



REVIEW

# Vaccines for SARS-CoV-2: Lessons from Other Coronavirus Strains

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## ABSTRACT

The emergence of the strain of coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and its impact on global health have made imperative the development of effective and safe vaccines for this lethal strain. SARS-CoV-2 now adds to the list of coronavirus diseases that have threatened global health, along with the SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) coronaviruses that emerged in 2002/2003 and 2012, respectively. As of April 2020, no vaccine is commercially available for these coronavirus strains. Nevertheless, the knowledge obtained from the vaccine development efforts for MERS and SARS can be of high value for COVID-19 (coronavirus disease 2019). Here, we review the past and ongoing vaccine development efforts for clinically relevant coronavirus strains with the intention that this information helps in the development of effective and safe vaccines for COVID-19. In addition, information from naturally exposed individuals and animal models to coronavirus strains is described for the same purpose of

helping into the development of effective vaccines against COVID-19.

**Keywords:** Coronavirus; COVID-19; MERS; SARS; Vaccine

### Key Summary Points

Current and past vaccine development efforts for SARS and MERS coronaviruses.

Current vaccine development for COVID-19 (SARS-CoV-2).

Correlates of protection and other immunologic features surrounding coronavirus strains.

Implications for developing effective vaccines for clinically relevant coronaviruses.

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## INTRODUCTION

Coronaviruses are a group of viruses that belong to the family *Coronaviridae* [1, 2]. These are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry. Their genome size is

relatively large for RNA viruses, between 27 and 34 kB [3]. Coronaviruses infect mammals and birds causing varied symptoms such as respiratory tract disease and diarrhea. In humans, coronavirus infections have been shown to be potentially lethal. This is the case of severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) coronaviruses.

In 2002–2003, the world experienced what would become the first of a series of lethal coronavirus infections. The disease denominated severe acute respiratory syndrome (SARS) would be characterized by high fever, eventually developing into shortness of breath and pneumonia [4]. Originating in southern China, the disease later would cause 8096 cases, resulting in 774 deaths in 26 countries [5]. Despite efforts from the scientific community, no vaccine became commercially available and SARS cases ceased to be reported from 2004 [4].

In September 2012, the world experienced the emergence of the Middle East respiratory syndrome (MERS) coronavirus. Originated in Saudi Arabia, the infectious disease is characterized by mild respiratory symptoms, but these could develop into acute respiratory distress syndrome and death [6]. The disease has affected 27 countries, resulting in 2494 cases and 858 deaths [7]. MERS cases are still being reported but no major outbreak has been declared since 2015 [8]. As in the case of SARS, no commercial vaccine is available for MERS.

Reasons for the lack of commercial and effective vaccines for SARS and MERS are varied. In the case of MERS, it is likely that the vaccine development was delayed because of the scarcity of suitable and cost-effective small animal models during pre-clinical experimentation. In addition, it is probable that a vaccine has not been delivered because of the low interest in investing in a vaccine for a disease that has produced relatively low and geographically centralized cases (compared with other more global and persistent infectious diseases such as influenza, HIV and tuberculosis). This last factor might have also contributed to the lack of a vaccine for SARS, in the sense that it was considered pointless to continue investing in a

vaccine for a disease whose cases ceased to be reported in 2004.

Coronavirus disease 2019 (COVID-19) is a current pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first cases were reported from Wuhan, China, in December 2019 [9, 10]. According to the World Health Organization (WHO), the disease has been reported in 213 countries and territories as of April 11, 2020, with evidence of ongoing local transmission [11]. According to the same organization, there are 1,669,595 confirmed cases and 106,138 deaths associated with the disease. Symptoms of COVID-19 are mild and include fever, cough and shortness of breath. Nevertheless, the disease might progress into severe pneumonia and multi-organ failure predominantly in elders and people with other underlying diseases [9, 12].

