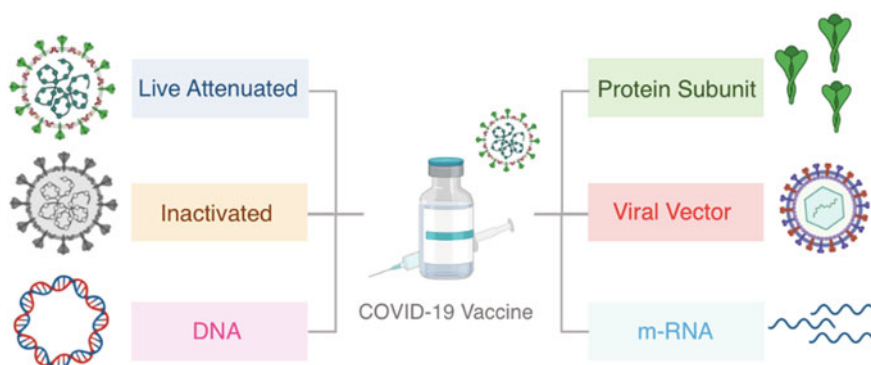# Chapter 5
Vaccine Development and Immune Responses in COVID-19: Lessons from the Past

Fataneh Fatemi, Zahra Hassani Nejad, and Seyed Ehsan Ranaei Siadat

Graphical Abstract

F. Fatemi (✉)
Protein Research Center, Shahid Beheshti University, Tehran, Iran
e-mail: f_fatemi@sbu.ac.ir

Z. Hassani Nejad
Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

S. E. R. Siadat (✉)
Sobhan Recombinant Protein, No. 22, 2nd Noavari St, Pardis Technology Park, 20th Km of
Damavand Road, Tehran, Iran
e-mail: ersiadat@sobhanbiotech.com

5.1 Introduction

Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first discovered in Wuhan, China, in late 2019 [1]. As of 24 December 2020, COVID-19 has spread to more than 200 countries and territories and has infected more than 78.7 million people and killed more than 1.7 million people globally and counting [2]. Elderly and those with underlying ailments such as diabetes, hypertension, heart disease, chronic respiratory disease, and cancer are at a higher risk of getting infected and exhibiting symptoms [3]. COVID-19 can be easily transmitted by inhalation of respiratory droplets and human-to-human contact [4]. Hence, most countries have implemented lockdowns and stringent social distancing policies.

Coronaviruses (CoVs) are enveloped, positive-sense single-stranded RNA (ssRNA) viruses that belong to the *Coronaviridae* family [5]. Currently, seven genera of CoVs that can cause infection in humans have been identified. Four of these viruses, including human (h)CoV 229E, hCoV OC43, hCoV NL63, and hCoV HKU1, are all associated with relatively mild symptoms [6]. The remaining three genera of CoVs are highly pathogenic and can sometimes be fatal. The potentially lethal CoVs, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and the newly discovered SARS-CoV-2 have caused or continue to cause severe illness in humans. Most human CoVs cause mild upper respiratory tract infections like common cold, unlike SARS-CoV, MERS-CoV, and SARS-CoV-2 which cause severe pneumonia [7, 8]. However, in comparison to SARS-CoV and MERS-CoV, SARS-CoV-2 has a lower fatality but higher transmission rate [8].

RNA viruses like CoVs evolve by mutations and homologous and non-homologous recombination, which facilitates the crossing of species barriers. It is still unclear as to how SARS-CoV-2 was first transmitted to humans. Its origin can be traced to bats, which is also the original host for other CoV infections in humans [9–11].

The genome and sub-genomes of a typical CoV include at least six open reading frames (ORFs). The first ORFs (ORF1a/b), which is about two-thirds of the whole genome length, encodes 16 NSPs (NSP1-16). The remaining one-third encodes at least four key structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein (Fig. 5.1) [12]. The S protein, which is expressed on the surface of the virus, is required for cell entry and plays a key role in eliciting immune response during disease progression [13]. The trimeric S protein consists of two subunits (S1 and S2), which mediate receptor binding (S1) and membrane fusion (S2). These subunits are further split into different functional domains. The S1 subunit includes a fragment called receptor-binding domain (RBD) that binds to angiotensin-converting enzyme 2 (ACE2), a receptor which is expressed in all organs [14], but primarily found in the lungs [15], brain [16], and gut [17]. After binding, the S protein is cleaved and activated by host transmembrane protease, serine 2 (TMPRSS2), for cell entry [18]. Studies based on the full-length genome phylogenetic analysis show that SARS-CoV-2 shares approximately 79% sequence identity
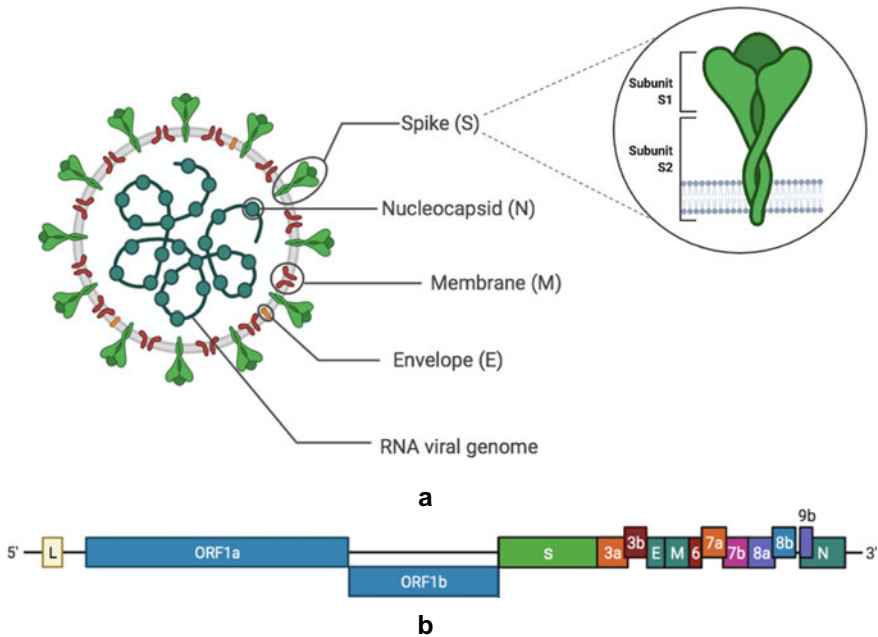
a

b

Fig. 5.1 Schematic representation of structure and genome of SARS-CoV-2 **a** SARS-CoV-2 is an enveloped RNA virus with four main structural proteins: spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins. **b** The single-stranded RNA genome of COVID-19 encodes two large overlapping open reading frames (ORFs) that makeup about two-thirds of the viral genome. These two ORFs encode 16 non-structural proteins (nsp1-nsp16). The 3′-end (the remaining one-third of the viral genome) encodes the four structural proteins (S, M, E and N) and accessory proteins

and has a similar cell entry mechanism and human receptor usage as SARS-CoV [10]. The SARS-CoV-2 genome shares about 50% sequence identity with MERS-CoV [9]. Therefore, previous investigations mostly on SARS-CoV and to some extent on MERS-CoV can provide insights into vaccination strategies for COVID-19.

