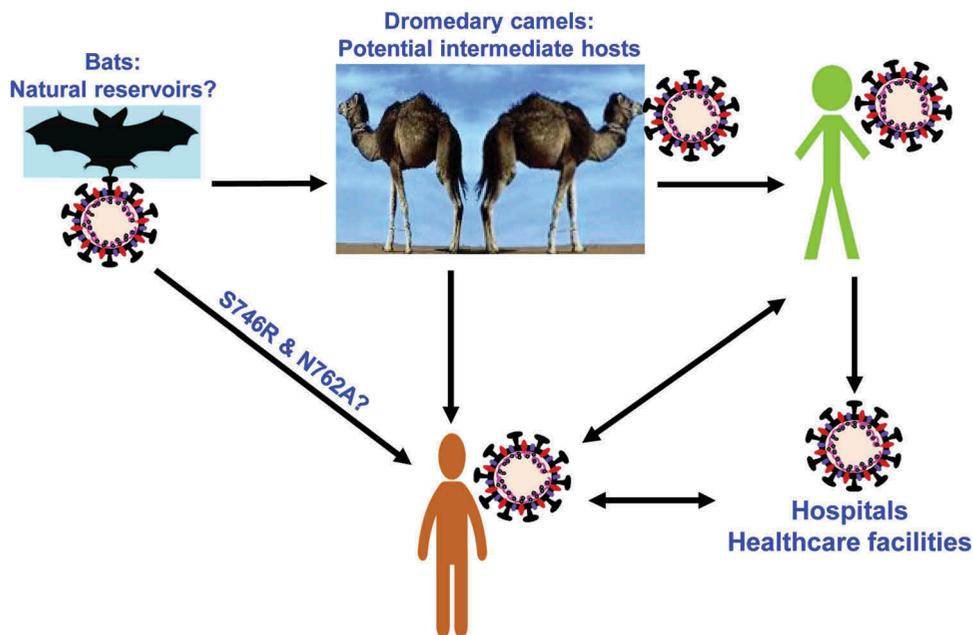
Figure 1. Potential MERS-CoV transmission routes and MERS-CoV-infection hosts.
Bats are the most likely natural reservoir of MERS-CoV, and dromedary camels are potential intermediate hosts. Human-to-human transmission of MERS-CoV may easily occur through healthcare facilities or within family clusters.
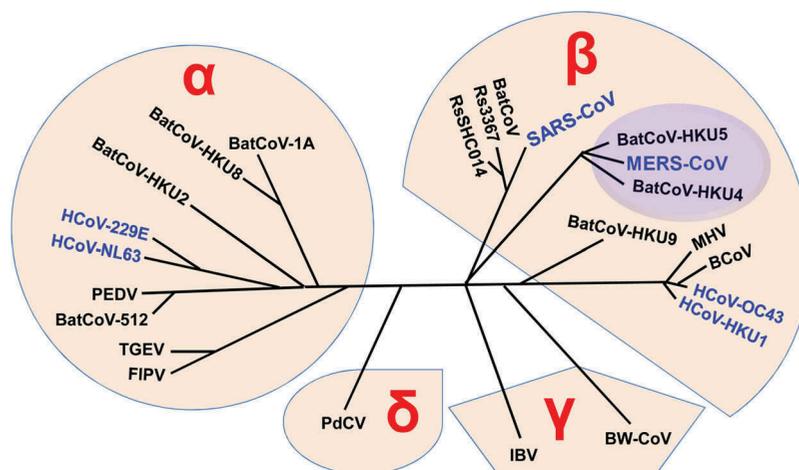

Figure 2. Classification of coronavirus genera.
The four coronavirus genera are α, β, γ, and δ coronaviruses. Each coronavirus genus contains different subclasses. Letters in blue indicate coronaviruses that have caused human infection.

[51–54]. In addition to the aforementioned major structural proteins, the MERS-CoV genome also encodes several accessory proteins, including 3, 4a, 4b, and 5, which, however, might not be essential for virus replication [27,43,47]. Recent studies have revealed that whole-genome consensus sequences of MERS-CoV from dromedary camels and humans are identical, further confirming MERS-CoV transmission from dromedary camels to humans [15,55].

MERS-CoV infection and resulting immune responses

MERS-CoV infection may trigger antigen-specific humoral immune responses and neutralizing antibodies in camels and humans [56–59]. MERS-CoV- or S-specific antibodies, including those with neutralizing activity, were identified in dromedary camels from MERS-affected regions, including Saudi Arabia, Jordan, Qatar and, the United Arab Emirates [13,55,56,60]. In addition, the seroprevalence of MERS-CoV-specific antibodies was shown to be significantly higher in individuals exposed to camels than that found among the general population [14]. Studies on 37 MERS-CoV-infected adult patients indicated that all survivals had serum IgG and neutralizing antibodies, and the levels of such antibodies were weakly but inversely correlated with viral loads in the lower respiratory tracts [61]. In South Korea, MERS-CoV-infected humans also demonstrated a clear kinetics of serologic responses, including robust antibody responses developed at the early stage of the disease onset, but delayed antibody responses with neutralizing activity associated with later, more severe stages of the disease [57]. The above studies suggest that humoral immune responses,
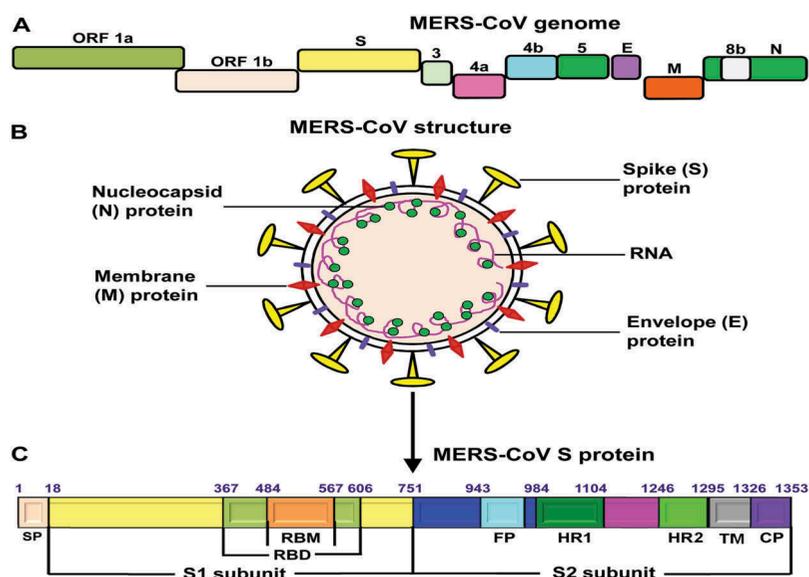
Figure 3. MERS-CoV genome and schematic structure of viral proteins.
(A) The MERS-CoV genome consists of 2 partially overlapping replicase open reading frames (OFR1a and 1b) and several downstream ORFs that encode viral functional structural proteins and other proteins with unknown function. (B) Schematic structure of major MERS-CoV structural proteins. (C) Schematic structure of MERS-CoV S protein. SP, signal peptide; RBD, receptor-binding domain; RBM, receptor-binding motif; FP, fusion peptide; HR1 and HR2, heptad repeat 1 and 2; TM, transmembrane domain; CP, cytoplasmic tail.

including neutralizing antibodies, play an important role in preventing MERS-CoV infection.

