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REVIEW

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Research progress and challenges to coronavirus vaccine development

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Abstract

Coronaviruses (CoVs) are nonsegmented, single-stranded, positive-sense RNA viruses highly pathogenic to humans. Some CoVs are known to cause respiratory and intestinal diseases, posing a threat to the global public health. Against this backdrop, it is of critical importance to develop safe and effective vaccines against these CoVs. This review discusses human vaccine candidates in any stage of development and explores the viral characteristics, molecular epidemiology, and immunology associated with CoV vaccine development. At present, there are many obstacles and challenges to vaccine research and development, including the lack of knowledge about virus transmission, pathogenesis, and immune response, absence of the most appropriate animal models.

KEYWORDS
animal model, coronavirus, receptor-binding domain, spike protein, vaccine

1 | INTRODUCTION

Coronaviruses (CoVs) are a large family of viruses with some causing mild to moderate illnesses like the common cold and others bringing severe diseases such as Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV). At the end of 2019, emerging infections caused by a novel CoV were reported in Wuhan, China. Most of the early reported cases came from the South China Seafood Wholesale Market in Wuhan, China, which is now closed and disinfected. The virus was identified as a new CoV and officially named by The International Committee on Taxonomy of Viruses as SARS-CoV-2 (previously provisionally named 2019 novel coronavirus or 2019-nCoV by World Health Organization [WHO]), and the disease caused by SARS-CoV-2 received its official name as COVID-19 later on February 11, 2020.[1] As of July 29, 2020, there were 16,341,920 confirmed COVID-19 cases worldwide, including 650,805 deaths. According to the WHO, 226,783 new cases and 4153 new deaths worldwide in the last 24 h.[2] Since the beginning of the new millennium, the rapid emergence and spread of CoVs have caused a grave loss of life and property. One of the most famous examples is the SARS-CoV, which first appeared during the winter of 2002 and caused a viral respiratory illness, namely the SARS.[3,4] The SARS epidemic had serious consequences in 29 regions and countries, with 8096 people being infected worldwide and the mortality rate reaching 9.6%.[5] Later in 2012, the MERS-CoV was first identified in a 60-year-old man who lived in the Kingdom of

Saudi Arabia and had acute pneumonia and subsequent renal failure.[6] From 2012 until January 15, 2020, the total number of laboratory-confirmed MERS-CoV infection cases reported globally to WHO is 2506, with 862 associated deaths.[7] In recent years, the CoV epidemics have exerted disastrous impacts on the global economy and human development.

A CoV is a positive-sense single-stranded RNA virus consisting of an enveloped virion that is around 80–120 nm in diameter. CoVs have the largest genome of all RNA viruses. There is a great deal of variation in overlapping open reading frames (ORFs) among CoVs, and the 50-terminal two-thirds of the genome contain two ORFs: ORF1a and ORF1b. The viral particles have a lipid-bilayer envelope membrane containing three glycoproteins on its surface, including the spike (S), envelope (E), and membrane (M) proteins, with the S protein as a receptor-binding, cytolytic and antigenic site, E as a smaller, envelope-bound protein, and M responsible for transmembrane transport of nutrients, budding and release of new viruses, and formation of the envelope membrane. The hemagglutinin-esterase protein is present in some CoV species.

The family *Coronaviridae* is classified into four genera (α, β, γ, and δ), and each can be further divided into multiple lineage subgroups. CoV is a common human pathogen, and 30%–60% of the Chinese population is positive for anti-CoV antibodies. As one of the main pathogenic viruses, human coronavirus (HCoV) strains HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 can cause cold like symptoms in adults and upper respiratory tract infections in children but rarely affects the lower respiratory tract. Clinical symptoms are characterized by fever, cough, shortness of breath, and development of pneumonia, which can progress to severe bilateral lung disease and respiratory failure.

Nearly 40% of the patients affected by SARS-CoV suffer from respiratory failure and require assisted ventilation. The pathologies of these patients' lungs mostly come from postmortem tissues, making it difficult to determine the time sequence of events leading to serious illness and death. Results of the studies focusing on a total of 24 human cases of CoV infections demonstrate the presence of diffuse alveolar damage with edema, apoptosis, and necrosis of the lung cells, and hyaline membrane formation, depending on the course of the disease before death.[8–10] In the case of severe acute respiratory illness caused by MERS-COV, patients largely experience such symptoms as coughing, fever, shortness of breath, diarrhea, nausea/vomiting, highly lethal pneumonia, and kidney infection in the most severe forms.

Despite the ongoing development of specific therapies or vaccines for human diseases caused by CoVs, no effective infection prevention or treatment method is currently available. In the recent CoV outbreaks, isolation remains the mainstay of virus containment. This underpins the urgent need for CoV therapies and vaccines to help control the spread of the virus from infected patients, thereby reducing the risk of any potential pandemic. The development of effective vaccine candidates depends on a more detailed understanding of the exact mechanisms of CoV transmission, pathogenesis, and immune response against CoV infection. The aim of this review is to describe current knowledge of the characteristics and immune responses to CoVs, recent advances made in developing animal models for CoVs, and the current state of CoV vaccine development. By understanding these research advances and actively facing the problems and challenges encountered, safe and effective vaccines may be achievable.