Although no vaccines are commercially available for SARS and MERS, past and current vaccine development efforts against these diseases might be of high value for the development of an effective vaccine for COVID-19. The present review aims to describe these efforts. Furthermore, we describe the possible implications of creating an effective vaccine against COVID-19 taking as a starting point results obtained from other clinically relevant coronavirus strains. We focused our review on active immunization approaches as this offers the possibility of a longer-term prevention for these diseases. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

## VACCINES FOR SEVERE ACUTE RESPIRATORY SYNDROME (SARS) CORONAVIRUS

After the SARS epidemic in 2002–2003, several laboratories around the world started to conduct vaccine development studies for preventing the disease. The majority of the subunit vaccines (vaccines based on a specific protein constituting the virus [13]) targeted the spike (S) glycoprotein of the virus. SARS-CoV uses this glycoprotein to bind and enter the host cells

[14]. Therefore, a vaccine that induces strong immune responses against this protein will have a significant effect on the deterrence of virus entry to the host cells during natural infection.

Vaccines based on a live-attenuated or inactivated virus, recombinant viral vectors, DNA, virus-like particles (VLPs) and soluble proteins were studied, mainly in pre-clinical studies. Live-attenuated and inactivated viruses are based on the use of the whole SARS-CoV as a vaccine. The virus has been rendered non-replicating, and infectivity has been greatly reduced by means of deleting components of the virus genome or by using physical or chemical methods [15]. In the case of recombinant viral vectors, viruses different from the SARS-CoV that are capable of host cell infection have been genetically engineered to express components of the SARS-CoV [16]. VLPs are non-infectious multiprotein structures formed from viral proteins that self-assemble into virus-like structures [17]. Table 1 summarizes these vaccines and the outcome from pre-clinical and clinical studies.

To our knowledge, only vaccines based on an inactivated SARS virus, DNA and soluble proteins based on the SARS S glycoprotein reached a clinical stage (phase I) [18–20]. It is desired in the field of vaccine development to know for certain if a vaccine can provide protection from virus infection and clinical signs. This is usually performed by exposing (challenging) vaccinated individuals and animal models to the virus in question. Due to the virulence of the SARS coronavirus, challenge studies in humans were not performed; therefore, the protective efficacy of the vaccines was not assessed.

## VACCINES FOR THE MIDDLE EAST RESPIRATORY SYNDROME (MERS) CORONAVIRUS

Several vaccines have been developed for MERS coronavirus since its emergence in 2012. As in the case of the SARS vaccines, most of the subunit vaccines for MERS are based on the S glycoprotein. Vaccines based on inactivated and live attenuated viruses, recombinant viral vectors, nanoparticles (conglomerate of antigenic

proteins of “nano” size [57]), DNA and soluble proteins have been developed and tested predominantly in animal models (Fig. 1; Table 2). To our knowledge, only a DNA-based vaccine has already been tested in clinical trials (phase I) [58] with other vaccines such as MVA (modified vaccinia virus Ankara) and adenoviruses being currently under study at that clinical stage [59, 60].

## VACCINE FOR CORONAVIRUS DISEASE 2019 (COVID-19)

Vaccines for COVID-19 are in pre-clinical development, and no clinical stage has been completed because of the recent emergence of the disease (as of April 13, 2020). Several entities from the globe have declared their intentions to develop a vaccine for COVID-19. According to the WHO, 41 candidate vaccines are being developed for COVID-19 as of March 13, 2020 [93]. Information about the nature of these vaccines under development is publicly scarce. Table 3 summarizes these current development efforts with information supplemented from other public sources. As of March 13, 2020, only one vaccine has started clinical trials (phase I). This vaccine is being developed by Moderna (USA), and it uses an mRNA-based vaccine expressing the SARS-CoV-2 S glycoprotein [94].

## TOWARD PROTECTIVE VACCINES FOR CLINICALLY RELEVANT CORONAVIRUS STRAINS

The vaccine development efforts for SARS and MERS coronavirus should help in the development of effective vaccines for COVID-19. This section aims to describe important information obtained from these experiments and to explore areas of opportunity for the development of effective vaccines for clinically relevant coronaviruses.

