

Review Article

Protective Immunity against SARS Subunit Vaccine Candidates Based on Spike Protein: Lessons for Coronavirus Vaccine Development

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The recent outbreak of the novel coronavirus disease, COVID-19, has highlighted the threat that highly pathogenic coronaviruses have on global health security and the imminent need to design an effective vaccine for prevention purposes. Although several attempts have been made to develop vaccines against human coronavirus infections since the emergence of Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2003, there is no available licensed vaccine yet. A better understanding of previous coronavirus vaccine studies may help to design a vaccine for the newly emerged virus, SARS-CoV-2, that may also cover other pathogenic coronaviruses as a potentially universal vaccine. In general, coronavirus spike protein is the major antigen for the vaccine design as it can induce neutralizing antibodies and protective immunity. By considering the high genetic similarity between SARS-CoV and SARS-CoV-2, here, protective immunity against SARS-CoV spike subunit vaccine candidates in animal models has been reviewed to gain advances that can facilitate coronavirus vaccine development in the near future.

1. Introduction

Before 2003, coronaviruses were known to cause only common cold in humans, but currently, they are the reason for three outbreaks in the 21st century: 2003 Severe Acute Respiratory Syndrome (SARS), 2012 Middle East Respiratory Syndrome (MERS), and 2019 Coronavirus Disease (COVID-19) [1]. Newly emerged virus, named SARS-CoV-2, was first discovered in December 2019, and in a short span of time, it has been announced as a global pandemic. On 2 June 2020, the virus spread has been noted in 213 countries and total confirmed cases climbed above 6.1 million with over 376000 deaths [2]. Despite numerous attempts to develop a vaccine against human coronavirus infection, there is no commercial vaccine available yet. Safety considerations and the degree of extensive diversity in antigenic variants are some of the potential reasons that limit coronavirus vaccine development [3].

Coronaviruses are enveloped positive-sense RNA virus, which are host-specific and can infect the human and a large number of animals [4]. Nucleotide substitution has been pro-

posed to be one of the most important mechanisms of viral evolution in nature, and it is not necessarily surprising for an RNA virus that is a measurably evolving population over a short time, to have distinct variants [5, 6]. Coronaviruses are phylogenetically classified into four major genera: *Alpha-coronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*, and all three highly pathogenic coronaviruses for human belong to the *Betacoronavirus* genus. All epidemiological, pathophysiological, and immunological researches, which have been done on *Betacoronaviruses* may shed light on the understanding of SARS-CoV-2. This newly emerged virus is genetically more closer to SARS-CoV than MERS coronavirus with the existence of 380 amino acid substitution differences in the encoded proteins [7–9]. Therefore, previous advances made in developing SARS-CoV vaccines could be exploited for designing a vaccine not only for current COVID-19 pandemic but also for other highly pathogenic coronaviruses, so-called universal vaccine. This vaccine can be effective against all strains of the virus as a consequence of cross-protective immunity against conserved antigens.

Moreover, the induced broad immunity can prevent the human from infection in the time of emerging a novel strain of the virus.

Inactivated virus and subunit vaccine technologies have been used to develop SARS-CoV vaccines. The inactive virus strategy is limited by safety considerations, as large quantities of the pathogenic virus are required directly in the vaccine preparation procedures. In contrast, the subunit vaccine that only relies on the antigen of interest by using recombinant technology is considered as a more reliable and safe technique. However, low immunogenicity might be a drawback in subunit vaccine development due to poor presentation to the immune system or incorrect folding of the antigens, but adjuvants can be involved in vaccination to boost immune responses and increase immunogenicity [10]. Alternatively, knowledge of the various viral proteins in inducing immune responses would facilitate subunit vaccine preparations [11]. The genome of coronaviruses includes a variable number of open reading frames that encode accessory proteins, non-structural proteins, and structural proteins [12]. Most of the antigenic peptides are located in the structural proteins [13]. Spike surface glycoprotein (S), a small envelope protein (E), matrix protein (M), and nucleocapsid protein (N) are four main structural proteins. Since S-protein contributes to cell tropism and virus entry and also it is capable to induce neutralizing antibodies (NAb) and protective immunity, it is recognized as the most important target in coronavirus vaccine development among all other structural proteins [3, 14–17]. Moreover, amino acid sequence analysis has shown that S-protein contains conserved regions among the coronaviruses, which may be the basis for universal vaccine development [18, 19]. This article reviewed the *in vivo* protective immunity of SARS-CoV S-protein vaccine candidates to provide an immunological evidence base that can aid coronavirus vaccine development in the future.

2. Search Strategy and Selection Criteria

References for this review were identified through searches of Scopus and PubMed for articles published between 2003 and 2020, using combinations of the terms “Severe Acute Respiratory Syndrome,” SARS, SARS-CoV, vaccin*, immuniz*, immunis*, innocul*, develop*, design*, immunogenicity, and “immune response.” The articles that indicated *in vivo* protective immunity study have been selected from the search result. The final reference list was generated based on this selection and relevant articles to the subtopics covered in this review, to highlight the immunological evidence base for coronavirus vaccine development and provide recommendations for navigating the limitations.