It is indisputable that the world will not return to normality until an anti-SARS-CoV-2 vaccine is developed. Therefore, a lot of efforts are being put into developing safe and effective vaccines against SARS-CoV-2. Some vaccines are currently in advanced clinical stages, and a few of them have already received emergency authorization in some countries. Here, we summarize the SARS-CoV-2-immune system interaction and provide an overview of previous efforts made on SARS-CoV and MERS-CoV vaccine development. In addition, we will discuss recent efforts made towards COVID-19 vaccine development.

## 5.2   COVID-19 and Immune System Interaction

5.2.1   Innate Immunity

The first line of defense against viral infections is known as the innate immunity. The innate immunity provides an initial, non-specific response, with no memory induced [19]. Due to its novelty, our understanding of the human immune response against SARS-COV-2 is in its infancy and much remains to be understood. However, the virus-host interactions in SARS-CoV-2 are most likely to recapitulate many of those that are seen in previously discovered CoVs due to their resemblance [19].

Upon binding of SARS-CoV-2 and entry to the cells through the ACE2 receptor, the innate immune system gets triggered [13]. The innate immune response is key for targeting and restricting infected cells and for the subsequent activation of the adaptive immune response. The host immune system recognizes the pathogen via pattern-recognition receptors (PRRs) signaling such as toll-like receptors (TLRs), a family of 11 transmembrane receptor proteins that recognize pathogen-associated molecular patterns (PAMPs) [20, 21]. TLRs are important sensor molecules that detect a wide range of microbial pathogens. For RNA viruses like CoVs, the receptors, TLR3 and TLR7, recognize the viral single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA) [22]. After PRR activation, downstream signaling cascade leads to the secretion of proinflammatory cytokines including type I/III interferons (IFNs), interleukin-1 (IL-1), IL-2, IL-6, IL-7, IL-18, and tumor necrosis factor-α (TNF-α), therefore, initiating the defense mechanism against viral infection [19]. Together with the pro-inflammatory cytokines, there will be a strong local inflammatory response that can lead to an influx of neutrophils and other myeloid cells into the lung [19] (Fig. 5.2). This is also seen in SARS-CoV and MERS-CoV infections indicating the importance of cytokine storm and lymphopenia in the COVID-19 pathogenesis [23, 24].

Cytokines induce the infiltration of immune cells to remove infectious viral agents. Most individuals infected with COVID-19 recover from the disease symptoms once the infiltrated immune cells clear the infection. However, dysregulation of proinflammatory cytokines can have detrimental consequences for the host and lead to pathogenesis [19]. Therefore, the activation of the innate immune system must be strictly modulated since excessive activation can cause systemic inflammation and tissue damage [25].

Similar to other CoVs, SARS-CoV-2 has several mechanisms to evade innate immune recognition, such as masking its antigenic epitopes, RNA shielding, and synthesizing viral proteins that hinder anti-viral responses [26, 27]. SARS-like CoVs encodes multiple proteins that antagonize IFN responses and are critical for promoting early viral pathogenesis [28, 29]. Similarly, severe cases of COVID-19 have shown impaired IFN-I/III signaling profile as compared to moderate or healthy cases [30]. Therefore, SARS-CoV-2 promotes prolonged survival via suppressing IFN-I/III signaling, which gives it adequate time to spread inside pneumocytes and alveolar cells [31].
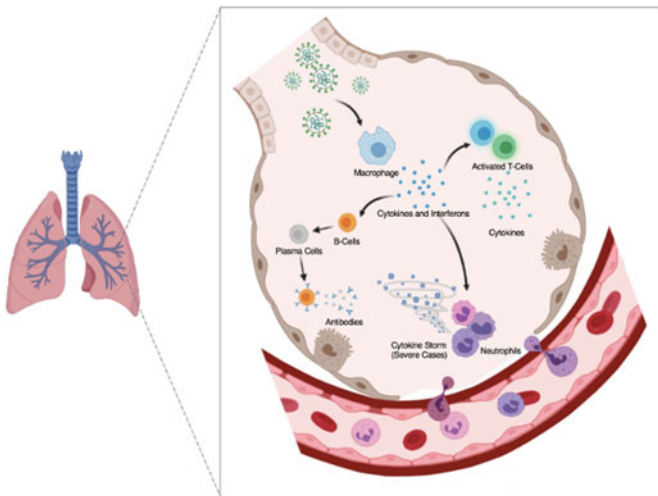
Innate Immunity

a
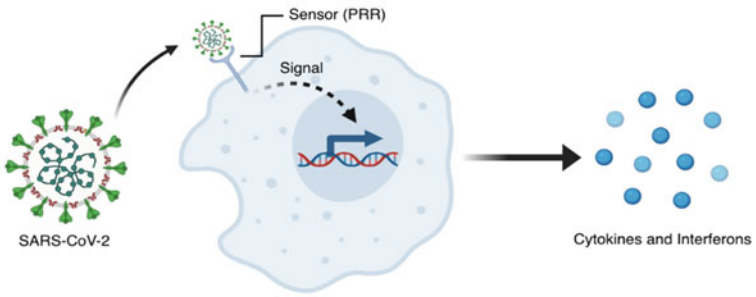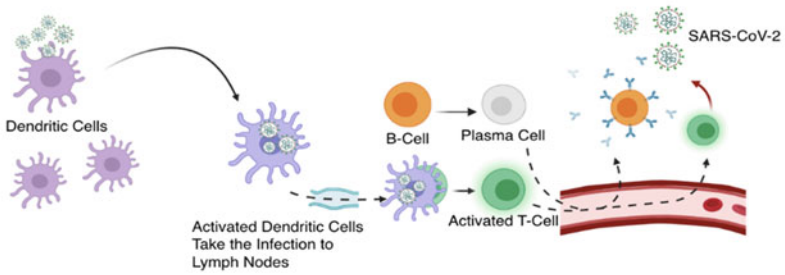
Adaptive Immunity

b

c

◄**Fig. 5.2** Overview of innate immunity (**a**), adaptive immunity (**b**), and cytokine storm (**c**) longs. **a** Innate immunity is initiated upon detection of the viral pathogen by the pattern recognition receptor (PRR). Following viral recognition, the expression of cytokines and interferons is induced. **b** Adaptive immunity is triggered when infected dendritic cells travel to lymph nodes to activate the T and B cells. The T cells and antibodies produced by the B cells attack viruses and virus-infected cells. **c** After viral infection, immune cells identify the virus and produce cytokines. These cytokines attract more immune cells which in turn produce more cytokines and lead to a cycle of inflammation that can eventually damage the lungs and cause respiratory failure

Another strategy to escape the innate immune recognition is the evolution of low genomic cytosine phosphate guanosine (CpG). Typically, the CpG motifs in genomes of RNA viruses are targeted and degraded by the zinc-finger antiviral protein (ZAP). Among the beta-CoVs, SARS-CoV-2 has the most severe CpG deficiency [27, 32]. Another way to protect mRNA is the processing of capping the 5' ends of the viral RNA. Degradation is decreased by capping since it prevents viral recognition by cytosolic PPRs. Similar to other CoVs, SARS-CoV-2 uses its own capping machinery to synthesize 2'-o-methyltransferase caps [33]. These RNA caps are indistinguishable from cellular mRNAs caps, thereby they do not get targeted for degradation. All and all, these mechanisms pave the way for widespread infection of the virus.