### Animal Models

The development of effective vaccines for SARS and MERS was slowed down by the lack of

**Table 1** Vaccine platforms for SARS-CoV

Vaccine	Target	Outcome	Reported side effects	Status	References
Inactivated SARS virus ( $\beta$ -propiolactone, formalin, UV irradiation)	All virus structural proteins	<u>Humans:</u> Induction of significant titers of neutralizing antibodies after two immunizations (100% seroconversion in participants). Vaccine was shown to be well tolerated with no severe adverse effects <u>Animal models:</u> Complete protection in mouse models after two doses (as measured by viral loads in lungs) Complete protection in rhesus macaques after two doses, as measured by viral shedding (also no evidence of adverse effects after challenge due to the vaccine)	<u>Humans:</u> Few cases of mild adverse effects, but that resolved in 24 h (local pain, erythema, abdominal pain and diarrhea). 4 out of 32 individuals reported reverted high levels of ALT (alanine aminotransferase) at a certain dose <u>Animal models:</u> Evidence of a degree of lung immunopathology in mouse models after challenge	Pre-clinical and phase I clinical trial	[18, 21–29]
Live attenuated/host-adapted SARS virus (E-deleted)	All genome (except the envelope protein)	Induction of neutralizing antibodies and CD4/CD8+ T cell responses in mice Protective against host-adapted SARS strains in mice (protected from lethal dose)	Not reported	Pre-clinical	[30–32]

**Table 1** continued

Vaccine	Target	Outcome	Reported side effects	Status	References
Live attenuated recombinant virus (parainfluenza virus, Vesicular Stomatitis Virus, Venezuelan equine encephalitis virus, Newcastle disease virus)	S glycoprotein, Nucleocapsid (N) protein	Induction of protection from viral replication in lungs (e.g., 236- to 1102-fold reduction during peak of infection) and virus shedding in African green monkeys  Short and long-term protection from lung viral replication in mice  Induction of long-term protection solely on the use of a CD4+ T cell peptide, cross-protective also for MERS-CoV in mice (protection from lethal dose)	Enhanced lung immunopathology after challenge when using the N protein encoded by the Venezuelan equine encephalitis virus	Pre-clinical	[33–37]
Recombinant modified vaccinia Ankara (MVA) virus	Spike (S) glycoprotein or nucleocapsid (N) protein	Induction of neutralizing antibodies and protection from SARS viral replication in lungs in mice  Induction of neutralizing antibodies in rabbits and rhesus monkeys  Induction of protection from virus shedding and viral replication from lungs in rhesus macaques  Not protecting from SARS viral replication and shedding in ferrets.  Induction of neutralizing antibodies against S but not N protein in this animal model	High levels of ALT, indicating hepatic lesion when expressing S protein and hepatitis after challenge with SARS in ferrets	Pre-clinical	[38–41]