In addition to B-cell-mediated antibody responses, cellular immune responses mediated by specific T cells may play a supplementary role. The absence of IFNα in a patient who died from MERS-CoV infection could have impaired the production of antiviral adaptive IL-12- and IFN-γ-mediated Th1 immune responses, suggesting that IFNα might be important in the induction of robust cellular immune responses during the initial stage of disease progression [62]. Moreover, MERS-CoV infection may drive IL-17 production in humans, as well as the expression of cytokines and chemokines, including IL-12, IFN-γ, IP-10, and RANTES, in dendritic cells [63], effectively modulating innate immune responses.

Vaccine-induced immune responses and criteria for evaluating MERS vaccines

MERS vaccines can also induce humoral and cellular immune responses. Specifically, a good MERS vaccine should be able to induce strong humoral immune responses, particularly neutralizing antibodies, in vaccinated animals and humans, completely protecting immunized subjects from MERS-CoV challenge. Depending on the immunization routes, MERS vaccination may activate B cells to produce systemic IgG and/or secretory IgA (sIgA) antibodies, both of which can bind to the virus and, respectively, mediate systemic and mucosal immune responses [64–66]. Serum IgA could also be induced upon vaccination, particularly through the mucosal or intranasal (i.n.) route [65]. Antibodies with neutralizing activity can then neutralize MERS-CoV infection by blocking virus–target cell binding via cellular receptor DPP4, thus inhibiting virus entry (Figure 4) [67,68]. It is likely that some B cells will become antigen-specific memory B cells capable of activation

by further boost immunization or other stimulation factors to induce rapid recall antibody responses [69], but this outcome has not been extensively studied in MERS-CoV-directed vaccines.

Immunization of MERS vaccines could induce antigen-specific T-cell immune responses as well. As such, CD4+ T cells can be activated to secrete Th1, including IL-2, IFN-γ, and TNFα, and/or Th2, such as IL-4, IL-5, and IL-10, cytokines, in turn promoting cytotoxic T cells, such as T lymphocytes or CD8+ T cells, to kill target cells infected with MERS-CoV (Figure 4) [65,70–72]. However, in animal models, neutralizing antibody alone was able to protect against challenge from MERS-CoV [64,73]; therefore, T-cell-mediated cellular immune responses, if needed, may play a supplementary role in preventing MERS-CoV infection [74,75].

An ideal MERS vaccine candidate should have high immunogenicity and strong potency, as judged by the ability to induce potent immune responses and neutralizing antibodies, as well as complete protection against MERS-CoV infection, with the lowest dosage and least injection time through an appropriate route. In addition, MERS vaccines need to maintain good safety without inducing virus-enhancing antibody or harmful immune responses, or causing immunopathological effects [27,75].

Current animal models for evaluating the *in vivo* efficacy of MERS vaccines

MERS vaccines need to be evaluated in appropriate animal models before proceeding to human clinical trials. Substantial progress has been made in the development of MERS animal models, including non-human primates (NHPs), such as rhesus macaques and common marmosets [76–79], as well as small animal models, such as hDPP4-transduced and transgenic (Tg)
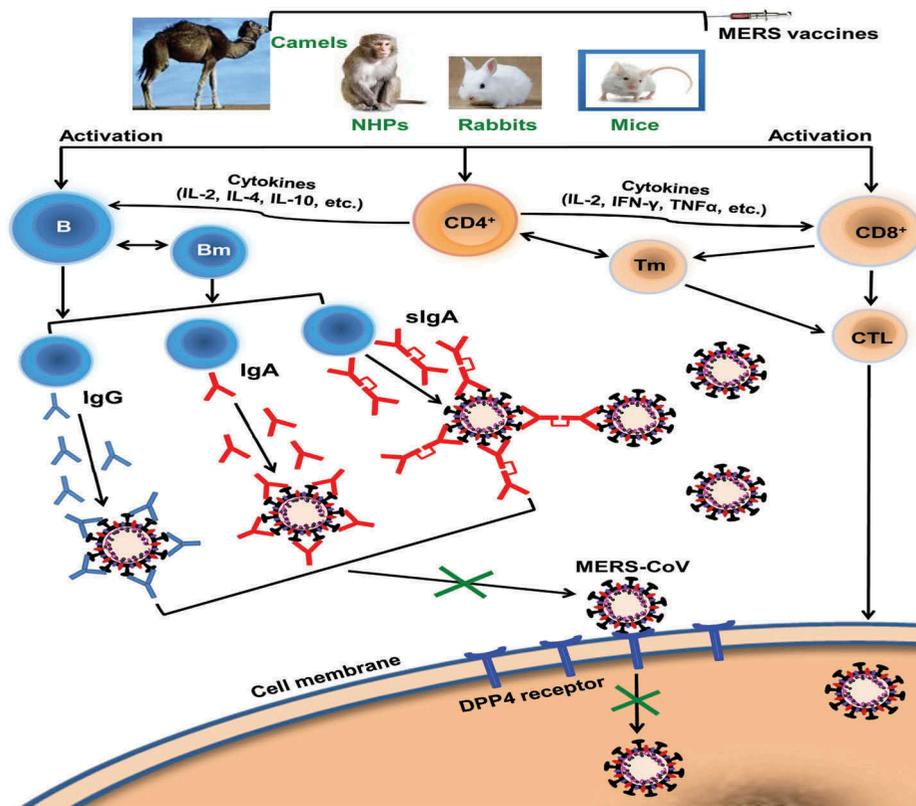
Figure 4. Schematic diagram of MERS vaccine-induced immune responses and neutralization.
Immunization of MERS vaccines may activate naïve B cells to differentiate into plasma cells and produce serum IgG, IgA, and/or secretory immunoglobulin A (sIgA) antibodies to bind MERS-CoV. Antibodies with neutralizing activity will block binding between MERS-CoV and its receptor dipeptidyl peptidase-4 (DPP4) at the cell surface, thus inhibiting virus entry into target cells. Naïve CD4+ and CD8+ T cells can also be activated to produce cytokines and/or function as cytotoxic T lymphocytes (CTLs) to destroy MERS-CoV-infected target cells. Some memory B (Bm) and T (Tm) cells may be activated after further stimulation or boost vaccination, and play a role in humoral and cellular immune responses.

mice [73,80–83]. Table 1 summarizes the currently available animal models, their characteristics, and potential applications for evaluating the efficacy of MERS vaccines.

NHP models have been initially established as an effective vehicle for MERS-CoV infection and vaccine evaluation. Rhesus macaques infected with MERS-CoV developed lower respiratory tract symptoms with mild-to-moderate interstitial pneumonia, and virus replication mainly occurred in alveolar pneumocytes. Also, clinical signs of disease and neutralizing antibodies were produced in these animals upon virus infection [77,78]. In contrast, marmosets, as a new MERS-CoV infection model, developed a much more severe disease with progressive severe pneumonia, leading to significant viral replication in the lungs and partial lethality [79]. However, other reports demonstrated a mild-to-moderate nonlethal respiratory disease in common marmosets with limited additional clinical signs upon inoculation with MERS-CoV [87]. Except for NHPs, camels infected with MERS-CoV may present upper respiratory tract symptoms with virus replication and shedding in the upper respiratory tract of inoculated dromedary camels [16].

Unlike SARS-CoV, which easily infects commonly used laboratory animals, including Syrian hamsters, ferrets, and mice [88–91], MERS-CoV does not normally infect these animal species because of the differences in binding viral receptor [92–95]. Recently, a number of small animal models were developed for MERS-CoV [73,80–83]. For example, after prior transduction with adenovirus 5 expressing human DPP4 (Ad5-hDPP4), mice became sensitive to MERS-CoV infection and developed pneumonia accompanied by clinical disease and histopathological changes in the lungs [83]. In addition, a humanized (HuDPP4) mouse model was established, in which mouse DPP4 was replaced by human DPP4 [86]. In particular, a human DPP4 transgenic (hDPP4-Tg) mouse model globally expresses the hDPP4 receptor, and it is fully permissive to MERS-CoV infection [73,81]. Infected animals developed progressive pneumonia and demonstrated significant weight loss and death upon virus infection. Virus replication was detected in lung and brain [80–82]. Thus, such small animal models provide an economical, readily available method of testing the efficacy of MERS-CoV candidate vaccines.