5.2.2 Adaptive (Active) Immune Response

5.2.2.1 Cell-Mediated Immune Response

After virus entry and the subsequent activation of the innate immune response, the adaptive immune response gets triggered to eliminate the virus. Adaptive immunization typically produces long-term immunity due to stimulation of the immune system by the exposure to an antigen. The adaptive immune system consists of three main lymphocytes: B cells (antibody-producing cells), CD4+ T cells (helper T cells), and CD8+ T cells (cytotoxic or killer T cells).

The CD4+ T cells are responsible for regulating CD8+ T cell responses, humoral immunity, as well as macrophage-mediated antiviral activity. In addition, the CD4+ T cells play a pivotal role in recruiting cells to infection sites. On the other hand, the CD8 + cells regulate viral infections via lysing the infected cells, secreting cytokines, and forming memory cells to provide protection if reinfection occurs. Nonetheless, these viral sensing mechanisms can often over induce the immune response and eventually lead to tissue damage [25].

5.2.2.2 Antibody-Mediated Immune Response

SARS-CoV-2 triggers a robust B cell response. The activation of B cells triggers antiviral antibody secretion and acts against the virus through a number of different mechanisms, including neutralization, opsonization and activation of complementary proteins [34]. Antibody-mediated immune response plays an essential role against CoV infections. The S protein, specifically the RBD subunit, is the main target for neutralizing antibody (NAb)-mediated inactivation of SARS-CoV-2 by inhibiting it from binding to ACE2 receptors. Therefore, NAbs remain even after infection to prevent the virus from re-infecting the host. In addition, NAbs are responsible for viral clearance during acute phases of the infection and modulate disease progression [35]. Most COVID-19 vaccine efforts focus on eliciting NAbs and CD4+ or CD8+ T cells. Therefore, the main role of a vaccine is to induce both arms of the adaptive immune system and to elicit adequate amounts of T and B cells.

5.3 Vaccine Development

5.3.1 Pre-clinical Studies

Vaccine development is a lengthy process and typically takes many years to develop a safe and effective vaccine. The first step in vaccine development involves basic laboratory research and computational modeling to identify natural and synthetic antigens that can be exploited as a vaccine candidate. Afterwards, the pre-clinical stage is initiated which involves evaluating the safety and the immunogenicity of the vaccine by testing it in various animal models. These studies provide insights into the cellular responses that might be expected in humans. Also, the safest starting dose and methods for administering the vaccine for the next phase of research will be determined through this stage of development.

There are various animal models for preclinical testing of SARS-CoV-2. These animal species have different degrees of susceptibility to SARS-CoV-2 depending on the binding affinity of the virus to the host ACE2 receptor or on host protease activities on the S protein [18]. Cats, ferrets, hamsters, mouse models, and non-human-primate models are all susceptible to SARS-CoV-2 and are usually used as animal models in pre-clinical studies [36]. The non-human primate models, particularly rhesus macaques, exhibit the highest binding affinity for SARS-CoV-2 among the animal models tested [37]. These species show viral shedding from the upper and lower respiratory tract. Nevertheless, their symptoms, clinical signs, and disease severity are different from humans [38, 39].

Mice are also a popular animal model for pathogenesis studies of many viruses. Nonetheless, conventional mice lack appropriate receptors to initiate CoV viral infection. The ACE2 receptor of these organisms does not bind well to the S protein of SARS-CoV-2 [40]. To circumvent this issue, transgenic mice expressing human

ACE2 have been developed. Previously these mice were tested with SARS-CoV and are now being tested with SARS-CoV-2. These mice have been shown to replicate SARS-CoV-2 in the lung and exhibit similar interstitial pneumonia as humans [41]. Another approach involves utilizing mouse-adapted CoV strains that could mimic and induce health conditions identical to human infection [42]. This strategy has been employed for SARS-CoV using conventional mouse strains without the requirement of transgenic mice expressing the human ACE2 receptor and is now being employed for SARS-CoV-2 [43, 44].

5.3.2   Clinical Studies

After assessing the immunogenicity and safety of the vaccine in animal models, progress is made to human clinical studies. The clinical-stage of development consists of at least three phases and progresses sequentially. After the clinical stage, the vaccine proceeds to regulatory approval and licensing. The phase I trial consists of short-term studies in which the vaccine is administered to a small number of healthy adult participants usually between 20 and 80 subjects to assess the safety, reactogenicity and the type of immune response the vaccine may produce. The optimal dose range and the desired route of administration are evaluated. In some cases, the participants are challenged with the pathogens under a controlled environment to find the true effect of the vaccine [45]. The preliminary information on efficacy and immunogenicity is analyzed and if satisfactory results are achieved, the trial advances to the next phase.

Following the completion of phase I trials, the phase II trials are conducted. In phase II, a large-scale (several hundred) of the target population will be tested. These trials are randomized, well-monitored, with a placebo control group. The purpose of this phase of testing is to examine the vaccine's safety, efficacy, and proposed doses.

The final phase in clinical evaluation before product licensing is the phase III trial, in which the vaccine is tested in a larger group of people. The designs of phase II and phase III are alike, but the target population of phase III is much larger. These trials are designed to evaluate the efficacy and safety of the final formulation. The immunogenicity (production of antibodies/cell-mediated immunity) is tested in this stage. After a successful phase III trial, the vaccine will go through licensing, as part of post-marketing surveillance.

In normal circumstances, vaccine development takes approximately 10–15 years [46]. The fastest vaccine development has been for mumps, which took about five years to get approved. The speed with which the COVID-19 vaccine has been developing is faster than conventional vaccines against other diseases and there is an overlapping of clinical trial phases and the whole process is compressed into 12–18 months.

5.4 Vaccine Platform Technologies

A variety of novel vaccine platform technologies have been established over the past decades. These platforms range from inactivated or live attenuated pathogens, protein subunit, nucleic acid-based (RNA or DNA), virus-like particle (VLPs), non-replicating, and replicating viral vectors, with all of them having different advantages and disadvantages (Table 5.1). Most viral vaccines that are currently available for human use are virus or protein-based. The virus-based vaccines employ inactivated or live attenuated viruses. These vaccines are highly effective for contagious diseases. However, their production is time-consuming and complicated. In addition, extensive safety testing is required to ensure that the virus does not revert to its infective state [47]. Protein-based vaccines exhibit more safety and are easier for mass production than whole-virus vaccines. However, their immunogenicity is lower and might need

Table 5.1 Vaccine production platforms and their advantages and limitations

Vaccine platform	Advantage	Disadvantage
Live-attenuated vaccine	High potency; long-lasting immune response; provides 'one shot' immunity	Requires low-temperature storage and transportation (cannot be used in countries with limited access to refrigerators); cannot be used in people with the weak immune system
Inactivated virus vaccine	It offers broad protection; strong immune response; safer than live-attenuated virus vaccine	Low production titer; might require multiple doses or an adjuvant
Viral vector-based vaccine	Induces strong T cell response without requiring an adjuvant; native antigens are preserved	Complicated manufacturing process; cannot be used in people with the suppressed immune system
RNA-based vaccines	Safe and well-tolerated; native antigen expression; low-cost and easy manufacturing	Lower immunogenicity; might require multiple doses; requires low-temperature storage and transportation
DNA-based vaccines	Low-cost and easy manufacturing; highly adaptable to the new pathogen; stable under room temperature	Requires expensive equipment; low immunogenicity; risk of genomic integration
Protein subunit vaccine	Can be used on almost everyone including people with weakened immune systems; does not have the live component of the viral particles; has fewer side-effects	Low immunogenicity; might require booster shots; requirement of adjuvant or conjugate
Virus-like particles (VLPs)	A well-established technology; safe and well-tolerated; native antigen expression; stable under room temperature	Low immunogenicity; might require multiple doses or an adjuvant

multiple doses. Nucleic acid-based vaccines have gotten considerable attention in the new generation vaccines field. One of their advantages is the short time required from the design to clinical trials and their potential for mass-production. But there may be a need for multiple doses to enhance immunity against the virus.