2 | CHARACTERISTICS OF CORONAVIRUS AND MAJOR STRUCTURAL PROTEINS

CoVs are large enveloped RNA viruses named for the spikes protruding from their surfaces. These active viruses express structural genes through a mechanism, specifically as a nested set of several subgenomic messenger RNAs (mRNAs), characterized by a common trailer sequence in 3′-end (3′-untranslated region [UTR]) and a conservative, capped leader sequence in 5′-end (5′-UTR). The unstructured gene is transcribed from the 5′-end into a polyprotein. Proteins are translated from five ORFs of each mRNA.[11] SARS-CoV has eight ORFs with unknown functions and four structural proteins, including envelope (E), matrix or membrane (M), spike (S), and nucleocapsid (N) proteins: the E protein plays a role in viral assembly; M is important for viral budding; N is associated with viral RNA packaging and responsible for coating viral genomic RNAs[11,12]; S is a glycoprotein that facilitates viral attachment and possible viral entry. The structure of MERS-CoV is similar to that of SARS-CoV. It mainly consists of envelope (E), matrix or membrane (m), spike (S), and Nucleocapsid (N) proteins. Particularly, the S protein serves as the main determinant of viral entry through receptor recognition and membrane fusion (Figure 1A). It is made of two subunits, with S1 containing the receptor-binding domain (RBD), and S2 providing epitopes for cross-reaction with other CoV homologous epitopes (Figure 1B).[13] In fact, the MERS-CoV S protein is capable of inducing a strong antibody response and/or cellular immune response in immunized animals, where S-specific neutralizing antibodies (NAbs) play a key role in preventing MERS-CoV infection. This article will also introduce the research progress regarding the development of MERS-CoV vaccines. The key vaccine targets in the MERS-CoV S protein are illustrated in Figure 1C.

3 | IMMUNE RESPONSES TO SARS-COV AND MERS-COV INFECTIONS

The immune pathogenesis of SARS remains unclear. SARS-CoV infection is found to be weak in human peripheral blood mononuclear cells (PBMCs). Viral replication in PBMCs appears to be self-limiting, possibly because of the presence of interferon-alpha (INF-α) in these cells.[14,15] Several studies have shown that reduced T-cell lymphocytosis and decreased CD4+ and CD8+ cell types can be observed in 94% of the patients affected by SARS-CoV. During the first 2 weeks after onset, Th1 cell-mediated immune and inflammatory responses were significantly increased by cytokines like interferons (IFNs) and

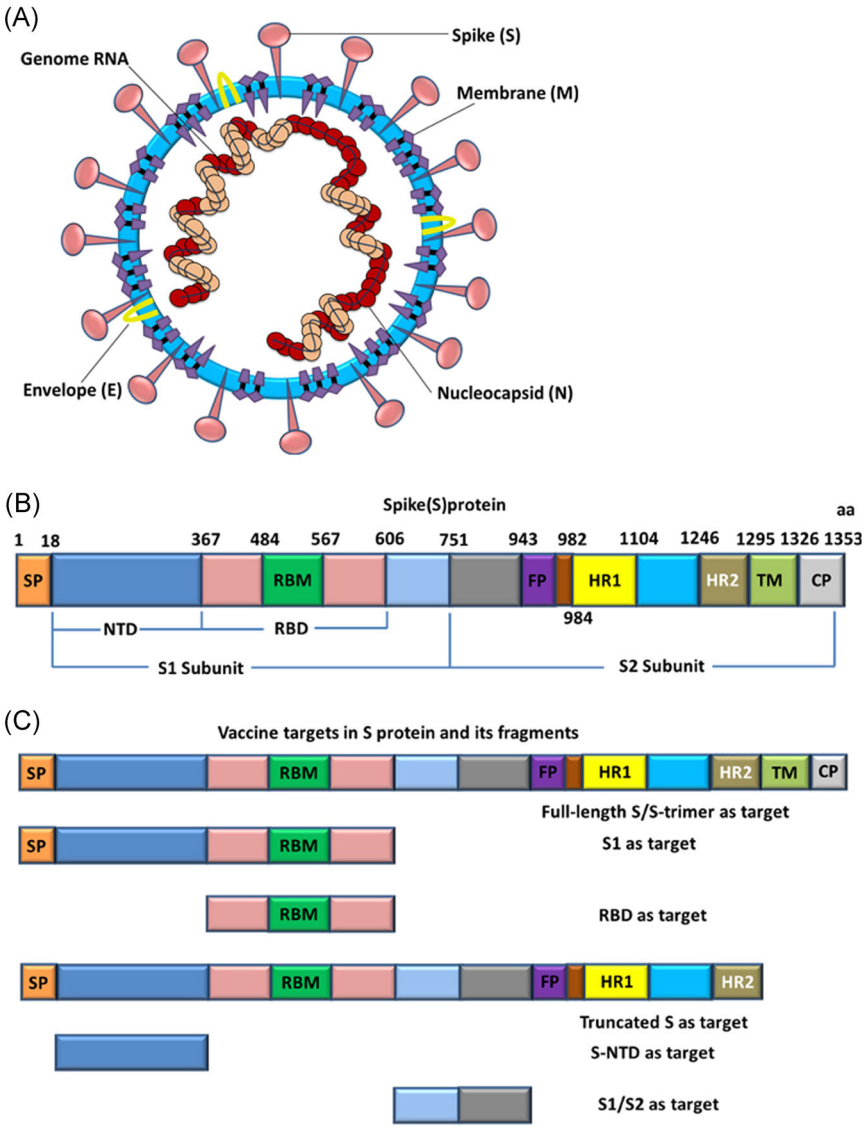

FIGURE 1 Middle East respiratory syndrome coronavirus (MERS-CoV) model and genomic composition. (A) Schematic structure of MERS-CoV virion and its major structural proteins. (B) Schematic structure of MERS-CoV S protein and its functional regions. S protein can be divided into S1 and S2 subunits. NTD, N-terminal domain. RBD, receptor-binding domain. RBM, receptor-binding motif. FP, fusion peptide. HR1 and HR2, heptad repeat 1 and 2 regions. TM, transmembrane. CP, cytoplasmic tail. (C) Key vaccine targets in MERS-CoV S protein and its fragments

neutrophil chemokines such as interleukin (IL)-8, IL-1, IL-6, and IL-12, not including TNFs, IL-2, IL-4, or IL-10. Also, accumulation of single cells/macrophages and neutrophils was detected in these patients.[11] Reghunathan et al.[16] examined PBMCs from SARS patients and healthy individuals using the DNA microarray technique and observed no significant upregulation of the MHC-1 genes or cytokines (including IFNs) in the cells, neither did they find any dramatically upregulated genes involved in complement-mediated cytolysis.[12] Most of the infected patients developed a humoral immune response to SARS-CoV, and it was detected that immunoglobulin G (IgG) and immunoglobulin M were decreased, respectively, 14 and 30 days after the onset of symptoms. The decrease in IgG antibodies lasted up to 210 days, and antiviral NAbs were present in the convalescent patients. The incidence was higher in the older age group, while children under the age of 12 did not develop the serious diseases seen in adults. Taken together these data may suggest that the quality of immune responses to SARS-CoV somewhat affects the outcome of viral infection.[11,17,18]