Current advances in MERS vaccine development

No vaccines against MERS-CoV are currently available for human use. Nevertheless, progress has been made since the emergence of MERS-CoV in 2012, and a number of MERS vaccines have been developed and tested in preclinical stages [64,66,70,72,84,96,97], two of which are scheduled for human clinical trials [98,99]. These vaccines are based on recombinant

Table 1. Current animal models being developed to evaluate the efficacy of MERS vaccines[a].

Animal models	Symptoms	Characteristics	Vaccines tested	Refs.
Rhesus macaques	Animals developed lower respiratory tract infection; MERS-CoV caused mild-to-marked interstitial pneumonia, with virus replication in alveolar pneumocytes; presented clinical signs; produced neutralizing antibodies	Lacked severe disease; MERS-CoV tropism limited to lower respiratory tract	DNA, DNA/protein, subunit vaccines	[70,77,78,84]
Common marmosets	Animals developed severe pneumonia; extensive lesions and high viral loads in lungs detected	Severe, partially lethal, disease model	Not identified	[79]
Camels	Animals developed upper respiratory tract infection with virus replication and clinical signs	MERS-CoV tropism extended to upper respiratory tract	DNA, viral vector vaccines	[16,64,70]
Rabbits	Infectious virus detected in lungs and upper respiratory tract	No significant histopathological changes or clinical signs of disease	Not identified	[85]
Ad5-hDPP4 mice	Animals developed pneumonia; showed clinical disease and histopathological changes in lungs	Transient expression; no mortality	Viral vector, subunit vaccines	[72,83]
Humanized (HuDPP4) mice	Expressed HuDPP4 in lungs; virus replication and pathology detected in lungs	No clinical signs of disease or mortality	Not identified	[86]
hDPP4-Tg mice	Animals developed progressive pneumonia; viral replication detected in lung and brain; produced neutralizing antibodies	Lethal disease model	Subunit vaccines; VRP vaccines	[73,80–82]

[a]Ad5-hDPP4: Adenovirus 5-human DPP4-transduced mice; hDPP4: human dipeptidyl peptidase-4; hDPP4-Tg mice: human DPP4-transgenic mice; MERS: Middle East respiratory syndrome; MERS-CoV: Middle East respiratory syndrome coronavirus; VRP: virus replicon particle.

virus; viral vectors, including modified vaccinia virus Ankara (MVA), adenovirus (Ad), and measles virus (MV); nanoparticles; DNA and DNA/protein; as well as subunit vaccines. Table 2 summarizes current MERS vaccines under development.

Recombinant MERS-CoV as vaccines

Unlike SARS vaccines that are usually developed based on attenuated or inactivated SARS-CoV, thus having a potential to recover virulence [11,105–108], MERS-CoV vaccines could be constructed based on recombinant viruses using reverse genetics. Accordingly, a recombinant MERS-CoV with expected marker mutations was generated using a panel of contiguous cDNAs spanning the entire viral genome and replicated to high titers with broad tissue tropism. Also, an engineered mutant MERS-CoV lacking the structural E protein was rescued and propagated in cells expressing the viral E protein in *trans* [46,47]. Using reverse genetics, it is possible to develop a replication-competent, propagation-defective MERS-CoV candidate vaccine, providing a platform for the design of live attenuated MERS-CoV vaccines. Since such recombinant MERS viruses still contain major virus components, their safety needs to be tested extensively, and their immunogenicity requires further evaluation in appropriate animal models.

Viral-vector-based MERS vaccines

Similar to viral vector-based SARS vaccines [109–111], MERS vaccines can also be constructed using viral vectors that express major MERS-CoV proteins, normally the S protein. Several such MERS vaccine candidates have been developed and/or tested for efficacy in hDPP4-expressing mouse models or camels [64,97,100,101].

Ad5 or Ad41 vector expressing full-length S or S1 protein of MERS-CoV-induced S-specific antibody responses and/or T-cell responses in a mouse model via the intramuscular (i.m.) or intragastric route, effectively neutralizing MERS-CoV infection *in vitro* [97,102]. Also, i.m. or subcutaneous (s.c.) vaccination of mice with a MVA-based full-length S vaccine elicited MERS-CoV-specific $CD8^+$ T-cell response and neutralizing antibodies, protecting hDPP4-transduced mice against MERS-CoV challenge [100,101]. Intranasally or intramuscularly administered MVA-S vaccine induced mucosal immunity, particularly the neutralizing antibodies, in dromedary camels, resulting in significant reduction of excreted infectious virus and viral RNA transcripts after MERS-CoV challenge [64]. Similarly, a recombinant MV-based MERS vaccine expressing full-length, or truncated, S protein of MERS-CoV induced robust MERS-CoV neutralizing antibodies and T-cell responses, protecting mice transduced with hDPP4 from MERS-CoV challenge [72].

Although able to elicit strong immune responses and/or protection, viral-vector-based vaccines might have some unwanted limitations in terms of safety and potency. For example, preexisting immunity to Ad in the general human population may cause some adverse effects by the induction of vaccine antigen-specific immune responses, thus reducing the overall efficacy of this vaccine type [112–114]. In addition, production of neutralizing antibodies against viral vectors themselves has been demonstrated in MV-S- and MVA-S-

Table 2. Summary of current vaccines being developed to prevent MERS-CoV infection[a].

Vaccine categories	Immunogenicity and protection	Immunization routes	Adjuvant needed	Potential limitations	Refs.
Recombinant MERS-CoV vaccines	Not indicated	N/A	N/A	Possibility of recovering virulence	[46,47]
Viral-vector-based vaccines	Induced antigen-specific humoral (IgG) and/or T-cell immune responses, and neutralizing antibody in mice; protected hDPP4-transduced mice against MERS-CoV challenge; reduced MERS-CoV excretion after virus infection in dromedary camels	i.g., i.m., s.c., or i.n.	No	Preexisting immunity; antivector responses; potential harmful immune responses by non-neutralizing epitopes of full-length S	[64,72,97,100–102]
Nanoparticles	Induced MERS-CoV neutralizing antibody in mice in the presence of adjuvants, particularly Matrix M1	i.m.	Yes: Alum, Matrix M1	Potential harmful immune responses by non-neutralizing epitopes of full-length S	[96]
DNA vaccines	Induced antigen-specific neutralizing antibody and cellular immunity in mice, NHPs and camels; protected NHPs from MERS-CoV challenge	i.m./AP system	No	Potential side effects; harmful immune responses by non-neutralizing epitopes of full-length S	[70]
DNA prime/protein-boost vaccines	Induced robust serum-neutralizing antibody in mice and NHPs; protected NHPs from MERS-CoV challenge	i.m./AP system	Yes: Ribi, Alum, AlPO$_4$	Potential harmful immune responses by non-neutralizing epitopes of full-length S	[84]
Subunit vaccines	Induced strong humoral and mucosal immune responses and potent neutralizing antibody in mice and/or rabbits; elicited T-cell responses in mice; protected hDPP4-mice and NHPs from MERS-CoV challenge	i.m., s.c., or i.n.	Yes: Alum, MF59, Montanide, Poly(I:C)	Need to maintain suitable protein conformation; require appropriate adjuvant, route, or dose	[65,66,71,73,74,103,104]

[a]Alum: aluminum hydroxide; AlPO$_4$: aluminum phosphate; AP system: AgilePulse® *in vivo* electroporation; hDPP4: human dipeptidyl peptidase-4; i.g.: intragastric; i.m.: intramuscular; i.n.: intranasal; s.c.: subcutaneous; MERS-CoV: Middle East respiratory syndrome coronavirus; S: spike.

based MERS vaccines [64,72]. Furthermore, full-length S protein of SARS-CoV encoded by the vectors can also induce non-neutralizing antibodies that may mediate enhancement of virus infection or cause harmful immune responses, such as inflammation and enhanced hepatitis [115–117], special attention should be drawn when developing MERS-CoV full-length S protein-based viral vectored vaccines.