The COVID-19 vaccine development is proceeding at an unprecedented speed. The efforts on vaccine development against COVID-19 started initially in China as soon as the disease was discovered. Since then, many countries have been directing their efforts towards the development of safe and effective vaccines against COVID-19. As of December 22, 2020, the worldwide SARS-CoV-2 vaccine landscape includes 233 vaccine candidates, out of which, 61 are in the clinical stage of development [48].

5.4.1   Live Attenuated Vaccines

Live attenuated and inactivated vaccine technology is one of the most traditional and effective approaches. Live attenuated vaccines provide a robust and long-lasting immune response due to preserving the native antigenic moiety. These vaccines have been commercially available for viruses like influenza, chickenpox, smallpox, polio, measles, and yellow fever virus [49]. Currently, attenuated virus strains are devised via deleting or mutating virulence genes. Thus, eliminating its ability to cause disease in vivo. Deletion of structural E protein [50–53] and non-structural proteins (NSP) [54, 55] has been used to devise vaccines against strains of several CoVs. The E protein is known to trigger inflammasomes and is correlated with intensified inflammation in the lung parenchyma [56]. Therefore, deletion or mutating the E protein can reduce the virulence of CoVs [56]. An alternative approach for the development of attenuated virus is known as codon deoptimization which involves hampering the translation of the viral protein during viral infection [57]. The development of a live attenuated vaccine for CoVs is challenging since they are known to recombine in nature with other CoVs, leading to new pathogenic strains [11]. In addition, live attenuated vaccines can have the potential to return to their virulent state [58]. Therefore, the safety of these vaccines should be thoroughly assessed in animal models before proceeding into the clinical stage. Currently, there is only one COVID-19 live attenuated vaccine in the clinical stage [48]. The COVI-VAC live attenuated vaccine, developed by Codagenix Inc. in collaboration with the serum institute of India is currently in phase I clinical trial (NCT04619628).

5.4.1.1   SARS-CoV and MERS-CoV Live Attenuated Vaccine

A number of effective SARS-CoV and MERS-CoV live attenuated vaccines have been developed and tested in vitro and in vivo. Nonetheless, all of these vaccines have remained in the pre-clinical trials [50–55, 57]. Most CoV live attenuated vaccines are designed through deletion of the E gene. Lamirande et al. developed a recombinant

SARS-CoV (rSARS-CoV) live attenuated vaccine lacking the structural E gene. The rSARS-CoV-Δ E elicited serum-NAbs and completely protected the upper and lower respiratory tract against challenge with homologous (SARS-CoV Urbani) and heterologous (GD03) virus in the Golden Syrian hamster model [50].

The nonstructural protein 16 (nsp16), a conserved 2'O methyltransferase (MTase) that encodes essential functions in immune modulation and infection [54], can be used as an alternative target for attenuation of CoVs. However, targeting the nsp16 gene alone can have the potential for reversion of the virus to its virulence state in aged animal models [59]. To overcome this issue, Menachery and colleagues designed a SARS-CoV vaccine by mutating the nsp16 gene in combination with another conserved attenuating mutation in nsp14, exonuclease (ExoN) activity. They evaluated this vaccine in the mouse model and noticed that combining the 2'O MTase mutation with a second attenuating mutation provides a vaccine strain that offers protection from heterologous virus challenge with no evidence of reversion to virulence state [54]. These results indicate that CoV 2'O MTase in parallel with other conserved attenuating mutations can be a suitable strategy for the production of live attenuated CoV vaccines. The dnsp16 can also serve as a target for live attenuated vaccine development against MERS-CoV. Menachery et al. devised a dnsp16 mutant MERS-CoV strain as an alive attenuated vaccine platform which showed robust protection from challenge with a mouse-adapted MERS-CoV strain [55]. Therefore, the viral 2'O-MTase activity can be a potential universal platform for vaccine development of human and animal CoVs.

5.4.2 Inactivated Vaccines

Inactivated vaccines are made non-infectious through chemical (e.g., formaldehyde) or physical (e.g., heat) methods [60, 61] and are a suitable option given their ability to induce robust immune responses and their feasibility for large-scale production [62]. However, inactivated vaccines' potency is less than that of live attenuated vaccines. Therefore, there may be a need for multiple doses over time to develop ongoing immunity against the disease. Inactivated vaccines have the risk of viral reactivation if improperly inactivated and deformation of immunogenic epitopes during the inactivation process can sometimes weaken the protection that inactivated viruses provide. In addition, inactivated viral vaccine studies have shown that CoVs may induce antibody-dependent enhancement (ADE) effect, recommending more attention when evaluating the safety of vaccines against these viruses [63, 64].

5.4.2.1 SARS-CoV and MERS-CoV Inactivated Vaccine

A few inactivated vaccines have been reported for SARS-CoV. Most of these vaccines induce high levels of specific NAbs in animal models[65−69]. Xiong and colleagues

formulated a formaldehyde inactivated SARS-CoV vaccine treated with formalde-hyde and supplemented with aluminum hydroxide, $Al(OH)_3$ [65]. Three doses of the vaccine induced specific IgM and IgG antibodies in BALB/c mice on day four and day eight, respectively, with no significant change in $CD4^+$ and $CD8^+$ levels [65]. Luo et al. reported an inactivated SARS-CoV Z-1 vaccine that elicits neutral-izing and protective antibody response in rhesus macaques upon challenge. They also examined whether the vaccine could trigger antibody-dependent enhancement (ADE). They found that low levels of antibodies induced by the inactivated SARS-CoV Z-1 vaccine might not induce ADE in rhesus macaques [66]. Takasuka et al. immunized mice with UV-inactivated SARS-CoV with or without an adjuvant (alum) [67]. They noticed that the UV-inactivated SARS-CoV virion induced a high level of humoral immunity and long-term antibody production and memory B cells even in the absence of an adjuvant. However, the serum IgG production was enhanced with the addition of alum to the vaccine [67].

Despite exhibiting potency in eliciting protective antibody responses, some UV and formaldehyde inactivated CoV vaccines that include the N protein are reported to cause eosinophil-related lung pathology in animal models upon SARS-CoV chal-lenge [68, 69]. In addition, adverse effects may arise from SARS-CoV N protein-specific T cells and Th2-skewed cytokine profile [68]. As a result, it is highly crucial to boost the protective S-specific immune response and decrease the potentially pathological anti-N response. In addition, some studies have shown that alum unadju-vanted or adjuvanted inactivated SARS-CoV vaccines provide inadequate protection in mice and induce eosinophilic pro-inflammatory pulmonary response post-viral challenge [68, 70]. Therefore, inactivated vaccines must be thoroughly evaluated prior to clinical studies.