Li, et al.[19] noted a rapid decline of peripheral T cell subsets in the patients with acute-phase SARS-CoV infection, in spite of which the subsets were restored to the normal level during recovery. Activated alveolar macrophages may contribute to the presence of proinflammatory cytokines, suggesting that they may relate to the pathogenesis of SARS.[8] Moreover, in the postmortem lung tissue samples from the SARS patients, macrophages and epithelial multinucleated giant cells were observed in the damaged alveoli. This suggests that intercellular transmission through syncytial formation may occur in SARS-CoV-infected patients. Thus, humoral responses may be insufficient to eliminate SARS-CoV, while T cell-mediated immunity appears to play a crucial role in clearing the infection.[12,20] MERS-CoV infection may stimulate humoral immune responses, including NAb induction, as well as cellular immune responses. Studies have shown that most patients have a strong IgG response within 3 weeks of onset. In another study, 14 patients who recovered from MERS presented with enhanced S1-specific serum IgG response, indicating the possible association between early antibody responses

and a longer incubation period, as well as a lower degree of disease severity. In addition, IL-6 and CXC-10 were elevated in the patients 1–2 weeks after onset, especially in severe cases. Another study of patients infected with MERS-CoV suggested that in a deceased patient, the impaired cellular (Th1) immune response might relate to the absence of INF-α. In addition, animal studies of INF-α and MyD88 knockout mice indicated that the clearance of MERS-CoV required effective T-cell and B-cell responses.[8,13,20–25] Moreover, Zhao et al.[25] pointed out that mice with B-cell defects could clear MERS-CoV, while those lacking T-cells failed to achieve MERS-CoV clearance, indicating the key role of T-cells in clearing the virus.

4 | ANIMAL MODELS AND VACCINES FOR COV INFECTION

An appropriate animal model to replicate the pathology of human CoV infections is the premise of studying the viral pathogenesis and testing vaccine candidates. So far, many animal models have been used to study the pathogenesis of CoV-induced infections and evaluate possible treatment methods. These studies have reviewed clinical symptoms, viral replication, and pathology in humans, non-human primates (NHPs), rabbits, macaques, hamsters, ferrets, and mice. Table 1 lists the animal model studies conducted against coronavirus in recent years.

It remains a major challenge to establish an accurate, reproducible, and predictive animal model for the evaluation of CoV vaccine candidates. The existing mouse models have been widely used to study HCoV-OC43 because this human CoV can bring the lethal outcomes to mice and induce neuronal cell death associated with viral persistence in the human brain.[37] However, after the mice have been inoculated with HCoV-OC43 isolates from the respiratory tract, the viral RNA can hardly be detected in the lungs,[36,38] indicating the limited value of this model in the study of virus-induced pathology as there is no replication of the respiratory tract pathology in humans. Hamsters, ferrets, and mice cannot be naturally infected with MERS-CoV due to the low level of mRNA and protein for the MERS-CoV receptor, dipeptidyl peptidase 4 (DPP4), and this greatly hinders their use as animal models.[26,28] Interestingly, ferrets are susceptible to experimental infection with SARS-CoV, which can effectively spread between animals in a contagious way. In ferrets, SARS-CoV replicates to high levels in the lungs and causes severe, progressively worsening pneumonia that starts in the small airways.[30,31] Although ferrets best mimic human disease, there are basically no reagents available to study the immune responses of ferret hosts. And further evaluation is needed to understand the poor efficacy and immunogenicity seen in modified Ankara vaccinia (MVA)-vectored, SARS-vaccinated ferrets compared to that observed in other animals immunized with a similar vaccine.[32] However, when inoculated with high doses of SARS-CoV (Tor2 strain) into the lungs of macaque monkeys, only slight clinical signs were observed.[34] For cynomolgus monkeys, there was reportedly no significant clinical disease or rapid virus clearance.[33] All these factors limit the development of this animal model. Therefore, it is questionable whether the clinical manifestations of CoV infection in macaques and marmosets provide a sufficient basis to evaluate the pathogenesis of SARS and assess the efficacy of relevant vaccines. Animal models of MERS-CoV infection were developed using dromedary camels.[39,40] But a recent study pointed out that dromedary camels were far from an ideal animal model for the study of human MERS-CoV infection because the virus mainly infected the nasal mucosa, inducing only minor symptoms; apart from this, their size and geographic availability also limited their practical use.[35,41]

The greatest difference between SARS-CoV and MERS-CoV is that SARS-CoV can infect several strains of mice, while MERS-CoV does not. It was recently reported that wild-type and innate immune-deficient mice were not susceptible to MERS-CoV.[26] In contrast, it was found that mice were susceptible to SARS-CoV, marking a significant advance in the SARS study.[42,43] Researchers discovered SARS-CoV in the stomach, intestine, duodenum, and the respiratory tract of infected mice.[44] Hamsters are considered a good model for SARS-CoV infection because of the high level of SARS-CoV replication.[44] Roberts et al.[29] investigated the ability of SARS-CoV to infect 5-week-old Golden Syrian hamsters and discovered that when administered intranasally, the virus replicated to high titers in the lungs and nasal turbinates. Peak replication in the lower respiratory tract was noted on Day 2 postinfection (p.i.) and was cleared by Day 7 p.i. Viral replication in epithelial cells of the respiratory tract was accompanied by cellular necrosis during the early stage of infection, followed by an inflammatory response with viral clearance, focal consolidation in the pulmonary tissue, and eventual pulmonary tissue repair. Compared to the mouse models where no significant pathologies have been observed, SARS-CoV replicates to higher titers and has longer durations in the respiratory tracts of hamsters.