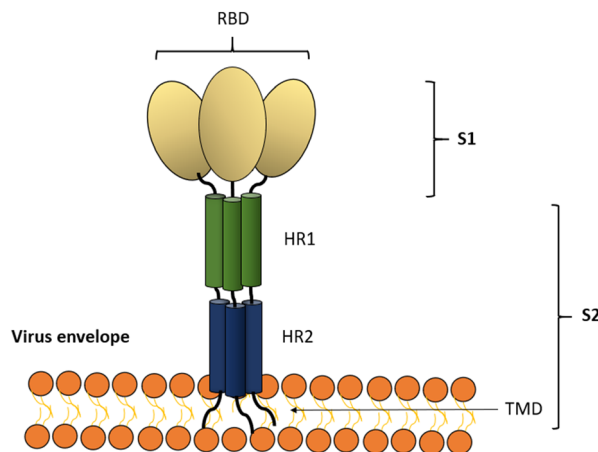
Table 1 continued

Vaccine	Target	Outcome	Reported side effects	Status	References
Recombinant non-replicating adenovirus (E-deleted)	S glycoprotein/nucleocapsid (N) protein	Superior cellular immune responses in lungs after intranasal and sublingual immunization compared with intramuscular immunization in mice Reduction of virus replication and virus shedding in mice and ferrets (incomplete protection)	In mice, re-direction of vector to the olfactory bulbs by intranasal administration	Pre-clinical	[42–44]
DNA-based vaccines	Full spike (S) glycoprotein or fragments	<u>Humans:</u> Induction of neutralizing antibodies (in 8/10 subjects) and T cell responses (in 10/10 subjects) after 2–3 doses. Well-tolerated <u>Animal models:</u> Induction of neutralizing antibodies and CD4+ CD8+ T cell responses in mice. Protection from lung viral replication dependent on humoral responses and not T cell responses	Evidence of a degree of lung immunopathology in mouse models after challenge	Pre-clinical and phase I clinical trial	[19, 23, 45, 46]
Soluble proteins/adjuvant	Full spike (S) glycoprotein or fragments	Protection from lung virus replication and pneumonia in hamsters and mice Induction of neutralizing antibodies in rabbits	Evidence of enhancement of virus entry on B cells by vaccine-induced serum in hamsters and mice (in vitro experiments)	Pre-clinical and phase I clinical trial	[20, 47–52]
Virus-like particles (VLPs)/adjuvant	Spike (S) glycoprotein	Induction of neutralizing antibodies and protection from SARS viral replication in lungs in mice	Evidence of a degree of lung immunopathology in mouse models after challenge	Pre-clinical	[23, 53]

**Table 1** continued

Vaccine	Target	Outcome	Reported side effects	Status	References
Combination of vaccine approaches (DNA/peptides, DNA/recombinant viral vector, viral vector/peptides)	S glycoprotein or fragments	Induction of significant titers of neutralizing antibodies and T cell responses	Not reported	Pre-clinical	[54–56]

The table summarizes the vaccines that were developed for SARS and the results obtained from pre-clinical and clinical studies. Vaccine platforms that have reached clinical trials are highlighted in italics. “Not reported side effects” means that side effects studies were not performed or did not result in moderate or severe effects



**Fig. 1** Scheme of the structure of the spike (S) glycoprotein of the MERS coronavirus used in vaccine development (modified from [61]). The MERS coronavirus S glycoprotein is used predominantly in vaccine development for coronaviruses. The S glycoprotein induces high titers of neutralizing antibodies, and the protein has been frequently exploited in subunit vaccination. In nature, the S glycoprotein binds to the host cell receptor DPP4 (dipeptidyl peptidase 4) through the receptor-binding domain (RBD) of the S glycoprotein [62]. The S glycoprotein can be divided into two subunits, S1 and S2. The subunit S1 contains the RBD. The subunit S2 contains heptad repeat regions (HR1 and HR2) that the virus uses for membrane fusion and entry to the host cell. The S glycoprotein is a class I fusion protein, and it exists as a trimer, as depicted. *DPP4* dipeptidyl peptidase 4, *S1* S1 subunit of S, *S2* S2 subunit of S, *RBD* receptor binding domain. *TMD* transmembrane domain

suitable animal models for testing these vaccines. Even though animal models develop immunologic responses to SARS and MERS coronaviruses, these animals show limited viral replication and clinical manifestations of disease (fever, cough, runny nose, shortness of breath, viremia, pneumonia), usually not leading to death [95]. This has produced certain limitations for evaluating protection conferred by the vaccines.