Nanoparticle-based MERS vaccines

Nanoparticles can be used as a delivery vehicle to develop MERS vaccines. Nanoparticles containing MERS-CoV full-length S protein can be prepared and purified from pellets of infected baculovirus insect cells. In the absence of adjuvants, these nanoparticles induced a lower level of MERS-CoV neutralizing antibodies in mice, while in the presence of adjuvants, such as aluminum hydroxide (Alum) or Matrix M1, such neutralizing antibodies were significantly increased and maintained. Also, Matrix M1 significantly promoted the production of neutralizing antibodies as compared with Alum [96]. Thus, adjuvants are required for MERS nanoparticle vaccines, and different adjuvants function differently in promoting the immunogenicity of these vaccines. Thus far, efficacy and protection have not been evaluated for this vaccine type in MERS-CoV challenge models.

DNA-based MERS vaccines

Like the full-length S gene of SARS-CoV, DNA encoding full-length S protein of MERS-CoV can also be utilized to develop MERS vaccines [70,118]. Indeed, i.m./electroporation of mice

and rhesus macaques with a synthetic DNA encoding full-length S protein of MERS-CoV elicited potent virus-neutralizing antibodies and cellular immune responses, as represented by the secretion of INF-γ, TNF-α, and/or IL-2 cytokines in CD4^{+} and/or CD8^{+} T cells, as well as the production of neutralizing antibodies in immunized camels. In addition, immunized NHPs were protected against MERS-CoV challenge without demonstrating clinical or radiographic signs of pneumonia [70]. Since such DNA vaccines encode MERS-CoV full-length S protein, the potential induction of virus-enhancing antibody and harmful immune responses is possible.

DNA prime/protein boosted MERS vaccines

In addition to a DNA-alone vaccination strategy, DNA priming followed by protein boosting could be used to develop MERS vaccines and, as a result, expand the immunogenicity and efficacy generated by DNA. In this combinational vaccination strategy, DNA was constructed to encode full-length S protein of MERS-CoV, while protein was expressed as the viral S1 subunit [84]. Results demonstrated that i.m./electroporation priming of full-length S DNA and i.m. boosting of S1 protein of MERS-CoV with Ribi or Alum (aluminum phosphate, AlPO$_4$) adjuvant in mice and rhesus macaques, respectively, induced robust neutralizing antibodies against MERS-CoV infection, conferring protection of NHPs against MERS-CoV-induced radiographic pneumonia. However, because of the containment of full-length S DNA in the vaccination regimen, the potential of vaccine-caused immunopathology needs to be investigated.

Subunit MERS vaccines

Protein-based subunit vaccines against MERS-CoV have been developed [66,67,71,103]. While some subunit vaccines are designed on the basis of the full-length S1 protein [84], the majority of them are based on viral RBD [66,67,71,103,119]. These RBD-based vaccines are evaluated for immunogenicity and protective immunity in a number of MERS-CoV animal models, including hDPP4-transduced and hDPP4-Tg mice, as well as NHPs [71,73,74,103,119,120]. The antigenicity and functionality of these RBD proteins have also been extensively investigated.

In general, subunit vaccines might not induce immune responses as strong as those induced by other vaccine types mentioned above. However, the immunogenicity of subunit vaccines could be significantly promoted in the presence of an ideal adjuvant via an appropriate route [65,74]. In addition, it is also essential to maintain a suitable conformation of the protein antigen in the vaccine, such as the MERS-CoV RBD proteins [66,67]. For example, both s.c. and i.n. immunization of MERS-CoV RBD protein adjuvanted with Montanide ISA51 or Poly(I:C) induced long-term, high titers of S-specific systemic IgG, IgA, and mucosal sIgA antibodies, potently neutralizing MERS-CoV infection [65]. After comparing several different RBD fragments of MERS-CoV S protein, a fragment containing residues 377–588 of RBD elicited the highest neutralizing antibody in mice and rabbits and was therefore identified as a critical neutralizing domain [66,68]. Moreover, since MF59 adjuvant improved the ability of RBD protein to elicit the highest titer of neutralizing antibodies of all adjuvants tested, it is considered an ideal adjuvant to use with RBD subunit vaccines [74]. Even low doses of the RBD antigen plus MF59 adjuvant elicited sufficient neutralizing antibodies against MERS-CoV infection [104]. In the presence of MF59 adjuvant, this RBD protein protected Ad5-hDPP4-transduced and hDPP4-Tg mice from MERS-CoV challenge [73,74]. Clearly, the identified critical neutralizing domain of MERS-CoV RBD protein maintained good conformational structure, strong antigenicity to bind specifically to MERS-CoV RBD-specific sera and neutralizing monoclonal antibodies, as well as intact functionality to interact with soluble and cell-associated hDPP4 receptors [66,68,121].

In terms of safety consideration, subunit vaccines should be accounted as the safest vaccine type. They do not contain viral genetic materials, but only include essential antigens for eliciting protective immune responses, thus excluding the possibility of recovering virulence or inducing adverse reactions [122–126]. Different from the vaccines based on the full-length S or S1 protein, RBD-based MERS subunit vaccines contain the major neutralizing epitopes and lack non-neutralizing immunodominant domains, thus having minimum risk to induce non-neutralizing antibodies with potential to cause harmful immune responses or enhancement of virus infection [27,66,75].

Summary and conclusions

MERS-CoV, a newly emerging infectious CoV and a new Category C Priority Pathogen, has caused high mortality in humans, thus posing continual threats to public health and global safety. Since the emergence of MERS-CoV in 2012, tremendous progress has been made in the development of MERS vaccines and the evaluation of their efficacy in suitable animal models. Presently, no MERS vaccines are available for human use. This review explains immune responses resulting from MERS-CoV infection, describes MERS vaccine criteria, and presents available small animal models to evaluate the efficacy of MERS vaccines. Current advances in vaccine development are summarized, focusing on specific applications and limitations of each vaccine category. These MERS vaccines were categorized as recombinant virus, viral vectors, nanoparticles, DNAs, DNAs/proteins, and subunit vaccines, denoting specific applications and limitations of each category. Taken together, this review provides valuable guidelines toward the development of an effective and safe MERS vaccine.

Expert commentary

Several MERS candidate vaccines in development have demonstrated the ability to induce immune responses and/or neutralizing antibodies that protect against MERS-CoV infection. Based on the limitations of some of these vaccine candidates, as discussed above, it might be fruitful to establish standards against which to measure the specific role of humoral and cellular immune responses relative to protection against MERS-CoV infection, and further evaluate the efficacy and correlation between immunogenicity and protection.