It has been reported that the MERS-CoV vaccine accompanied with alum or MF59 adjuvant can induce NAbs [71]. However, these vaccines can also induce eosinophil-related lung pathology on the challenge with the virus [71]. Inactivated vaccines can still be a suitable platform for CoV vaccine development since the incorporation of suitable inactivation techniques and adjuvants can overcome the bottleneck of lung pathology. Iwata-Yoshikawa and co-workers have shown that UV-inactivated SARS-CoV adjuvanted with TLR agonists could elicit protective antibodies and can reduce eosinophilic responses in the BALB/c mouse model [72]. In addition, inactivated MERS-CoV inactivated with formaldehyde and adjuvanted with a combined alum and unmethylated CpG adjuvant can decrease or prevent pulmonary immunopathology and enhance protective immunity against MERS-CoV in mice post-challenge [73].

5.4.2.2   COVID-19 Inactivated Vaccine

As of now, there are eight vaccine candidates based on inactivated SARS-CoV-2 going through clinical trials. Among these vaccines, the CoronaVacc (also known as PiCoVacc) developed by Sinovac Biotech Ltd in China is the most advanced with published preclinical results [74]. CoronaVacc vaccine is produced in Vero cells and

inactivated using β-propiolactone [74]. This vaccine has been tested on non-human primates, rats, and mice, demonstrating potency, safety, and good immunogenicity with vaccine-induced NAbs that neutralize representative strains of SARS-CoV-2 [74]. Data published from pre-clinical trials in macaque and mice models demonstrate that adequate specific IgG response and NAb titer levels were achieved with no notable cytokine changes and ADE in the macaques [74]. In addition, no pathological changes in vaccinated macaques' vital organs were observed after the SARS-CoV-2 challenge [74]. CoronaVacc has completed its phase I/II clinical trial and is currently in phase III of the clinical trial [48]. Results from phase I/II show that the vaccine is well-tolerated and induces NAbs with a seroconversion rate of 90% [75].

The SARS-CoV-2 inactivated vaccine developed by Sinopharm Inc. in collaboration with Wuhan Institute of Biological Products works through propagating WIV04 strain from different COVID-19 patients in Vero cells doubly inactivated with two rounds of β-propiolactone exposure [76]. In their phase I and II studies, different dosage and injection timelines were tested. All the vaccinated patients who received different vaccination regimens had produced NAbs with little to no adverse effects in their phase I/II studies [76]. In addition, Sinopharm Inc. is developing another COVID-19 vaccine called BBIBP-CorV, with $Al(OH)_3$ as an adjuvant [77]. This vaccine has a similar manufacturing process as the other Sinopharm Inc. vaccine except that the HB02 strain is used instead of the WIV04 strain. The BBIBP-CorV has been tested in pre-clinical models and has demonstrated efficacy in non-human primates when immunized with two doses of BBIBP-CorV with no disease enhancement upon SARS-CoV-2 challenge [77]. The results from their I/II clinical trial demonstrate the vaccine's efficacy, tolerability, and good humoral response in all participants 42 days post-immunization in all tested doses [78]. Both of these Sinopharm Inc. inactivated vaccines are currently in Phase III clinical trial.

Three vaccine manufacturing and academic/research institutions in Iran are in the process of developing inactivated COVID-19 vaccines. The alum adjuvanted inactivated vaccine developed by Shifa-Pharmed, a part of the state-owned pharmaceutical conglomerate, has recently started its clinical trial in Iran. In the Phase I clinical trial, they enrolled 56 participants, each receiving two shots of the vaccine within a period of two weeks. This company has not disclosed any of its pre-clinical results, and its Phase I clinical results are to be announced roughly a month after the second shot [79].

5.4.3 Viral Vectored Vaccines

Viral vector vaccines consist of a recombinant virus (often attenuated) in which genes encoding antigens of interest (usually S gene for CoVs) have been cloned using recombinant DNA techniques. The viral vector elicits antigen-specific humoral and cell-mediated immune responses through antigen presentation [80]. The most commonly employed non-replicating vectors are the adenovirus (Ad), measles virus, and vesicular stomatitis virus vectors [81]. These vectors mimic natural viral infection

and elicit the production of the target viral proteins inside host cells. The main advantage of vector-based vaccines is their ability to induce both humoral and innate immune responses [82]. In addition, vector-based vaccines provide stronger cellular immune responses compared to the recombinant protein vaccines. One of the main drawbacks of using recombinant viruses is the possibility of genome integration with the host cell genome. Moreover, the manufacturing process of viral vector vaccines is very complex and includes optimizing cellular systems and eliminating contaminants that can potentially impede the efficiency of viral vectors [81].

5.4.3.1   SARS-CoV and MERS-CoV Viral Vectored Vaccines

Some studies have assessed the efficacy of the adenovirus-based SARS-CoV vaccine. Gao et al. and Liu et al. have shown that Ad vector expressing S1 can induce potent NAbs responses in rhesus macaques and rats [83, 84]. However, these experiments were done in vitro and whether these vaccines provide protection against SARS-CoV challenge in vivo is still questionable. The potency of Ad vaccine expressing the SARS-CoV S protein has been compared with the whole inactivated SARS-CoV vaccine by See and co-workers. It was found that these vaccines provide immunity in mice when challenged with SARS-CoV. Nevertheless, the NAb response is stronger in the whole-inactivated virus vaccine than the adenovirus-based vaccine [85].

A number of Ad-based MERS-CoV vaccines have been developed. MERS-CoV vaccines based on human Ad type 5 and type 41 (Ad41) expressing S or S1 protein have demonstrated the induction of NAb in mice [86, 87]. An example is the Ad5-MERS-S vaccine which works with S protein nanoparticles. This vaccine provides protection in hDPP4-transduced mice against viral challenge. In addition, heterologous immunization with Ad5/MERS prime and S protein nanoparticles boost has demonstrated enhanced Th1/Th2 responses than the Ad5- or nanoparticles-alone homologous prime-boost vaccines [88].

5.4.3.2   COVID-19 Viral Vectored Vaccines

Currently, 18 viral vectored COVID-19 vaccines are undergoing clinical trials. Out of these vaccines, the ChAdOx1nCoV-19 and now designated AZD1222 developed by Oxford University in collaboration with AstraZeneca is the most clinically advanced viral-vector based COVID-19 vaccine. This vaccine consists of a replication-deficient chimpanzee Ad vector ChAdOx1, containing the S glycoprotein gene. The AZD1222 is designed via the deletion of E1 and E3 genes. The deletion of E1 inhibits replication and deletion of E3 allows integration of larger genetic cargo into the viral vector [89, 90]. The AZD1222 has demonstrated high NAb levels in 91% of participants following the first dose. Participants receiving booster dose had a high NAb response, therefore, indicating the need for a two-dose regimen to enhance the NAb response [89]. Phase I/II results demonstrate its acceptable safety profile with no serious adverse events with induction of binding and NAb antibodies as well as production

of interferon-γ enzyme-linked immunospot responses with higher antibody titers after the second dose of vaccine [89, 91]. In addition, no severe adverse effects were seen during this phase. [89] The interim analysis from the AZD1222 vaccine's phase I/II clinical trial showed that AZD1222 has an efficacy of about 70% [92].

Another viral vector-based vaccine is the Ad5-nCoV co-developed by CanSino Biological Inc. and the Beijing Institution of Biotechnology. The ad5-nCoV is designed similarly to the AZD1222. The full-length S gene along with the plasminogen activator signal peptide gene is cloned into the Ad5 vector missing the E1 and E3 genes. Deletion of E1 inactivates the replication potential of the vaccine, and deletion of E3 allows for the addition of large genes (up to 8 kb) [90]. Phase-III safety studies of this vaccine have shown success with both groups of vaccinated participants developing NAb responses in 47–59% of the volunteers and seroconversion of binding antibodies in 96–97% of them [93].