Another interesting finding is that immune-deficient mice are capable of clearing SARS-CoV infection.[45] These immune-deficient mice have highly inducible inflammatory cytokines, indicating that in a mouse model, no adaptive immune response or NK cell is needed for virus clearance. This also demonstrates the significant role of the innate immune response in controlling the virus, which speculatively is related to the IFN pathway. STAT1 is important to INF regulation, with STAT1-deficient mice infected with SARS-CoV providing additional evidence for the importance of innate immunity.[46] Results of a study showed that SARS-CoV replicated to high titers in the respiratory tract after BALB/c mice were administered nasally; the peak of virus replication appeared on Day 1 or 2 after injection, but the virus was cleared within a week and none of the BALB/c mice developed severe disease.[42] This is significantly different from the case of STAT1-deficient mice subject to intranasal inhalation of SARS-CoV.[47] In STAT1-deficient mice lacking IFN-α/β signaling, a higher severity of infection is observed, with bronchiolitis progressing to interstitial pneumonia and mediastinitis—a histopathological feature of those who died of atypical pneumonia.[46] The results for the Stat1$^{-/-}$ animals highlight the importance of innate immunity in

TABLE 1 Summary of published reports of experimental infection of animal models

Animal species	Virus	Immunization strategy		Clinical manifestations	Mean histological lesion	Ref.
		Route	Dosage			
C57BL/6 mice (3 weeks old	HCoV-OC43	IC	10 $TCID_{50}$	Loss of weight, apathy, ruffled fur, humped posture, and wasting	This acute infection targeted neurons, which underwent vacuolation and degeneration while infected regions presented strong microglial reactivity and inflammatory reactions.	Coleman et al.[26]
BALB/c mice (3 weeks old)			10^5 $TCID_{50}$			
BALB/c mice, WT 129S6/SvEv mice and innate immune-deficient 129/$STAT1^{-/-}$ mice	MERS-CoV	IN	1200 $TCID_{50}$	None found	Analysis of the lungs shows that in 129S6/SvEv and the innate immune-deficient 129/$STAT1^{-/-}$ mice there are only minor signs of pathological lesions or inflammatory response to the infection. In BALB/c mice infected with the high dose of MERS-CoV, no cytopathic effect nor signs of MERS-CoV infection (apoptotic cells, syncytia formation) were noted	Zhao et al.[27]
Human Dipeptidyl Peptidase 4 Transgenic Mice	MERS-CoV (HCoV-EMC/2012 strain)	IN	$10^{4.3}$ $TCID_{50}$	Significant weight loss from Day 6 after infection, and all of the infected mice died by Day 10	Presence of inflammatory tissue damage in the kidney, liver, and spleen, with mild inflammatory responses in the lungs but no significant changes in the intestines, hDPP4 transgenic mice exhibited mild inflammation in the lungs with focal exudation and hemorrhage.	Wit et al.[28]
Syrian hamsters	MERS-CoV (isolate HCoV-EMC/2012)	Aerosols	$4 \times 10^2 - 10^6$ $TCID_{50}$	None found	No histological detection	Roberts et al.[29]
Golden Syrian Hamsters	SARS-CoV (Urbani strain)	IN	10^3 $TCID_{50}$	Neither weight loss nor clinical signs of disease were observed	Nasal turbinates, trachea, and bronchi showed swelling and blebbing of the luminal cytoplasm. Small ulcers were noted in the nasal passages, and focal loss of cilia was noted in the trachea	ter Meulen et al.[30]
Ferrets	SARS-CoV (HKU-39849)	IT	10^3 $TCID_{50}$ or 10^4 $TCID_{50}$	Lethargy and mortality in ferrets	Viral replication was accompanied by multifocal pulmonary lesions affecting about 5%–10% of the surface area of the lung. Histologically lesions consisted mainly of mild alveolar damage as well as peribronchial and perivascular lymphocyte infiltration.	Martina et al.[31]

(Continues)

TABLE 1 (Continued)

Animal species	Virus	Immunization strategy		Clinical manifestations	Mean histological lesion	Ref.
		Route	Dosage			
Cat	SARS-CoV	IT	$1 \times 10^3 \pm 0.51$ $TCID_{50}$	Neither of the cats showed clinical signs of infection, but both had seroconverted by Day 28.	Cats had multifocal pulmonary consolidation.	Weingartl et al.[32]
Rhesus macaque (2–3 years old)	MERS-CoV (hCoV-EMC)	IT	6.5×10^7 $TCID_{50}$	The rectal temperature of the infected rhesus macaques increased to 40.5°C at 1–2 days postinfection, and thereafter turned to normal	MERS-CoV induces lesions that are primarily observed in the lungs, with varying degrees of inflammation, interstitial pneumonia, pulmonary oedema, hemorrhaging, degeneration and necrosis of pneumocytes and bronchial epithelial cells, and the infiltration of inflammatory cells.	McAuliffe et al.[33]
Rhesus macaque	SARS-CoV	IT	$10^{6.3}$ $TCID_{50}$	None found	None found	Rowe et al.[34]
Common marmoset (2–3 years old)	MERS-CoV (hCoV-EMC)	IT	5×10^6 $TCID_{50}$	The infected common marmosets showed manifest symptoms of viral infection, including severe respiratory symptoms, drastically water intake decrease and overt weight loss, and the maximum bodyweight loss were about 11%.	Exudative pathological changes were found, exhibiting hemorrhage, widespread pulmonary oedema, and a large number of inflammatory cells	McAuliffe et al.[33]
Cynomolgus monkeys	SARS-CoV	IT	10^7 PFU	The cyno/IT animals developed a mild cough and slightly decreased activity on Days 2 and 3 after virus challenge; these findings quickly resolved and the animals were asymptomatic until Days 8 to 10 when sneezing was noted	Gross examination of the lungs of cyno/IT animal 17087 revealed a few scattered pleural adhesions.	Adney et al.[35]
Dromedary Camels	MERS-CoV (strain HCoV-EMC/2012)	IT/IN/CJ	10^7 $TCID_{50}$	Minor clinical signs of disease, consisting of rhinorrhea and a mild elevation in body temperature	Viral antigen was detected within the epithelial cells of the nasal turbinates, larynx, trachea, bronchi, and bronchioles, but not the alveoli.	St-Jean et al.[36]