Several studies addressed the issue of the lack of a suitable animal model by using transgenic animal models that rendered these animals more permissive to coronavirus infection. By these means, the animal models allowed systemic virus replication and the analysis of more severe and clinically relevant symptoms of the



**Table 2** Vaccine platforms for MERS-CoV

Vaccine	Target	Outcome	Reported side effects	Status	References
Live attenuated MERS-CoV (E envelope-deleted)	All virus genome components (except E)	Not yet tested in vivo	Not yet tested in vivo	In vitro	[63]
Chemically or physically-Inactivated virus (MERS-CoV, rabies virus)	S glycoprotein and S1 subunit	Induction of high titers of neutralizing antibodies, protection from lung viral loads and lung pathologic damage in humanized mice	Enhanced lung eosinophil infiltrations after challenge	Pre-clinical	[64–66]
<i>Replication-deficient viral-vectored vaccines (poxvirus, adenovirus, measles, rabies)</i>	<i>S glycoprotein or S1 subdomain (containing the receptor-binding protein)</i>	<i>Induction of both neutralizing antibody responses and long-term T cell responses in animal models</i> <i>Protection from lung viral replication and MERS-CoV lethal dose in humanized mice</i> <i>Protection from viral shedding in camels</i>	<i>Use of S1 subunit in an adenovirus-based vaccine induced lung pathology in mice after challenge</i>	<i>Phase I</i>	[59, 67–76]
Soluble protein vaccines/adjuvant	S glycoprotein and fragments	Induction of neutralizing antibodies and T cell responses in animal models Protection from lung viral loads and from lethal dose in humanized mice Protection from virus shedding in camels and alpacas Partial protection in rhesus macaques (vaccine did not completely prevent pneumonia)	Not reported	Pre-clinical	[77–87]



**Table 2** continued

Vaccine	Target	Outcome	Reported side effects	Status	References
Nanoparticles	S glycoprotein	Induction of neutralizing antibody titers and reduction of viral replication in lungs of mice	Not reported	Pre-clinical	[88, 89]
<i>DNA-based vaccines</i>	<i>S glycoprotein and subunits</i>	<u>Humans:</u> <i>Induction of neutralizing antibodies (48% of participants) and T cell responses (76% of participants) after three doses (phase I clinical trial)</i>  <u>Animal models:</u> <i>Partially protective in non-human primates (observance of lung viral loads and pneumonia)</i> <i>Induction of neutralizing antibodies and T cell responses in mice. Also, protective from lung viral loads</i>	<i>Moderate and mild symptoms in phase I clinical trial</i>	<i>Phase I/IIa (South Korea) and phase II (Middle East)</i>	[58, 76, 90–92]
Combination vaccines (protein and DNA)	S glycoprotein and subunits	Induction of long-lived neutralizing antibodies and protection from pneumonia in non-human primates	Not reported	Pre-clinical	[78]

The table summarizes the vaccine development efforts (past and current) for MERS-CoV and the results obtained from the pre-clinical and clinical studies. Vaccine platforms that have reached clinical trials are highlighted in italics. “Not reported side effects” means that side effects studies were not performed or did not result in moderate or severe effects

SARS and MERS diseases [31, 43, 68, 69, 76, 96]. For example, transgenic mice were created to express the human cell receptor of SARS-CoV (human angiotensin-converting enzyme 2). This allowed the enhancement of infection sensitivity and the evaluation of protection from lethal doses of the virus [31]. Other approaches involved the use of mouse-adapted

coronavirus strains that could replicate and induce health conditions that resemble human infection (e.g., severe pneumonia, viremia leading to death) [30–32, 47]. Transgenic mouse models for SARS and MERS are now commercially available.