5.4.4 MRNA Vaccines

Over the past decade, there has been a lot of effort and investment in enabling mRNA to become a promising therapeutic tool for vaccine development and for cancer prophylaxis and therapy [94]. In this approach, the antigen-encoding mRNA is complexed with a carrier that can efficiently deliver it to the cytoplasm of host cells for protein translation and post-translation modification [95]. RNA vaccines utilize lipid- and polymer-based nanoparticles or protamine, for increased efficacy [96]. These vaccines are synthesized in vitro transcription and are non-infectious, making them ideal for rapid and inexpensive production. In addition, unlike DNA vaccines, RNA vaccines are able to synthesize viral proteins without the risk of integration with the host cell genome. Also, DNA vaccines require special devices for administration, whereas RNA vaccines can be administered through various ways including intravenous injection. Nonetheless, RNA vaccines do have their own demerits such as having low immunogenicity and instability concerns.

No RNA vaccine studies for SARS-CoV or MERS-CoV have been previously reported. Nonetheless, eight RNA vaccines for SARS-CoV-2 are currently in the clinical stage with two of them receiving emergency authorization in the US. The first mRNA-based vaccine for COVID-19 to get approved is the BNT162b1 vaccine developed by BioNTech company in collaboration with the Pfizer company. This vaccine exploits a lipid nanoparticle (LNP)-formulated nucleoside-modified mRNA that encodes the RBD of SARS-CoV-2 S protein [97]. The mRNA in this vaccine is modified with single nucleoside incorporations of 1-methylpseudouridine, which reduces the immunogenicity of the mRNA in vivo and enhances its translation [98]. The results from phase I/II showed that the BNT162b1 elicited RBD-binding and NAbs. This vaccine has been reported to trigger the production of T helper type 1 (1_H1)-skewed T cell immune responses with RBD-specific CD8$^+$ and CD4$^+$ T cells and robust release of immune-modulatory cytokines such as IFN$_y$, [97, 99] which is essential for several antiviral responses and inhibits replication of SARS-CoV-2

[99]. On 18, November 2020, it was announced that the BNT162b2 vaccine exhibits more than 95% effectiveness in preventing the disease in participants of 16 years or older [100]. This result was based on examining a total of 178 confirmed COVID-19 cases, out of which 162 cases were in the placebo group and the remaining were in the BNT162b2 group [100]. Also, nine severe COVID-19 cases were in the placebo group with one of them being in the BNT162b2 group. No severe adverse effects were detected among the 43,000 enrolled participants [100].

Another leading mRNA vaccine is the mRNA-1273, co-developed by researchers at the National Institute of Allergy and Infectious Diseases (NIAID) and Moderna (Cambridge, MA). The mRNA-1273 vaccine recently received FDA approval for emergency use. Moderna started its clinical testing just two months after sequence identification of SARS-CoV-2. This vaccine is made out of synthetic mRNA encapsulated in LNPs that encodes for the full-length, pre-fusion stabilized S protein of SARS-CoV-2. Two proline substitutions in the vaccine mRNA at amino acids 986 and 987, located in the central helix of the S2 subunit, keep the protein in its prefusion conformation [101]. The mRNA is also modified to increase the half-life and its translation, as well as to prevent the activation of interferon-associated genes upon cell entry [102].

Their phase I clinical trial report showed that NAbs were detected in 45 participants after two doses of immunization. In addition, antibody titers were higher than convalescent serum after two doses of vaccination in immunized individuals. There were some systemic adverse effects such as headache, fatigue, myalgia, chills, and pain after the second dose of vaccination, particularly with the highest dose. However, no grade 4 adverse effects were reported [102, 103]. Based on these results, they concluded that 100 μg dosage can lead to acceptable immune response. In addition, the vaccine was tested in elderly participants (55 or older) and their results showed that 100 μg doses can lead to higher binding and NAb titers as compared to 25 μg dose, and the adverse effects were moderate in these elderly participants. On 16 November 2020, the results from their phase III trial were reported. Out of 95 participants who had symptomatic COVID-19, five were from the vaccinated group and the remaining participants were from the placebo group. The vaccine efficacy was estimated to be 94.5% without any significant safety concerns [104].

Both Moderna's and Pfizer-BioNTech vaccines have shown efficacy levels near 95%. Unlike the Pfizer vaccine which needs to be kept at −75 °C, Moderna's vaccine does require really cold temperatures. Moderna's vaccine can be kept at about −20 °C and can be kept in a refrigerator for 30 days before it expires. Therefore, Pfizer's vaccine can be more suitable for places with established infrastructure like hospitals.

5.4.5 DNA-Based Vaccine

DNA-based vaccines have a lot of potential due to their ability to induce humoral and cell-mediated immune responses, low-cost manufacturing, and their long shelf life, which makes them suitable for use in endemic areas [105, 106]. DNA vaccines consist

of genes or fragments of viral antigens that are transferred to the host cells via DNA plasmid vectors. Once the genetic material is translocated to the host cell's nucleus, the transcription of the gene is triggered. The antigen-presenting cells (APCs) are the main target cells to acquire the genetic material [107]. One advantage of DNA-based vaccines is that the native conformation and post-translational modification will be recapitulated since the antigens are produced in the target cells. One of the disadvantages associated with this type of vaccine is its low immunogenicity and that the DNA molecule must be able to first cross the nuclear membrane barrier and then get transcribed. Therefore, enhancing the efficacy of these vaccines by using an adjuvant or a multiple shot might be required. In addition, a major safety concern is the integration of DNA vaccines with the host DNA, which may cause mutagenesis and oncogenesis [81].

5.4.5.1 SARS-CoV and MERS-CoV DNA Vaccines

A number of DNA-based vaccines for SARS-CoV have been developed [108–111]. All of these vaccines have shown to produce antibody and cell-immune responses. Among the S, E, and N antigens, the S protein-based SARS-CoV DNA vaccine has shown to induce a protective immune response. Yang and colleagues have reported that a DNA vaccine encoding full-length S protein can induce T cell and NAb responses in the mouse model. In addition, alternative forms of the S protein were analyzed by DNA immunization. All of these vaccines were able to elicit strong immune responses mediated by the CD4+ and CD8+ cells. [108]. Furthermore, the expression vector encoding a form of S that includes its transmembrane domain induced NAb production [108]. Prime-boost strategies can be employed to augment the strength of the S protein-based SARS-CoV DNA vaccine. For instance, DNA vaccine augmented with recombinant S protein booster has shown to induce higher NAbs titers than DNA or protein subunit vaccine alone [112]. In addition, combining DNA and whole-inactivated SARS-CoV vaccines can increase the antibody response and induce a better Th1-skewed immune response [113].

A few MERS-CoV DNA vaccines have been developed. Muthmani and co-workers have devised a full-length S protein-based MERS-CoV DNA vaccine, GLS-5300 or INO-4700 that can induce strong cellular immunity and NAbs in mice, macaques, and camels. Vaccinated macaques were immune against MERS-CoV challenge without exhibiting histopathological or radiological evidence of pneumonia [114]. Therefore, a phase I study based on the GLS-5300 vaccine was done to assess its safety and immunogenicity in humans. The vaccine showed robust immunogenicity in 85% of participants after two vaccinations. In addition, the vaccine was well tolerated with no serious adverse events were reported [115].