In addition to efficacy, safety is an important issue for any MERS vaccine. Experience garnered from SARS vaccine studies has demonstrated that vaccines based on the full-length S protein of SARS-CoV may induce non-neutralizing antibodies with enhancing effect on virus infection or harmful immune responses, or cause immunopathological effect, such as inflammation and increased severity [115,116]. Thus, when developing MERS vaccines based on the full-length S protein, precautions should be taken against the induction of harmful immune responses and/or virus-enhancing antibodies potentially resulting from its non-neutralizing epitopes in the immunodominant domains. Concomitantly, the immunopathological effects of these MERS vaccines should be investigated. Other safety tests, such as toxicity experiments, are also recommended before moving a MERS vaccine candidate to human clinical trials or patient use.

One may argue that no virus-enhancing antibody induced by full-length S protein of MERS-CoV has been reported so far. Indeed, there had been no report on antibody-mediated enhancement of SARS-CoV infection for 8 years since the virus was first identified in Guangdong Province, China in 2003. However, Jaume et al. [117] reported in 2012 that a SARS vaccine based on the full-length S protein could induce in mice virus-neutralizing antibodies tested in Vero E6 cell culture, and virus-enhancing antibodies, via an FcγR-dependent manner, detected in cultures of THP-1, Raji, and Daudi cells that express FcγR. This finding suggests that virus-enhancing antibodies induced by the full-length S protein of MERS-CoV may be detectable if an appropriate assay system is used.

Therefore, it would be especially important to investigate the potential of these MERS vaccines to induce virus-enhancing antibodies and harmful immune responses, and to cause immunopathological effects before moving a MERS vaccine candidate into human clinical trials. A lesson should be learned from the development of SARS vaccines – a shift from developing vaccines based on the full-length S protein at the beginning to developing RBD-based vaccines at the end.

Different from the full-length S protein, RBD of MERS-CoV S protein contains a critical neutralizing domain and lacks immunodominant domains with non-neutralizing epitopes, thus is safe and highly immunogenic to induce potent neutralizing antibodies and protective immunity against MERS-CoV infection. In comparison with other vaccine categories, such as recombinant viruses and viral vectored vaccines, subunit vaccines, including those based on the RBD, maintain higher safety profile due to the absence of viral genetic materials from the infectious viruses. The major conformational neutralizing epitopes in MERS-CoV RBD may attribute to RBD's ability to induce neutralizing antibodies against both wild-type and mutant viral strains, since a viral strain with mutations in one epitope may still be sensitive to the neutralizing antibodies induced by other epitopes in RBD [75,84], demonstrating RBD's capacity to elicit broad-spectrum neutralizing antibodies and cross-protective immunity. Therefore, similar to RBD-based SARS vaccines [11,127–133], subunit vaccines based on MERS-CoV RBD have the greatest potential for further development as an effective and safe vaccine candidate. It is noted that in addition to RBD, other regions, such as N-terminal domain, in S1 fragment of MERS-CoV may also possess some neutralizing epitopes [119,134]. Thus, combining RBD and S1 N-terminal domain in a subunit vaccine may result in synergistic effect in inducing broadly cross-neutralizing antibodies against divergent MERS-CoV strains.

At present, two full-length MERS-CoV S candidate vaccines, one based on MVA and the other on DNA, have been scheduled for clinical trials [98,99]. With the continual increase and extensive research of promising MERS vaccines in preclinical studies, more and more candidates with high efficacy and strong safety should be pushed forward to clinical trials for prevention of MERS-CoV infection.

Five-year view

In the next 5 years, more robust, affordable small animal models should be developed to help evaluate the efficacy of MERS vaccines. Comprehensive studies on the efficacy and safety of MERS vaccines are expected. Since MERS-CoV RBD-based subunit vaccines induce strong immune responses and neutralizing antibodies and maintain the highest safety profile, such vaccines are expected to increase in number, and with investment from government and Big Pharma, it is further expected that such vaccines will be brought to clinical trials in an expeditious manner and, upon approval, be used for preventing MERS-CoV infection in humans and for building biodefense stockpiles.

Key issues

- Since its first identification in Saudi Arabia in 2012, MERS-CoV has infected at least 1638 persons worldwide, including 587 deaths, as of 2 February 2016, with Saudi Arabia and South Korea having the first and second largest MERS cases, respectively.

- MERS-CoV uses bats and dromedary camels as the most likely natural reservoirs and intermediate transmission hosts. Human-to-human transmission has been confirmed. Therefore, as a newly added Category C Priority Pathogen, MERS-CoV poses a threat to public health and global safety, highlighting the importance of developing effective and safe MERS vaccines.

- Among the four major structural proteins of MERS-CoV, S protein is the most important in viral pathogenesis. MERS-CoV depends on the S protein (S1 and S2 subunits) to bind the cellular receptor DPP4 through the RBD, followed by mediating MERS-CoV entry into target cells. As such, the viral S protein and RBD are major vaccine targets.

- Similar to MERS-CoV infection, MERS vaccines can trigger antigen-specific humoral, mucosal, and/or cellular immune responses. While cellular immune responses might be required to clear or kill virus, humoral immune responses, particularly neutralizing antibodies, play critical roles in protecting against MERS-CoV infection.

- Several animal models, including small animal models expressing hDPP4 receptor, have been developed to evaluate the efficacy of MERS vaccines.

- No MERS vaccines are available for human use. MERS vaccines under development are in preclinical stages, some of which are scheduled for human clinical trials. These vaccines are categorized as recombinant virus, viral vectors, nanoparticles, DNAs, DNAs/proteins, and subunit vaccines, the majority of which are based on the viral S protein.

- In addition to the major neutralizing epitopes in RBD, the full-length S protein also contains some immunodominant domains with non-neutralizing epitopes that can induce non-neutralizing antibodies, some of which may mediate enhancement of viral infection or harmful immune responses, or cause immunopathological effects, as those induced by the full-length S protein of SARS-CoV.

- The RBD in the S1 subunit of MERS-CoV S protein contains major neutralizing epitopes and lacks immunodominant domains with non-neutralizing epitopes, thus having much less risk to induce virus-enhancing antibody or harmful immune responses and better potential than full-length S protein to be developed as an effective and safe MERS vaccine.

Financial & competing interests disclosure

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Ki M. 2015 MERS outbreak in Korea: hospital-to-hospital transmission. Epidemiol Health. 2015;37:e2015033.

2. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814–1820.

   •• First report on the isolation of Middle East respiratory syndrome coronavirus (MERS-CoV) in humans.

3. WHO. Middle East respiratory syndrome coronavirus (MERS-CoV). 2016. [cited 2016 Feb 2]. Available from: http://www.who.int/emergencies/mers-cov/en/.

4. MERS-CoV daily update. 2016. [cited 2016 Feb 24]. Available from: http://www.moh.gov.sa/en/CCC/PressReleases/Pages/default.aspx

5. Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005;310(5748):676–679.

6. Huang YW, Dickerman AW, Pineyro P, et al. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. MBio. 2013;4(5):e00737–13.

7. Lu G, Wang Q, Gao GF. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends Microbiol. 2015;23(8):468–478.

8. Yang Y, Du L, Liu C, et al. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. Proc Natl Acad Sci U S A. 2014;111(34):12516–12521.

9. Yang Y, Liu C, Du L, et al. Two mutations were critical for bat-to-human transmission of Middle East respiratory syndrome coronavirus. J Virol. 2015;89(17):9119–9123.

10. Memish ZA, Mishra N, Olival KJ, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis. 2013;19(11):1819–1823.

11. Du L, He Y, Zhou Y, et al. The spike protein of SARS-CoV-a target for vaccine and therapeutic development. Nat Rev Microbiol. 2009;7(3):226–236.

12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003;302(5643):276–278.

13. Alagaili AN, Briese T, Mishra N, et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. MBio. 2014;5(2):e00884–14.

14. Muller MA, Meyer B, Corman VM, et al. Presence of Middle East respiratory syndrome coronavirus antibodies in Saudi Arabia: a nationwide, cross-sectional, serological study. Lancet Infect Dis. 2015;15(5):559–564.