Abbreviations: CJ, conjunctival infection; HCoV, human coronavirus; IC, intracerebral; IN, intranasal inoculation; IT, intratracheal inoculation; MERS-CoV, Middle East respiratory syndrome coronavirus; PFU, plaque-forming unit; SARS-CoV, severe acute respiratory syndrome coronavirus; $TCID_{50}$, tissue culture infective dose; WT, wild type.

controlling SARS-CoV infection and suggest potential therapeutic strategies that augment the innate immune response in the context of INF action.[46]

In selecting animal models for MERS-CoV, the first consideration is transgenic mice presenting with hDPP4 expression, namely the small animal models currently available for severe or fatal MERS-CoV infection with respiratory symptoms and viremia.[47] Although MERS-CoV grows as much in the lungs as in the brains of these transgenic mice, they have been proved to be highly useful for studying the pathogenesis of MERS-CoV infection and evaluating the efficacy of MERS vaccines and therapeutic agents.[27] Another approach to establish a mouse model for MERS-CoV infection is to transduce mice with adenovirus vectors expressing the human host-cell receptor DPP4.[25] This technology has been used to prove that polyclonal IgG antibody derived from Tc cattle can prevent and alleviate virus replication.[48] Another distinct advantage of this method lies in its quick adaptation to other viruses that may appear in the future, especially in the absence of a suitable mouse model.

5 | ADVANCES IN THE DEVELOPMENT OF CORONAVIRUS VACCINES

At the time of the outbreak of SARS, various monoclonal antibodies (mAbs) against SARS-CoV came out one after another. The mAbs were used in diagnosis, clinical treatment, and basic research.[49] Although mAbs produced antiviral outcomes in MERS-CoV infection cases based on cell cultures and animal models, the treatment window for mAbs is usually narrow and large-scale production takes considerable time and resources—all this limits the large-scale application of mAbs for disease prevention in high-risk areas affected by MERS-CoV. Vaccine is still the best protection against MERS-CoV.[50]

Considering its important role in mediating virus entry and as a major target for NAbs, the S glycoprotein containing an RBD has become the main target for MERS-CoV immunogen selection and vaccine design.[50] We now know that the S protein of SARS-CoV binds to the cell receptor ACE2 that recognizes the S protein at the amino acid residues 318–510 (aa318–510),[51] and DPP4 (also known as CD26) acts as the cell receptor of MERS-CoV.[52] These regions are designated as RBDs. Therefore, the S protein, especially the RBD of the S protein, is the main target of NAb responses. At the same time, these targets are also applied to the development of SARS-CoV vaccines.[53–55] Reportedly, immunization of mice with an RBD-based vaccine by the intramuscular (i.m.) route confers long-term resistance against SARS-CoV infection.[56] In the past few years, some progress has been made in the research and development of CoV vaccines. Various methods have been applied and over 20 vaccine candidates have been reported, including vaccines based on DNA, recombinant protein subunits, recombinant viral vectors, virus-like particles (VLPs), inactivated virions, and live attenuated vaccines. Vaccine Production Platforms and Technologies are listed in Table 2.

6 | DNA VACCINES

DNA immunization has developed into a safe and stable technology for vaccination, which can prevent a range of infectious diseases.[49] It can produce stable antigen expression and induce humoral and cellular immune responses at a relatively low manufacturing cost. Yang et al.[57] showed that in a mouse model, a DNA vaccine encoding the S protein of SARS-CoV could produce T-cell and NAb responses, as well as protective immunity. These expression vectors elicit strong immune responses mediated by CD4 and CD8 cells.[58] Moreover, antibody responses in mice vaccinated with an expression vector encoding a form of S that includes its transmembrane domain elicited NAbs.[57] A study in rhesus macaques indicated that MERS-CoV DNA vaccine expressing the full-length S protein of MERS-CoV EMC/2012 administered via i.m. injection with electroporation (EP) at 0, 3 and 6 weeks induced S-specific NAb response as well as T cell responses producing IFN-g, TNF -a, and to a lesser extent, IL-2 in both low- and high- dose groups.[59] In Phase I clinical trial, the DNA vaccine against SARS-CoV was proved to be able to induce NAbs and effective T cell responses in the human body.[60]

DNA vaccines expressing the full-length S protein of MERS-CoV or small protein fragments can both effectively combat the virus. A study in rhesus monkeys showed that a DNA vaccine that expressed the full-length S protein of MERS-CoV EMC/2012 could produce S-specific NAb and T-cell responses if delivered by i.m. EP at 0, 3, and 6 weeks, while the induction of IFN-g, TNF-α, and IL-2 was reduced to a lower level in both high- and low-dose groups.[59] This also suggests that T-cell responses may play a role in the protection against MERS-CoV. On this basis, Inovio Pharmaceuticals and GeneOne Life Science Inc have conducted Phase I clinical trials of MERS-CoV DNA vaccines.[59,61]

DNA priming and protein-boosting is another strategy for the development of MERS-CoV vaccines. Immunogens based on the full-length S DNA and the S1 subunit protein exhibit strong serum neutralizing activity against several MERS-CoV strains in mice and NHPs. From a serological analysis and isolation of mouse mAbs, it was found that NAbs were produced against both RBD and non-RBD portions of S1 and S2 subunits.[62] This is the first time that researchers have demonstrated the protective immunity induced by the MERS-CoV S DNA and gene–protein combined immunization, as well as the effectiveness of any immunization regimens as protection in NHP models.