Non-human primates have also been used for testing protection in coronavirus

**Table 3** Vaccines under development for COVID-19 according to the WHO as of March 13, 2020 (adapted from [93])

Vaccine	Target	Producing entity (country)
Inactivated virus/alum	All structural proteins of the virus	Sinovac Biotech (China)
Virus-like particle (VLP)	Unknown	Medicago (Canada), ExpreS2ion Biotechnologies ApS (Denmark), Griffith University (Australia)
Protein nanoparticles/Matrix-M	S glycoprotein	Novavax/Emergent Biosolutions (USA)
Non-replicating viral vector (adenovirus, chimeric chimpanzeeadenovirus, MVA*)	S glycoprotein/unknown	Altimmune (USA), University of Oxford (UK), CanSino Biologics/Beijing Institute of Biotechnology (China), Vaxart (USA), Greffex (USA), Janssen Pharmaceutical (Belgium), GeoVax/BravoVax (USA/China)
*Vaccine expressing a VLP		
RNA-based vaccine, RNA vaccine encoding VLP	S glycoprotein/unknown	Moderna/NIAID (USA), CureVac (Germany), Imperial College London (UK), Arcturus Therapeutics/Duke-NUS Medical School (USA), Fudan University/Shanghai JiaoTong University/RNACure Biopharma (China), China CDC/Tongji University/Stermina (China), BioNTech/Fosum Pharma/Pfizer (Germany/China/USA)
DNA-based vaccine	S glycoprotein/unknown	Inovio Pharmaceuticals (USA), Zydyus Cadila (India), Takis Biotech/Applied DNA Sciences/Evvivax (USA/Italy), Sanofi Pasteur/BARDA (France/USA)
Protein subunit	S glycoprotein and peptides/unknown	WRAIR/USAMRIID (USA), Clover Biopharmaceuticals Inc./GSK (China/UK), Vaxil Bio (Israel), Generex/EpiVax, EpiVax/University of Georgia (Canada/USA), Sanofi Pasteur (France), University of Queensland (Australia), Baylor College of Medicine/New York Blood Center (USA), Fudan University (China), iBio Pharma/CC-Pharming (USA/China), AJ Vaccines (Denmark), Heat Biologics/University of Miami (USA)
Live attenuated virus	All proteins of the virus	Codagenix/Serum Institute of India (USA/India)
Replicating viral vector (measles, horsepox)	Unknown	Zydyus Cadila (India), Pasteur Institute/Themis/University of Pittsburg (France/USA), Tonix Pharma/Southern Research (USA)
Unknown	Unknown	University of Pittsburgh (USA), University of Saskatchewan (Canada), MIGAL Galilee Research Institute (Israel), Peter Doherty Institute (Australia), Academy of Military Medical Sciences (China), Tulane University (USA), Flinders University/Vaxine Pty Ltd (Australia)

vaccination. In the case of MERS, rhesus macaques allow virus replication but they do not seem to develop severe clinical symptoms (increased respiratory rate, fever, cough) [97, 98]. Common marmosets have been shown to display severe symptoms of disease, leading to death [99, 100], but information about their use in active vaccination experiments is scarce. In the case of SARS, the virus shows varied lung pathologies and limited clinical symptoms in cynomolgus macaques and African green monkeys [101, 102]. This symptomatology, although limited, has allowed analyzing the conferring of protection from vaccine candidates.

### Correlates of Protection

It is generally accepted that neutralizing antibodies against the SARS and MERS S glycoproteins play a predominant role in the protection against these coronaviruses [103, 104]. Neutralizing antibodies are antibodies that bind and neutralize virus infection of host cells [105]. Vaccinated animals and passive immunization approaches focusing on the MERS and SARS S glycoproteins induced high titers of neutralizing antibodies that correlated with protection [39, 43, 45, 49, 53, 106, 107]. Nevertheless, it is still in doubt whether adaptive T cell responses (another arm of the immune system) can also play a role in conferring protection, with few studies addressing this issue. As an example, a group of researchers demonstrated that specific CD4+ and CD8+ T cell peptides against SARS-CoV could be exploited to provide protection in mice [37, 108, 109]. Another study pointed out that the protection against SARS-CoV in mice induced by a DNA vaccine was due only to antibody responses (depletion of CD4/CD8+ T cells and adoptive T cell transfer did not have an effect on protection) [45]. In MERS, vaccination efficacy in non-human primates has been correlated to the induction of CD8+ T cell responses (in addition to neutralizing antibodies) when using DNA-based vaccines [78]. It is then likely that protection induced by vaccination might be dependent on different factors such as the selected vaccination platform,

antigens, animal models and routes of vaccination.