In addition to full-length S protein DNA vaccines, the S1 subunit can also serve as a suitable target for DNA vaccine development against MERS-CoV. In a study done by Al-Amri and co-workers, the immunogenicity of full-length S-based and S1-based MERS-CoV vaccines was compared by using the same expression vector. It was found that plasmids expressing full-length pS1 immunization elicited a balanced

Th1/Th2 response and higher levels of all IgG isotypes compared to pS vaccination. Based on these results, it can be concluded that vaccines expressing S1-subunit of the MERS-CoV could be a more suitable target than full-length S protein [116].

5.4.5.2 COVID-19 DNA Vaccines

Thus far, five SARS-CoV-2 DNA vaccines are under clinical trials. The most clinically advanced SARS-CoV-2 DNA vaccine is the Invivio's vaccine, INO-4800, which has published results on MERS-CoV and SARS-CoV-2 DNA vaccines. This vaccine employs a plasmid pGX9501 designed to encode the full-length SARS-CoV-2 S protein and is administered intradermally via electroporation of the skin by a device called CELLECTRA [117, 118]. Pre-clinical studies of this vaccine have shown that it can induce NAb that inhibits the binding of SARS-CoV-2 S protein to the ACE2 receptor and elicits Th1-skewed immune responses in animal models [117, 119].

5.4.6 Protein Subunit Vaccine

Protein subunit vaccines are based on recombinant antigenic proteins or synthetic polysaccharides [120]. These recombinant proteins are synthesized in various expression systems, including insect cells and mammalian cells (CHO cells), yeast, or plants [121–123]. They are easy to manufacture and safer compared to some viral vector vaccines and inactivated or live-attenuated virus vaccines that include infectious components of the virus. Protein-subunit vaccines provide a strong immune response targeted towards key parts of the virus without including any virulent components of the virus [120]. Therefore, eliminating concerns of virulence recovery or pre-existing immunity [124]. One of the limitations associated with these vaccines is their low immunogenicity. Thus, an adjuvant or a booster shot may be required to potentiate the vaccine-induced immune response and to enhance the immunomodulatory cytokine response [125].

   Among the SARS-CoV-2 structural proteins, the prime target for subunit vaccines is the S protein especially RBD, S1, and S2 as in the case of MERS and SARS vaccines. This is because the S protein is regarded as the most suitable antigen to induce the production of NAbs. The S protein of CoVs, particularly the RBD, is known to induce NAbs and T cell immune responses [126–128]. Therefore, RBD is a promising target for COVID-19 vaccine development, and previous investigations from using RBD-base vaccines for SARS-CoV and MERS-CoV can provide information based on the design of RBD-based vaccines against SARS-CoV-2. The S protein is a dynamic protein and has two conformational states: pre-fusion state and post-fusion state. In order to trigger good quality antibody responses, the antigen must maintain its surface chemistry of the original pre-fusion spike protein [129]. It is known that recombinant S protein vaccines could have improper epitope confirmation unless mammalian cells are utilized for their production [130].

5.4.6.1 SARS-CoV and MERS-CoV Protein Subunit Vaccine

None of the SARS-CoV protein subunit vaccines have proceeded to the clinical phase despite showing potent antibody responses and protective immunity against infection in animal models. Previous studies indicate that vaccines based on the full-length S protein, trimeric S protein, and its antigenic fragments including S1, RBD, and S2 subunit can all provide protection against SARS-CoV. He et al. showed that immunized mice with full-length recombinant S protein or its extracellular domain vaccines develop increased titers of anti-S antibodies with strong NAbs activities against SARS-CoV [131]. Even though full-length S protein vaccines have the ability to induce strong immune responses, some in vitro studies have found that immunization with SARS-CoV full-length S protein can cause antibody-mediated enhancement viral infection, raising safety concerns for the development of these types of vaccines against CoVs [132, 133].

The S protein RBD-based vaccines have shown high-titer NAbs with no apparent adverse effects [134–136]. In addition, RBD-based vaccines can induce S-specific antibodies that can last for almost a year [134] and can induce the production of RBD-specific IFN-γ which induce cellular immune responses in mice [135]. Therefore, the RBD serves as a promising vaccine target for inducing NAbs against viral infection. Guo et al. investigated the immune responses against the S2 domain of SARS-CoV in BALB/c mice and noticed that the S2 domain could elicit specific cellular and humoral responses with little NAb against infection by SARS-CoV [137].

Besides the S protein, the N and M proteins have been also utilized as the target antigen in developing subunit vaccines against SARS-CoV [123, 138]. Liu et al. have shown that the N protein of SARS-CoV is immunogenic in the mouse and macaque models. They devised a recombinant N (rN) protein vaccine formulated with ISA/CpG adjuvants. This vaccine-elicited potent Th1 immune responses and the immunodominant B-and T-cell epitopes of the rN protein were present in both mice and macaques [138]. Zheng and colleagues formulated a plant-expressed SARS-CoV rN protein-based vaccine. This vaccine also induced potent humoral and cellular responses in mice [123]. The N-based subunit vaccines have also shown their efficacy in eliciting specific antibody responses. However, the protective efficacy of non-S protein-based SARS-CoV vaccines is still unclear [139, 140].

Most protein subunit vaccines against MERS-CoV are based on the RBD of the S protein. The MERS-CoV RBD-based vaccines have shown potent immunogenicity and elicited strong neutralizing antibodies, cell-mediated immunity, and protective effect against MERS-CoV challenge [127, 141, 142]. Lan et al. evaluated a recombinant RBD protein vaccine in the rhesus macaque model and noticed robust immunological responses including the production of NAbs after rRBD vaccination. In addition, the rRBD vaccine reduced tissue impairment and clinical side-effects in monkeys [127]. Tai and co-workers have reported that the RBD of MERS-CoV in its native trimeric form can induce strong RBD-specific NAb in mice against challenge [141]. In addition, the recombinant RBD from various MERS-CoV strains can elicit NAbs in animals that cross-neutralize with different human and animal MERS-CoV

strains [142]. Therefore, the RBD domain is a promising target for protein subunit vaccines against CoVs.

5.4.6.2   COVID-19 Protein Subunit Vaccine

Similar to SARS-CoV and MERS-CoV, COVID-19 protein subunit vaccines account for most of the vaccines that are currently under development. Most COVID-19 protein subunit vaccines contain full length or portions of the SARS-CoV-2 S protein. Currently, there are 18 COVID-19 subunit vaccines in clinical trials. An example is the NVX-CoV2373 vaccine developed by Novavax. NVX-CoV2373 is a nanoparticle-based immunogenic vaccine based on recombinant expression of the CoV trimeric full-length S protein stabilized in the prefusion conformation [143]. This recombinant protein is optimized in the baculovirus (Sf9) insect cell-expression system. During the pre-clinical studies, it was shown that low-dose NVX-CoV2373 supplemented with the Matrix-M1^{TM} adjuvant induces NAbs and high levels of anti-S protein antibodies which block the hACE2 receptor-binding domain in mice and non-human primate [144]. The vaccine also elicits CD4$^+$ and CD8$^+$ T cells, CD4$^+$ T helper cells and induced the production of antigen-specific germinal center (GC) B cells in the spleen [144]. More importantly, vaccinated non-human primates had little to zero viral shedding in either upper or lower respiratory tracks [145]. In Phase I-II trial, the vaccine-induced binding and NAbs in all participants. In addition, compared to the placebo group and the unadjuvanted 25 μg dose group, both adjuvanted regimens induced higher levels of NAb titers [146]. Currently, the NVX-CoV2373 vaccine is being evaluated in the Phase III trial.