7 | RECOMBINANT PROTEIN SUBUNIT VACCINES

Recombinant full-length S proteins have been reported to be highly immunogenic and able to elicit an efficacious protective immune response.[63] Antibody responses can be enhanced using tailored subunit reconstruction with adjuvant combinations.[64,65] In the study of SARS-CoV vaccines, the S1 subunit, especially the RBD, has been identified as a primary target for NAbs in mice, NHPs, and humans.[66,67] A recombinant fusion protein (designated RBD-Fc) containing

TABLE 2 Overview of vaccine production platforms for CoV

Platform	Target	Existing, licensed human vaccines using the same platform	Advantages	Disadvantages
RNA vaccines	S protein	No	No infectious virus needs to be handled, vaccines are typically immunogenic, rapid production possible.	Safety issues with reactogenicity have been reported.
DNA vaccines	S protein	No	No infectious virus needs to be handled, easy scale-up, low production costs, high heat stability, tested in humans for SARS-CoV-1, rapid production possible.	Vaccine needs specific delivery devices to reach good immunogenicity.
Recombinant protein vaccines	S protein	Yes for baculovirus (influenza, HPV) and yeast expression (HBV, HPV)	No infectious virus needs to be handled, adjuvants can be used to increase immunogenicity.	Global production capacity might be limited. Antigen and/or epitope integrity needs to be confirmed. Yields need to be high enough.
Viral vector-based vaccines	S protein	Yes for VSV (Ervebo), but not for other viral vectored vaccines	No infectious virus needs to be handled, excellent preclinical and clinical data for many emerging viruses, including MERS-CoV	Vector immunity might negatively affect vaccine effectiveness (depending on the vector chosen).
Live attenuated vaccines	Whole virion	Yes	Straightforward process used for several licensed human vaccines, existing infrastructure can be used.	Creating infectious clones for attenuated coronavirus vaccine seeds takes time because of the large genome size. Safety testing will need to be extensive
Inactivated vaccines	Whole virion	Yes	Straightforward process used for several licensed human vaccines, existing infrastructure can be used, has been tested in humans for SARS-CoV-1, adjuvants can be used to increase immunogenicity.	Large amounts of infectious virus need to be handled (could be mitigated by using an attenuated seed virus). Antigen and/or epitope integrity needs to be confirmed.

Abbreviations: MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus.

193-amino acid RBD (residues 318–510) and a human IgG1 Fc fragment can induce highly potent antibody responses in immunized rabbits.[66] At a serum dilution of 1:10 and the antibody can recognize the RBD in the S1 region and completely inhibit SARS-CoV infection. Rabbit antiserum can effectively block the binding of the S1 RBD to ACE2.[66] Similarly, the MERS-CoV vaccines based on RBD-containing subunits can produce NAbs in some small animal models.[68,69] A recent study showed that the 377–588 residues of the MERS-CoV S-protein RBD were capable of stimulating strong humoral and cellular responses, and the stimulation could be enhanced by adding the MF59 adjuvant.[70] Apart from this, purified coronavirus spike protein nanoparticles are viewed as stable immunogens and sources of effective NAb responses to MERS-CoV.[71,72] Despite all that, more research is needed to evaluate the immunogenicity, safety, and effectiveness of nanoparticles for further development.

8 | VIRAL VECTORED VACCINES

Viral vectored vaccines are mainly based on vectors having the ability to produce strong humoral and cellular immune responses, such as the MVA virus, adenovirus, and measles virus (MV). In the case of MERS-CoV, the most promising candidate is the MVA-based, full-length S MERS-CoV vaccine (MVA-S) developed by the German Center for Infection Research.[73] MVA-S is sufficient to stimulate robust NAb responses in mice and reduce viral replication in the lower respiratory tract.[73] Meanwhile, MVA-S can induce mucosal immunity and reduce virus shedding in camels.[61] More importantly, MVA is used as a vector to express the S protein fragments of different lengths, thereby inducing immune responses to MERS-CoV in different cases.[40]

Another vaccine is based on recombinant adenovirus vectors. Researchers have demonstrated that human adenovirus Type 5 or 41 vector-based vaccines carrying the S protein of MERS-CoV can induce antigen-specific IgG and NAbs in serum.[74] Besides, it is reported that a replication-deficient chimpanzee adenovirus (ChAdOx1) containing the MERS-CoV S glycoprotein antigen, MERS001, can produce NAbs and cellular immune responses in mice.[75]

In susceptible mice, MV vectors expressing the SARS-CoV full-length S protein can stimulate the induction of NAbs with the highest titer and effectively protect animals from SARS-CoV infection.[76] The Th1-biased response induced by recombinant MV—a typical live attenuated virus—represents an ideal feature of antiviral vaccines.[76]

In addition to the main viral vectored vaccines mentioned earlier, a novel bivalent vaccine against MERS-CoV and rabies virus (RV) has been developed using the replication-incompetent P-gene-deficient RV (RVΔP).[77] This vaccine is considered a promising bivalent vaccine candidate because it can safely and effectively induce mice to produce MERS-CoV- and RV-specific NAbs.[77] A recent study showed that the recombinant vaccine using RV as a virus vector and expressing the MERS-CoV S1 (spike) protein on the RV surface could elicit a very high antibody response, and compared to granular element method-particle vectored vaccines, the cellular immune level was significantly higher.[78] These findings provide a theoretical basis for the development of MERS-CoV vaccines.