15. Briese T, Mishra N, Jain K, et al. Middle East respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. MBio. 2014;5(3):e01146–14.

16. Adney DR, van Doremalen N, Brown VR, et al. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerg Infect Dis. 2014;20(12):1999–2005.

17. Sabir JS, Lam TT, Ahmed MM, et al. Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. Science. 2016;351(6268):81–84.

18. Choi JY. An outbreak of Middle East respiratory syndrome coronavirus infection in South Korea, 2015. Yonsei Med J. 2015;56(5):1174–1176.

19. Lee SS, Wong NS. Probable transmission chains of Middle East respiratory syndrome coronavirus and the multiple generations of secondary infection in South Korea. Int J Infect Dis. 2015;38:65–67.

20. Oboho IK, Tomczyk SM, Al-Asmari AM, et al. 2014 MERS-CoV outbreak in Jeddah–a link to health care facilities. N Engl J Med. 2015;372(9):846–854.

21. Assiri A, McGeer A, Perl TM, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. N Engl J Med. 2013;369(5):407–416.

22. Memish ZA, Al-Tawfiq JA, Alhakeem RF, et al. Middle East respiratory syndrome coronavirus (MERS-CoV): A cluster analysis with implications for global management of suspected cases. Travel Med Infect Dis. 2015;13(4):311–314.

23. Memish ZA, Zumla AI, Al-Hakeem RF, et al. Family cluster of Middle East respiratory syndrome coronavirus infections. N Engl J Med. 2013;368(26):2487–2494.

24. Omrani AS, Matin MA, Haddad Q, et al. A family cluster of Middle East respiratory syndrome coronavirus infections related to a likely unrecognized asymptomatic or mild case. Int J Infect Dis. 2013;17(9):e668–672.

25. Abroug F, Slim A, Ouanes-Besbes L, et al. Family cluster of Middle East respiratory syndrome coronavirus infections, Tunisia, 2013. Emerg Infect Dis. 2014;20(9):1527–1530.

26. NIAID Emerging Infectious Diseases/Pathogens. 2015. [cited 2015 Feb 25]. Available from: https://www.niaid.nih.gov/topics/biodefenserelated/biodefense/pages/cata.aspx

27. Zhang N, Jiang S, Du L. Current advancements and potential strategies in the development of MERS-CoV vaccines. Expert Rev Vaccines. 2014;13(6):761–774.

28. Woo PC, Lau SK, Lam CS, et al. Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. J Virol. 2012;86(7):3995–4008.

29. Menachery VD, Yount BL Jr, Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med. 2015;21(12):1508–1513.

30. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013;503(7477):535–538.

31. Ma Y, Zhang Y, Liang X, et al. Origin, evolution, and virulence of porcine deltacoronaviruses in the United States. MBio. 2015;6(2):e00064.

32. Wang L, Byrum B, Zhang Y. Detection and genetic characterization of deltacoronavirus in pigs, Ohio, USA, 2014. Emerg Infect Dis. 2014;20(7):1227–1230.

33. Marthaler D, Raymond L, Jiang Y, et al. Rapid detection, complete genome sequencing, and phylogenetic analysis of porcine deltacoronavirus. Emerg Infect Dis. 2014;20(8):1347–1350.

34. Hu H, Jung K, Vlasova AN, et al. Isolation and characterization of porcine deltacoronavirus from pigs with diarrhea in the United States. J Clin Microbiol. 2015;53(5):1537–1548.

35. Bonavia A, Zelus BD, Wentworth DE, et al. Identification of a receptor-binding domain of the spike glycoprotein of human coronavirus HCoV-229E. J Virol. 2003;77(4):2530–2538.

36. Hofmann H, Pyrc K, van der Hoek L, et al. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. Proc Natl Acad Sci U S A. 2005;102(22):7988–7993.

37. Lau SK, Woo PC, Yip CC, et al. Coronavirus HKU1 and other coronavirus infections in Hong Kong. J Clin Microbiol. 2006;44(6):2063–2071.

38. Zhong NS, Zheng BJ, Li YM, et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet. 2003;362(9393):1353–1358.

39. McIntosh S Assessing the South Korea MERS outbreak: could it happen elsewhere. 2015. [cited 2015 Jul 30]. Available from: http://www.medicalnewstoday.com/articles/297535.php

40. Su S, Wong G, Liu Y, et al. MERS in South Korea and China: a potential outbreak threat? Lancet. 2015;385(9985):2349–2350.

41. Chan JF, Li KS, To KK, et al. Is the discovery of the novel human betacoronavirus 2c EMC/2012 (HCoV-EMC) the beginning of another SARS-like pandemic? J Infect. 2012;65(6):477–489.

42. Chan JF, Lau SK, Woo PC. The emerging novel Middle East respiratory syndrome coronavirus: the "knowns" and "unknowns". J Formos Med Assoc. 2013;112(7):372–381.

43. van Boheemen S, de Graaf M, Lauber C, et al. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. MBio. 2012;3(6):pii: e00473–12.

44. Wang Q, Qi J, Yuan Y, et al. Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26. Cell Host Microbe. 2014;16(3):328–337.

45. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature. 2013;495(7440):251–254.

• **First report on the identification of MERS-CoV's receptor dipeptidyl peptidase 4 (DPP4).**

46. Scobey T, Yount BL, Sims AC, et al. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. Proc Natl Acad Sci U S A. 2013;110(40):16157–16162.

47. Almazán F, DeDiego ML, Sola I, et al. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. MBio. 2013;4(5): e00650–13.

48. Li F. Receptor recognition mechanisms of coronaviruses: a decade of structural studies. J Virol. 2015;89(4):1954–1964.

49. Lu L, Liu Q, Zhu Y, et al. Structure-based discovery of Middle East respiratory syndrome coronavirus fusion inhibitor. Nat Commun. 2014;5:3067.

50. Gao J, Lu G, Qi J, et al. Structure of the fusion core and inhibition of fusion by a heptad-repeat peptide derived from the S protein of MERS-CoV. J Virol. 2013;87:13134–13140.

51. Wang N, Shi X, Jiang L, et al. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell Res. 2013;23(8):986–993.

52. Chen Y, Rajashankar KR, Yang Y, et al. Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. J Virol. 2013;87(19):10777–10783.

53. Lu G, Hu Y, Wang Q, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature. 2013;500(7461):227–231.

54. Li F, Li W, Farzan M, et al. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science. 2005;309(5742):1864–1868.

55. Haagmans BL, Al Dhahiry SH, Reusken CB, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis. 2014;14(2):140–145.

56. Reusken CB, Ababneh M, Raj VS, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013. Euro Surveill. 2013;18(50):20662.

57. Park WB, Perera RA, Choe PG, et al. Kinetics of serologic responses to MERS coronavirus infection in humans, South Korea. Emerg Infect Dis. 2015;21(12):2186–2189.

58. Hemida MG, Perera RA, Al Jassim RA, et al. Seroepidemiology of Middle East respiratory syndrome (MERS) coronavirus in Saudi Arabia (1993) and Australia (2014) and characterisation of assay specificity. Euro Surveill. 2014;19(23):pii:20828.

59. Park SW, Perera RA, Choe PG, et al. Comparison of serological assays in human Middle East respiratory syndrome (MERS)-coronavirus infection. Euro Surveill. 2015;20:41.

60. Reusken CB, Haagmans BL, Muller MA, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis. 2013;13 (10):859–866.

61. Corman VM, Albarrak AM, Omrani AS, et al. Viral shedding and antibody response in 37 patients with Middle East respiratory syndrome coronavirus infection. Clin Infect Dis. 2016;62(4):477–483.