5.5   Other Vaccine Platforms

5.5.1   Virus-like Particles (VLPs)

Virus-like particles also known as VLPs are composed of some key structural viral components that are either admixed or co-expressed in a manner that resembles the conformation of the native virus. VLPs are non-infectious and non-replicating due to the lack of genetic materials [147]. Compared to protein subunit vaccines, VLP vaccines have better immunization responses. Also, the manufacturing process of VLP vaccines is simpler and more convenient than inactivated or attenuated vaccines since the inactivation step is skipped and there is no need for the live virus. Currently available VLP-based vaccines marketed for human use are against human papillomavirus and hepatitis B virus [148].

VLPs for CoVs are formed when the viral protein S, M, and E, with or without N, are co-expressed in eukaryotic cells [149]. The N protein encapsulates the viral genome into virions and does not have an essential role in SARS-CoV-2 VLPs

assembly. The S protein present on the surface of the VLPs allows them to bind and fuse into ACE2$^+$ cells similar to the native virus and elicits immune response [150]. Similar to subunit and inactivated viral vaccines, VLPs usually require multiple doses and an adjuvant because of their poor immunogenicity.

5.5.1.1   SARS-CoV and MERS-CoV VLP Vaccines

VLP vaccines have shown satisfactory results in eliciting both humoral and cellular immunity in SARS-CoV and MERS-CoV preclinical studies. Lokugamage et al. devised chimeric VLPs composed of the S protein of SARS-CoV and mouse hepatitis virus E, M and N proteins that elicit the production of NAb responses and reduce SARS-CoV virus shedding in mice lung [151]. In addition, no apparent lung pathology in the chimeric-VLP-treated mice was reported when compared to the negative control mice [151]. Another study done on chimeric Sf9 cell-based VLPs consisting of SARS-CoV full-length S protein along with M1 protein of influenza virus expressed in the baculovirus insect cells has shown that chimeric VLPS can induce NAbs and provide protection against challenge in mice [152]. However, possible adverse effects of CoV VLP vaccines should be examined carefully. For instance, Tseng et al. employed the same chimeric VLPs as Lokugamage et al. and observed pulmonary immunopathology post SARS-CoV challenge [69]. Wang and colleagues have constructed recombinant baculovirus co-expressing the S, E, and M genes of MERS-CoV. The assembled VLPs were able to elicit robust antibody response and Th1-mediated immunity in rhesus macaques and can serve as a promising vaccine candidate [153]. In addition, they devised a chimeric canine parvovirus (CPV) VLP expressing the RBD of MERS-CoV self-assembled into chimeric spherical VLP (sVLP). The sVLP vaccine was shown to induces RBD-specific antibody response and T-cell immunity in mice [154].

5.5.1.2   COVID-19 VLP Vaccines

Currently, only two VLP-based COVID-19 vaccines are in the clinical trial. None of them has publicly reported their vaccine studies until now. One of them is a plant-derived VLP called CoVLP vaccine developed by Medicago, a Canadian pharmaceutical company. The CoVLP vaccine is composed of recombinant S protein expressed as VLPs. Medicago exploits living plants as bioreactors to produce VLPs. A synthetic gene containing a fragment of SARS-CoV-2 genes is transfected to a species of tobacco by means of a bacterial vector. The expressed VLPs can then be purified by various purification techniques. The Phase I results showed that after receiving two doses of Medicago's COVID-19 adjuvanted vaccine, all the participants developed NAbs antibody responses [155]. This vaccine has been tested with two separate adjuvants: GSK's propriety pandemic adjuvant technology and Dyanavax's CpG 1018^{TM}. These adjuvants improved humoral and cellular responses

compared to the non-adjuvanted formulation. Medicago's COVID-19 vaccine candidate is currently in Phase II/III clinical trials and the company has estimated to hold a production capacity of 10 million doses a month [156]. The second VLP vaccine is being developed by the Serum Institute of India, which recently entered phase I/II trial in Australia.

5.5.2 Bacillus Calmette Guerin (BCG) Vaccine

Although several COVID-19 vaccines are under clinical trials with some of them being in advanced stages, scientists have been also testing existing licensed vaccines to fight COVID-19. One example is the Bacillus Calmette Guerin (BCG) vaccine, which contains an attenuated strain of the bovine tubercle bacillus *Mycobacterium bovis*, which is an old age vaccine used for the prevention of tuberculosis. Currently, about 100 million children are vaccinated annually worldwide. However, the vaccine does not exhibit satisfactory results for the adult pulmonary tuberculosis but provides a broad protection against other diseases [157]. This BCG vaccine has the ability to exert a potent nonspecific immunity (off-target protection) against viral and bacterial infections [158]. The increase in immunogenicity against pathogens induced by this vaccine is due to heterologous effects on adaptive immunity, such as T cell-mediated cross-reactivity, as well as trained immunity [159].

To see whether BCG-induced immunity could influence the adaptive immune response against SARS-CoV-2, Urbán et al. conducted a study in which the T-cell and B-cell epitopes of the BCG strain Pasteur 1173P2 were compared with T-cell and B-cell epitopes of SARS-CoV-2 to find similar epitopes that might induce adaptive cross-immunity and to explain the protective qualities of BCG vaccination against SARS-CoV-2. They discovered shared MHC-I restricted T-cell epitopes and B-cell epitopes between SARS-CoV-2 and BCG-Pasteur which might induce cross-immunity. Their results suggest that BCG can be a potential preventive immunotherapy against COVID-19 and to enhance innate immunity [160].

BCG vaccine has shown to offer protection against COVID-19 and reduced mortality in countries with a routine BCG vaccination program, even when the BCG vaccination was performed during childhood [161]. For instance, in South Asian regions where the BCG vaccine is administered at birth, delayed or less infection rate and less deaths due to COVID-19 infections have been reported [162]. A few other studies have also suggested that there is a correlation between BCG and the SARS-CoV-2 epidemic [163, 164], while others have disbelieved its relation to COVID-19 mortality and morbidity [165]. Therefore, whether the BCG vaccine is effective against COVID-19 is highly debatable and further elucidation on the use of the BCG vaccine as a preventive therapy against SARS-CoV-2 infection is still required.

5.6 Conclusion

Coronaviruses are expected to continue to cross species barriers and cause severe illness in humans. Undoubtedly COVID-19 will not be the last pandemic of the century due to changes in climate and the increased interactions of humans with animals. Therefore, the development of novel and effective technology platforms is required to expedite vaccine development. With record numbers of human causalities and confirmed cases being reported daily, an anti-SARS-CoV-2 vaccine is imperative. While vaccines against SARS-CoV-2 are being developed at a fast pace, the special nature of this virus and safety concerns make the development of these vaccines very challenging. Some individuals get infected with SARS-CoV-2 while remaining asymptomatic, whereas some exhibit severe illness and succumb to the disease. Given the variability of host immune responses to SARS-CoV-2, there is no guarantee that vaccination could provide uniform long-lasting immunity in whoever gets vaccinated. However, with the increasing accumulation of knowledge about SARS-CoV-2 and massive efforts from the scientific community to develop anti-SARS-CoV-2 vaccines, the COVID-19 pandemic will come to an end eventually.

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