Few animal experiments have addressed the question of whether a certain vaccination regimen could induce long-term protection. In the case of SARS, viral vectors and protein-based vaccines employing the S glycoprotein have shown a certain level of protection from infection (in at least 75% of mice) after 4–12 months of vaccination [34, 35, 49]. In the case of MERS, protein-based vaccines and a combination of DNA and protein-based vaccines have been shown to induce a certain level of long-term protection in mice and macaques [78, 79]. In general, these studies focus on the induction of persistent neutralizing antibodies during vaccination, while the contribution of T-cell responses is not frequently addressed.

### Natural Infection of Coronavirus Strains

Exposure to SARS and MERS coronaviruses can also shed light on the possible mechanisms of protection. In humans, rapid and strong neutralizing antibody responses are highly correlated to the severity of the disease and the recovery of the patient. Nevertheless, T cell responses might also play a role in the elimination of the virus during infection, therefore also likely supporting the recovery of the patient [110–114]. From these studies, the induction of both arms of the immune response is likely necessary for the effective elimination of the virus and recovery from the disease.

In vaccine development, it is ideal that a vaccine provides long-term protection. Whether long-term protection can be achieved by means of vaccination or exposure to coronaviruses is under debate, and more information is needed in this regard. For SARS-CoV, memory T cells, but not B cells, could be detected 6 years after infection in human survivors [115]. Nevertheless, a certain level of neutralizing antibodies can be tracked until 24 months after infection, opening the possibility of a certain level of protection during this time frame due to this humoral response [116]. In the case of MERS, little is known about the generation of memory B cells, but neutralizing antibodies are

known to persist for 34 months [117]. In the case of memory T cells, CD4+ and CD8+ T cell responses have been detected 24 months after infection [114].

Other human coronaviruses can offer some clues about the induction of long-term protection. In the cases of human coronaviruses HCoV-229E and HCoV-OC43, these respiratory viruses do not cause long-lived protection as challenge experiments suggest, even when antibody responses are detected in the long run [118, 119].

### Safety

Studies about the safety of vaccines against SARS and MERS are relatively scarce. There is a concern about the induction of antigen-dependent enhancement (ADE) and other adverse effects derived from vaccination or natural re-exposure. ADE is a phenomenon that occurs when non-neutralizing antibodies against proteins of a virus enhance virus entry to host cells, also enhancing virus infectivity [120]. ADE has been already observed in cats vaccinated against a species-specific coronavirus (feline infectious peritonitis coronavirus) [121]. In the case of SARS, antibody-dependent enhancement and other adverse effects induced by vaccination in animal models have raised some concern.

It has been found that certain vaccine platforms expressing the SARS S glycoprotein or using a whole inactivated virus induced lung immunopathology and hepatitis after challenge in some animal models [23, 29, 38, 40]. Furthermore, MERS coronavirus vaccination has been shown to induce pulmonary infiltration after challenge in mice when using an inactivated MERS-CoV vaccine [66]. Of note, some SARS-CoV-infected animal models are not protected from MERS-CoV (and vice versa), and they might develop adverse effects after secondary infection [96, 122]. Other studies concerning passive antibody transfer in mice and non-human primate experimentation have not found evidence of ADE or pathologic effects [22, 26, 33, 39, 55, 123, 124].

The induction of ADE using the S glycoprotein has been tackled by using truncated

versions of the same protein. It is believed that the use of the receptor-binding domain (RBD) or the S1 subunit of the S glycoprotein can lead to the induction of neutralizing antibodies while avoiding ADE. The idea is to focus the induction of antibodies to relevant S regions for efficient virus neutralization and, by doing so, to avoid the induction of potential non-neutralizing antibodies targeting other regions of the S protein. Even though there seems to be little direct evidence proving this idea, vaccines based on these regions have already been tested, allowing the induction of high titers of neutralizing antibodies and a certain level of protection in small animals and non-human primates [49, 50, 56, 78].