62. Faure E, Poissy J, Goffard A, et al. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside? PLoS One. 2014;9(2):e88716.

63. Chu H, Zhou J, Wong BH, et al. Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. Virology. 2014;454–455: 197–205.

64. Haagmans BL, Van Den Brand JM, Raj VS, et al. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science. 2016;351(6268):77–81.

•• **A report about the protective immunity of a viral vector modified vaccinia virus Ankara (MVA)-based, spike-protein-expressing vaccine against MERS-CoV challenge in dromedary camels.**

65. Ma C, Li Y, Wang L, et al. Intranasal vaccination with recombinant receptor-binding domain of MERS-CoV spike protein induces much stronger local mucosal immune responses than subcutaneous immunization: Implication for designing novel mucosal MERS vaccines. Vaccine. 2014;32(18):2100–2108.

66. Ma C, Wang L, Tao X, et al. Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments – the importance of immunofocusing in subunit vaccine design. Vaccine. 2014;32(46):6170–6176.

•• **A report about the identification of a critical neutralizing domain fragment in MERS-CoV receptor-binding domain (RBD) as a vaccine target.**

67. Du L, Kou Z, Ma C, et al. A truncated receptor-binding domain of MERS-CoV spike protein potently inhibits MERS-CoV infection and induces strong neutralizing antibody responses: implication for developing therapeutics and vaccines. PLoS One. 2013;8(12): e81587.

68. Zhang N, Tang J, Lu L, et al. Receptor-binding domain-based subunit vaccines against MERS-CoV. Virus Res. 2015;202:151–159.

69. Ise W. Development and function of follicular helper T cells. Biosci Biotechnol Biochem. 2015;80(1):1–6.

70. Muthumani K, Falzarano D, Reuschel EL, et al. A synthetic consensus anti-spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. Sci Transl Med. 2015;7(301):301ra132.

•• **A report about the protective efficacy of a MERS-CoV full-length spike-based DNA vaccine in NHPs.**

71. Lan J, Deng Y, Chen H, et al. Tailoring subunit vaccine immunity with adjuvant combinations and delivery routes using the Middle East respiratory coronavirus (MERS-CoV) receptor-binding domain as an antigen. PLoS One. 2014;9(11):e112602.

72. Malczyk AH, Kupke A, Prufer S, et al. A highly immunogenic and protective Middle East respiratory syndrome coronavirus vaccine based on a recombinant Measles virus vaccine platform. J Virol. 2015;89(22):11654–11667.

73. Tao X, Garron T, Agrawal AS, et al. Characterization and demonstration of value of a lethal mouse model of Middle East respiratory syndrome coronavirus infection and disease. J Virol. 2015;90(1):57–67.

74. Zhang N, Channappanavar R, Ma C, et al. Identification of an ideal adjuvant for receptor-binding domain-based subunit vaccines against Middle East respiratory syndrome coronavirus. Cell Mol Immunol. 2015. doi:10.1038/cmi.2015.03.

75. Du L, Jiang S. Middle East respiratory syndrome: current status and future prospects for vaccine development. Expert Opin Biol Ther. 2015;15(11):1647–1651.

76. van Doremalen N, Munster VJ. Animal models of Middle East respiratory syndrome coronavirus infection. Antiviral Res. 2015;122:28–38.

77. De Wit E, Rasmussen AL, Falzarano D, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. Proc Natl Acad Sci U S A. 2013;110(41):16598–16603.

78. Yao Y, Bao L, Deng W, et al. An animal model of MERS produced by infection of rhesus macaques with MERS coronavirus. J Infect Dis. 2014;209(2):236–242.

79. Falzarano D, De Wit E, Feldmann F, et al. Infection with MERS-CoV causes lethal pneumonia in the common marmoset. PLoS Pathog. 2014;10(8):e1004250.

80. Li K, Wohlford-Lenane C, Perlman S, et al. Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human dipeptidyl peptidase 4. J Infect Dis. 2016;213(5):712–722.

81. Zhao G, Jiang Y, Qiu H, et al. Multi-organ damage in human dipeptidyl peptidase 4 transgenic mice infected with Middle East respiratory syndrome-coronavirus. PLoS One. 2015;10(12): e0145561.

82. Agrawal AS, Garron T, Tao X, et al. Generation of a transgenic mouse model of middle East respiratory syndrome coronavirus infection and disease. J Virol. 2015;89(7):3659–3670.
   • **First report on the generation of a MERS-CoV transgenic mouse model.**

83. Zhao J, Li K, Wohlford-Lenane C, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc Natl Acad Sci U S A. 2014;111(13):4970–4975.

84. Wang L, Shi L, Joyce MG, et al. Evaluation of candidate vaccine approaches for MERS-CoV. Nat Commun. 2015;6:7712.
   • **A report about employing DNA priming and protein boosting strategy in developing MERS vaccines.**

85. Haagmans BL, Van Den Brand JM, Provacia LB, et al. Asymptomatic Middle East respiratory syndrome coronavirus infection in rabbits. J Virol. 2015;89(11):6131–6135.

86. Pascal KE, Coleman CM, Mujica AO, et al. Pre- and postexposure efficacy of fully human antibodies against Spike protein in a novel humanized mouse model of MERS-CoV infection. Proc Natl Acad Sci U S A. 2015;112(28):8738–8743.

87. Johnson RF, Via LE, Kumar MR, et al. Intratracheal exposure of common marmosets to MERS-CoV Jordan-n3/2012 or MERS-CoV EMC/2012 isolates does not result in lethal disease. Virology. 2015;485:422–430.

88. Roberts A, Vogel L, Guarner J, et al. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J Virol. 2005;79(1):503–511.

89. Chu YK, Ali GD, Jia F, et al. The SARS-CoV ferret model in an infection-challenge study. Virology. 2008;374(1):151–163.

90. Darnell ME, Plant EP, Watanabe H, et al. Severe acute respiratory syndrome coronavirus infection in vaccinated ferrets. J Infect Dis. 2007;196(9):1329–1338.

91. Roberts A, Paddock C, Vogel L, et al. Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans. J Virol. 2005;79(9):5833–5838.

92. De Wit E, Prescott J, Baseler L, et al. The Middle East respiratory syndrome coronavirus (MERS-CoV) does not replicate in Syrian hamsters. PLoS One. 2013;8(7):e69127.

93. van Doremalen N, Miazgowicz KL, Milne-Price S, et al. Host species restriction of Middle East respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase 4. J Virol. 2014;88 (16):9220–9232.

94. Cockrell AS, Peck KM, Yount BL, et al. Mouse dipeptidyl peptidase 4 is not a functional receptor for Middle East respiratory syndrome coronavirus infection. J Virol. 2014;88(9):5195–5199.

95. Coleman CM, Matthews KL, Goicochea L, et al. Wild-type and innate immune-deficient mice are not susceptible to the Middle East respiratory syndrome coronavirus. J Gen Virol. 2014;95(Pt 2):408–412.

96. Coleman CM, Liu YV, Mu H, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine. 2014;32(26):3169–3174.
   • **A report on the development of nanoparticle-based MERS vaccines.**

97. Kim E, Okada K, Kenniston T, et al. Immunogenicity of an adeno-viral-based Middle East respiratory syndrome coronavirus vaccine in BALB/c mice. Vaccine. 2014;32(45):5975–5982.

98. FDA approves first in-human study of MERS vaccine. 2015. [cited 2015 Nov 23]. Available from: http://vaccinenewsdaily.com/stories/ 510649306-fda-approves-first-in-human-study-of-mers-vaccine.