9 | VIRUS-LIKE PARTICLE VACCINES

CoV vaccines based on VLPs are safe and well-tolerated.[79] Composed of one or more protein subunits, VLPs can be easily recognized by the immune system. Moreover, the highly structured protein particles assembled by the single or multiple structural proteins of the virus retain the natural conformation of the virus antigen protein, so it has the function of stimulating the innate and adaptive immune response of the host.[79] MERS-CoV-like particles constructed by the baculovirus expression system are structurally similar to the natural virus, and the VLP vaccine can induce specific IgG antibodies targeting the MERS-CoV RBD, with the endpoint titer reaching as high as 1:1,280.[80] Chimeric virus-like particles containing the SARS-CoV S protein and the influenza matrix protein 1 are found to protect mice from SARS-CoV and provoke strong immune responses.[81] In general, these VLP vaccines need appropriate adjuvants to improve their efficacy when injected.[80–82]

10 | LIVE ATTENUATED AND INACTIVATED VACCINES

Vaccines based on chemically inactivated SARS-CoV particles have been evaluated in hamsters, mice, ferrets, and NHPs. In these animal models, NAbs provide protective immunity at varying levels.[83,84] A study reveals that SARS-CoV can induce Th2 immunopathological changes in mice, which indicates that the components of SARS-CoV can produce hypersensitivity reactions. Further studies have shown that the immunopathology leading to eosinophilia is at least partly related to viral nucleoproteins.[83] Moreover, in different animal model systems, the oligomeric immunization with the SARS-CoV S protein also presents an increase in eosinophils after a virus attack.[82] These suggest that vaccines based on inactivated SARS-CoV or MERS-CoV should be carefully evaluated for potential side effects before they are ready for public use.

Live attenuated vaccines present antigens to the immune system in a way similar to the natural infection to induce stronger immune responses and highly effective protection against viruses. A candidate strain of live attenuated MERS-CoV vaccine has been developed using a MERS-CoV strain with robust replication but reproductive defects.[85]

In conclusion, further research is needed for this kind of inactivated vaccines, especially when it comes to eosinophilia following viral challenge in different animal model systems.

11 | RNA VACCINES

mRNA vaccine has multiple advantages, such as favorable immunogenicity, short research and development cycle, and is suitable for the prevention of infectious diseases. It is considered to have

breakthrough potential by the industry. In recent years, the platform for the development of gene vaccines has been verified in terms of immunogenicity and effectiveness of vaccines.[86,87] mRNA as the technological basis of therapeutics and vaccines is characterized by great flexibility with respect to production and application. Any protein can be encoded and expressed by mRNA, in principle enabling the development of prophylactic and therapeutic vaccines fighting diseases as diverse as infections and cancer as well as protein replacement therapies. In addition, a highly efficient and nontoxic RNA vector has been developed,[88] which in some cases allows the expression of antigens to be prolonged in vivo. Some vaccine formulations contain new adjuvants, while others cause a strong response in the absence of adjuvants.

Exogenous mRNA can be recognized by many kinds of natural immune receptors on the cell surface, cell body, and cell interior, and has a natural immune-stimulating effect (Figure 2). This feature of mRNA has potential advantages for vaccination, because in some cases, it can provide an adjuvant activity to promote the maturation of dendritic cells, thus triggering strong T and B cell immune responses. It has been shown that by purifying in vitro transcribed mRNA and introducing modified nucleosides, as well as by complexing mRNA with various carrier molecules, the immunostimulatory spectrum of mRNA can be formed.[89,90]

Because of the poor stability of mRNA, it is always a concern that it is easy to be destroyed by nuclease in vivo. Therefore, there are very high technical requirements for preparation and targeted delivery. A variety of in vitro and in vivo transfectors have been developed, which can promote the uptake of mRNA by cells and protect it from degradation. Once the mRNA is transferred to the cytoplasm, the translation machine will produce a protein modified after translation, thus producing a protein with correct folding and full functions. This feature of mRNA pharmacology is particularly beneficial for vaccines and protein replacement therapies, which require cytoplasmic or transmembrane proteins to be delivered to the right cell compartment for proper presentation or function.[91]

At present, the mRNA vaccine mRNA-1273 developed by the National Institute of Allergy And Infectious Diseases of the United States and Moderna company is a new type of lipid nanoparticles wrapped mRNA encoding s protein (Clinical Trial Registration No.: nct04283461) which has entered the clinical trial.[92] However, there is no report on the effectiveness of the mRNA-1273 vaccine in animal experiments, and it has entered clinical trials directly. The latest research shows that mRNA-1273 induces both potent neutralizing antibody and CD8 T cell responses and protects against SARS-CoV-2 infection in the lungs and noses of mice without evidence of immunopathology. mRNA-1273 is currently in Phase 2 clinical trial with a trajectory toward Phase 3 efficacy evaluation.[91] In addition, because there is no mRNA vaccine product in the market, there is no ready-made experience for mass production and production process of the mRNA vaccine, and large-scale industrial production also needs to be further explored.[93]