The use of adjuvants has also been considered for avoiding the potential undesired effects of coronavirus vaccination. Adjuvants are substances that potentiate and modify the immunogenicity and protection efficacy of the vaccines [125]. A group of researchers found that by using a chemical adjuvant (a delta inulin-based polysaccharide), lung immunopathology previously observed in mice after SARS challenge experiments were no longer observed [126]. It was hypothesized that the adjuvant helped to avoid an exacerbated Th2-polarized response after challenge causative of the adverse effects. This is a case where an adjuvant not only enhanced vaccine-induced protection but also helped to minimize adverse effects due to coronavirus vaccination.

### Areas of Opportunity in the Development of Vaccines for Coronaviruses

Experiments directly comparing vaccine platforms and approaches for coronaviruses are scarce. A group of scientists discovered that, at a certain dose, DNA and MVA-based vaccines encoding the S glycoprotein of MERS develop similar levels of neutralizing antibodies [106]. In another study, vaccines based on heterologous prime-boost vaccination (DNA followed by protein-based vaccination) induced higher neutralizing antibody titers and protection than homologous prime-boost vaccination approaches; this is in mice and non-human primates

[127]. Nevertheless, a different number of immunizations were performed using different subunits of the S glycoprotein. Therefore, it is difficult to determine whether a given vaccine platform would be more immunogenic and protective than others solely on terms of the vaccine platform used. This information could potentially lead to the selection of ideal vaccine platforms for coronaviruses.

Although inactivated viruses, DNA and viral vector-based vaccines have been tested for SARS and MERS coronavirus in clinical trials, other vaccine platforms are yet to be tested. An mRNA-based vaccine, a relatively recent technology, is being tested for COVID-19 in phase I clinical trials [128] with other companies starting clinical trials soon as of April 11 of the year of this publication [129]. Due to the relatively new emergence of such vaccine platforms, their performance with coronavirus diseases is still to be known.

Different routes of vaccination could also help in the development of effective vaccines for coronaviruses. Considering that SARS and MERS-CoV are respiratory viruses, inducing memory responses in the respiratory tract would be advantageous. A group of researchers has found that inducing cellular and antibody responses in the respiratory tract by means of intranasal vaccination might induce higher protection levels in mice [37, 43].

Even though targeting the spike glycoprotein in vaccine development could be ideal for inducing neutralizing antibodies, the selection of other antigens might also offer some advantages. For example, the nucleocapsid (N) protein is more conserved between SARS and MERS coronavirus strains [130]. In addition, the N protein induces long-lived memory T-cells in humans [131]. Peptides derived from this protein have been shown to induce cross-protection against SARS and MERS in mouse experiments [37]. Therefore, the N protein could serve as a potentially viable alternative to provide cross-protective and long-term T-cell immunity against coronaviruses.

## CONCLUSIONS

The vaccine development efforts for coronavirus strains such as SARS and MERS can help to direct the vaccine development efforts for COVID-19. The development of highly effective and safe vaccines for COVID-19 should consider aspects such as the possibility of ADE and other adverse effects previously observed with SARS and MERS. Even though these features have only been seen in some animal models and vaccination regimens, the possibility is still there to be considered for COVID-19. In addition, these vaccine development efforts should address the possibility of the short-term immunogenicity derived from neutralizing antibodies, as also previously observed for SARS and MERS-CoV after natural infection.

The possibility of exploiting T cell responses for coronavirus vaccination should also be considered (along with B cell responses). These responses have been shown to be persistent and protective in animal models. Furthermore, there is evidence of long-term persistence in humans. Strategies such as adjuvantation, tailoring of the S glycoprotein, different routes of vaccination and the use of unexplored vaccine platforms for enhancing immunogenicity and preventing potential undesired effects should also be considered. It is worth mentioning that employing the N protein of the coronavirus for vaccination could have several benefits. As previously mentioned, there is the potential of providing long-term cross-protection when employing this antigen. Of note, the evidence of short-term immunogenicity and protection in coronavirus-exposed individuals does not mean that an effective vaccine is not possible. Vaccines for other now eradicated diseases that lack naturally acquired immunity prove this point (e.g., smallpox). The emergence of COVID-19 should also serve for elevating our comprehension and expertise in the abatement of pathogenic microorganisms of global health importance.

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