99. München. Drying out the reservoir. 2015. [cited 2015 Dec 28]. Available from: https://www.en.uni-muenchen.de/news/news archiv/2015/sutter_mers_camels.html

100. Song F, Fux R, Provacia LB, et al. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. J Virol. 2013;87(21):11950–11954.

101. Volz A, Kupke A, Song F, et al. Protective efficacy of recombinant modified vaccinia virus Ankara delivering Middle East respiratory syndrome coronavirus spike glycoprotein. J Virol. 2015;89 (16):8651–8656.

102. Guo X, Deng Y, Chen H, et al. Systemic and mucosal immunity in mice elicited by a single immunization with human adenovirus type 5 or 41 vector-based vaccines carrying the spike protein of Middle East respiratory syndrome coronavirus. Immunology. 2015;145(4):476–484.

103. Lan J, Yao Y, Deng Y, et al. Recombinant receptor binding domain protein induces partial protective immunity in Rhesus Macaques against Middle East respiratory syndrome coronavirus challenge. EBioMedicine. 2015;2(10):1438–1446.

104. Tang J, Zhang N, Tao X, et al. Optimization of antigen dose for a receptor-binding domain-based subunit vaccine against MERS cor-onavirus. Hum Vaccin Immunother. 2015;11(5):1244–1250.

105. Tang L, Zhu Q, Qin E, et al. Inactivated SARS-CoV vaccine prepared from whole virus induces a high level of neutralizing antibodies in BALB/c mice. DNA Cell Biol. 2004;23(6):391–394.

106. Takasuka N, Fujii H, Takahashi Y, et al. A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. Int Immunol. 2004;16(10):1423–1430.

107. Zhou J, Wang W, Zhong Q, et al. Immunogenicity, safety, and protective efficacy of an inactivated SARS-associated coronavirus vaccine in rhesus monkeys. Vaccine. 2005;23(24):3202–3209.

108. Jimenez-Guardeno JM, Regla-Nava JA, Nieto-Torres JL, et al. Identification of the mechanisms causing reversion to virulence in an attenuated SARS-CoV for the design of a genetically stable vaccine. PLoS Pathog. 2015;11(10):e1005215.

109. Faber M, Lamirande EW, Roberts A, et al. A single immunization with a rhabdovirus-based vector expressing severe acute respiratory syndrome coronavirus (SARS-CoV) S protein results in the production of high levels of SARS-CoV-neutralizing antibodies. J Gen Virol. 2005;86(Pt 5):1435–1440.

110. Liniger M, Zuniga A, Tamin A, et al. Induction of neutralising antibodies and cellular immune responses against SARS corona-virus by recombinant measles viruses. Vaccine. 2008;26(17):2164–2174.

111. Kobinger GP, Figueredo JM, Rowe T, et al. Adenovirus-based vac-cine prevents pneumonia in ferrets challenged with the SARS coronavirus and stimulates robust immune responses in macaques. Vaccine. 2007;25(28):5220–5231.

112. Pandey A, Singh N, Vemula SV, et al. Impact of preexisting adeno-virus vector immunity on immunogenicity and protection con-ferred with an adenovirus-based H5N1 influenza vaccine. PLoS One. 2012;7(3):e33428.

113. McCoy K, Tatsis N, Korioth-Schmitz B, et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. J Virol. 2007;81(12):6594–6604.

114. Haut LH, Ratcliffe S, Pinto AR, et al. Effect of preexisting immunity to adenovirus on transgene product-specific genital T cell responses on vaccination of mice with a homologous vector. J Infect Dis. 2011;203(8):1073–1081.

115. Czub M, Weingartl H, Czub S, et al. Evaluation of modified vaccinia virus Ankara based recombinant SARS vaccine in ferrets. Vaccine. 2005;23(17–18):2273–2279.

116. Weingartl H, Czub M, Czub S, et al. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. J Virol. 2004;78(22):12672–12676.

117. Jaume M, Yip MS, Kam YW, et al. SARS CoV subunit vaccine: anti-body-mediated neutralisation and enhancement. Hong Kong Med J. 2012;18(Suppl 2):31–36.

118. Yang ZY, Kong WP, Huang Y, et al. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature. 2004;428(6982):561–564.

119. Mou H, Raj VS, van Kuppeveld FJ, et al. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. J Virol. 2013;87(16):9379–9383.

120. Du L, Zhao G, Kou Z, et al. Identification of a receptor-binding domain in the S protein of the novel human coronavirus Middle East respiratory syndrome coronavirus as an essential target for vaccine development. J Virol. 2013;87(17):9939–9942.

121. Du L, Zhao G, Yang Y, et al. A conformation-dependent neutralizing monoclonal antibody specifically targeting receptor-binding domain in Middle East respiratory syndrome coronavirus spike protein. J Virol. 2014;88(12):7045–7053.

122. Chlibek R, Pauksens K, Rombo L, et al. Long-term immunogenicity and safety of an investigational herpes zoster subunit vaccine in older adults. Vaccine. 2016;34(6):863–868.

123. Berkowitz EM, Moyle G, Stellbrink HJ, et al. Safety and immunogenicity of an adjuvanted herpes zoster subunit candidate vaccine in HIV-infected adults: a phase 1/2a randomized, placebo-controlled study. J Infect Dis. 2015;211(8):1279–1287.

124. Durando P, Fenoglio D, Boschini A, et al. Safety and immunogenicity of two influenza virus subunit vaccines, with or without MF59 adjuvant, administered to human immunodeficiency virus type 1-seropositive and -seronegative adults. Clin Vaccine Immunol. 2008;15(2):253–259.

125. NIH. 2012. [cited 2012 Apr 3]. Available from: https://www.niaid.nih.gov/topics/vaccines/Pages/typesVaccines.aspx#subunit.

126. WHO. Subunit vaccines. 2015. [cited 2015]. Available from: http://vaccine-safety-training.org/subunit-vaccines.html

127. Du L, Zhao G, Li L, et al. Antigenicity and immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. Biochem Biophys Res Commun. 2009;384(4):486–490.

128. Du L, Zhao G, Chan CC, et al. Recombinant receptor-binding domain of SARS-CoV spike protein expressed in mammalian, insect and E. coli cells elicits potent neutralizing antibody and protective immunity. Virology. 2009;393(1):144–150.

129. Jiang S, Bottazzi ME, Du L, et al. Roadmap to developing a recombinant coronavirus S protein receptor-binding domain vaccine for severe acute respiratory syndrome. Expert Rev Vaccines. 2012;11 (12):1405–1413.

130. He Y, Zhu Q, Liu S, et al. Identification of a critical neutralization determinant of severe acute respiratory syndrome (SARS)-associated coronavirus: importance for designing SARS vaccines. Virology. 2005;334(1):74–82.

131. Du L, Zhao G, He Y, et al. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. Vaccine. 2007;25(15):2832–2838.

132. He Y, Li J, Li W, et al. Cross-neutralization of human and palm civet severe acute respiratory syndrome coronaviruses by antibodies targeting the receptor-binding domain of spike protein. J Immunol. 2006;176(10):6085–6092.

133. Chen WH, Du L, Chag SM, et al. Yeast-expressed recombinant protein of the receptor-binding domain in SARS-CoV spike protein with deglycosylated forms as a SARS vaccine candidate. Hum Vaccin Immunother. 2014;10(3):648–658.

134. Corti D, Zhao J, Pedotti M, et al. Prophylactic and postexposure efficacy of a potent human monoclonal antibody against MERS coronavirus. Proc Natl Acad Sci U S A. 2015;112(33):10473–10478.