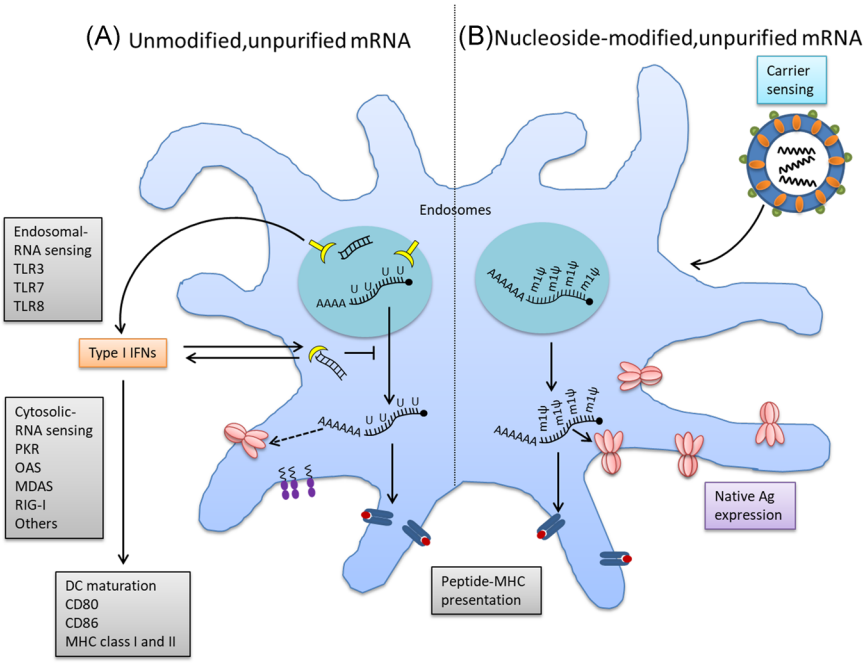

FIGURE 2 Innate immune sensing of mRNA vaccines. Innate immune sensing of two types of mRNA vaccine by a dendritic cell (DC), with RNA sensors, are shown in yellow, DC maturation factors in purple, antigen in red, and peptide-major histocompatibility complex (MHC) complexes in navy blue and red. An example of a lipid nanoparticle carrier is shown at the top right. Unmodified, unpurified (A) and nucleoside modified, purified by fast protein liquid chromatography (FPLC). (B) mRNAs were selected for illustration of two formats of mRNA vaccines where known forms of mRNA sensing are present and absent, respectively. Dotted arrows indicate reduced antigen expression. MDA5, interferon-induced helicase C domain-containing protein 1; PKR, interferon-induced, double-stranded RNA-activated protein kinase; Ag, antigen; IFN, interferon; mRNA, messenger RNA; OAS, 2′-5′-oligoadenylate synthetase; TLR, Toll-like receptor

12 | CONSIDERATIONS IN VACCINE DEVELOPMENT

Eosinophilic immunopathology and antibody-dependent enhancement of infection (ADEI) are real challenges to the development of SARS-CoV and MERS-CoV vaccines. Particularly, when the antibody level is relatively low, vaccines for CoVs like SARS-CoV may cause ADEI in human promonocyte cell cultures, resulting in the cytopathic effect and increased levels of TNF-α, IL-4, and IL-6.[40,94] Studies have shown that VLPs or inactivated viruses can induce eosinophilic immunopathology in both young and old mice.[82,83] Interestingly, it is reported in another study that toll-like receptor agonist adjuvants can prevent such immunopathology of the lungs in SARS-CoV infection. Therefore, it is of great importance to identify an appropriate adjuvant that extends the duration of the vaccine-induced immune response and CoV-induced lung immunopathology after natural infection.[95]

The immune effects of adjuvants, such as alum, CPG, and Adva (a new delta-inulin-based polysaccharide adjuvant), are reflected by the immunization of recombinant CoV S protein RBD and inactivated whole virus vaccines.[96] While all effective vaccines provide protection against lethal infection, the use of adjuvants can significantly increase serum NAb titers and reduce lung virus titers on Day 3 postchallenge. Whereas unadjuvanted or alum-formulated vaccines are associated with the significant increase in the eosinophilic immunopathology of the lungs on Day 6 postchallenge, this does not occur in mice immunized with vaccines formulated with delta inulin adjuvant particles. The effective protection provided by vaccines containing delta inulin adjuvant particles against eosinophilic immunopathology is associated with the enhancement of T-cell IFN-γ-recall responses rather than the reduction of IL-4 responses. All this suggests that immunopathology is primarily the result of inadequate vaccine-induced Th1 response, which addresses the significance of an appropriate adjuvant for sustained IFN-γ responses.

Another consideration is the cell substrate used for manufacturing all of these vaccines. Vero cells, in which SARS-CoV grows rapidly, offer a promising solution to the development of an effective SARS vaccine. It was previously reported that a licensed poliovirus vaccine was prepared with Vero E6 cells.[97]

13 | SUMMARY

The radical problem and challenge we face today is the constant emergence of new animal and human CoVs. The convenient transportation network has increased the risk of exposure to more and more pathogens. In addition, the failure in early detection of a new virus may lead to a large outbreak, just as the attack of 2019-nCoV (a novel CoV) that took place in Wuhan, China during late 2019.

2019-nCoV is a new type of β-coronavirus from the subgenus Sarbecovirus. It is 79% homologous to SARS-CoV and 50% to MERS-CoV. By drawing lessons from the existing research and findings with regard to the development of SARS-CoV and MERS-CoV vaccines, it may facilitate the research and development of effective vaccines for 2019-nCoV infection.

Therefore, the rise of coronavirus in recent years, especially the emergence of SARS-CoV2 in 2019, has aroused the widespread concern of the whole human society. The vaccine for coronavirus is urgently needed. A candidate mRNA vaccine for SARS-CoV2 has entered human clinical trials, and other vaccine candidates are soon to follow.

In conclusion, the development of effective vaccines requires a comprehensive understanding of viruses in animals and humans in terms of pathogenesis, transmission, and immune responses.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Conceptualization: Geng Li and Qin Li; methodology: Peiwen Zhou; Validation, Zonghui Li; formal analysis: Yaohua Fan; investigation: Jianguo Wu; Resources, Geng Li and Jianguo Wu; Writing—original Draft Preparation: Peiwen Zhou, Linqing Xie, and Dong An; writing—review and editing, Qin Li and Xiaohong Liu; supervision: Jianguo Wu; project administration: Xiao Wang; funding acquisition: Geng L and Yiwei Li.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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