3. Coronavirus Spike Protein: Promising Immunogen for Universal Vaccine Development

The trimetric S-protein of coronaviruses is the reason for the crown shape of the viral particles, from which the name of the virus was given [20]. This structural protein belongs to class I viral fusion proteins and plays essential roles in the cell

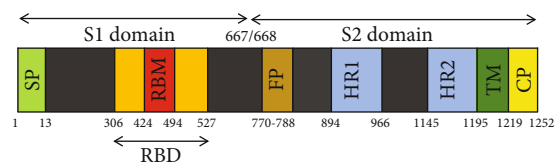


FIGURE 1: Schematic structure of the spike protein. Abbreviations: SP: signal peptide; RBD: receptor-binding domain; RBM: receptor motif binding; FP: fusion peptide; HR1: heptad repeat domain 1; HR2: heptad repeat domain 2; TM: transmembrane domain; CP: cytoplasmic domain. Numbers indicate amino acid sequence.

receptor binding, host tissue tropism and, pathogenesis. S-protein is cleaved at the S1/S2 site by host cell proteases during infection. Following cleavage, the protein is divided into an S1-ectodomain that recognizes a cognate cell surface receptor and an S2-membrane-anchored protein involved in viral entry [21]. The S1-protein contains a receptor-binding domain (RBD), which recognizes the angiotensin-converting enzyme 2 (ACE2) as one of the main receptors in the host [22]. RBD contains receptor-binding motif (RBM) that makes all the contacts with ACE2 and RBD core that is mostly conserved among coronaviruses [21, 23]. Binding of the RBD to the ACE2 receptor provokes S2 conformational changes [24]. S2 contains membrane-anchoring, fusion peptide, and two heptad repeat domains (HR1 and HR2), which plays a key role in virus assembly and entry (Figure 1) [25–27]. The studies showed that vaccines based on S-protein can evoke the immune system to induce humoral and cellular responses and protect vaccinated animals from SARS viral challenges (Table 1) [17, 28–32].

In general, vaccine development for pathogens with a high rate of antigenic changes in their surface antigens such as SARS-CoV is difficult and complicated due to the emergence of vaccine escape variants over time. Hence, identification of conserved antigenic determinant on S-protein, which can induce cross-protective immune responses, may have implications in the development of an effective vaccine against all pathogenic coronaviruses. There is a limited number of SARS vaccine studies based on conserved antigens in which the potential protective immunity was not evaluated. For example, Zhang et al. identified a highly conserved antigenic determinant on S2 (amino acid 803 to 828) by using sera from convalescent SARS patients that could induce the S2-specific antibody with *in vitro* neutralizing activity against the SARS-CoV pseudovirus [33]. Other laboratory results indicated that peptides from the HR2 region of the S2 domain can block SARS-CoV infection *in vitro* [34, 35]. However, the potential protection was not evaluated by challenge experiments, and further investigation is required to check *in vivo* neutralizing activity and also cross-protective immunity of these antigens for broad immune responses. Moreover, recent genome analysis disclosed that S2 and RBD core domains of SARS-CoV-2 are highly conserved, supporting the idea of universal vaccine design [8, 9, 18, 36–38]. It is notable that functional sites of S2 domain might be buried under S1 in the native state of the virus structure, and it may affect the accessibility of the S2 antigens for the

TABLE 1: In vivo immunological responses against SARS-CoV spike-based vaccine candidates.

Vaccine type	Vaccine compound	Production system	Adjuvant	Route	Animal model	Humoral	Immune response cellular	protective immunity	Side effect	Note	Reference
Protein based	S-protein Residues 14-762	Insect cells	Saponin: QS21 Ribi: MPL+TDM	S.C.	Mice	✓	✓	✓	N/A	The better significant protection obtained with the QS21.	[31]
	RBD-Fc (193 aa) Residues 318- 510	Mammalian 293T cells	Freund's complete adjuvant. Boost: Freund's incomplete adjuvant	I.M.	Mice	✓	N/A	✓	Only one vaccinated mouse from five had mild alveolar damage in the lung tissues.		[17]
	RBD (193 aa)	Mammalian 293T cells	Sigma adjuvant system	S.C.	Mice	✓	N/A	✓			[28]
	Residues 318-510	Insect cells				✓	N/A	✓	N/A		
		<i>E. coli</i>				✓	N/A	✓			
	RBD (193 aa) Residues 318-510	(CHO)-K1 cells	Freund's complete Boost: Freund's in-complete	S.C.	Mice	✓	✓	✓	N/A	Virus replication detected in two of five mice.	[70]
	RBD (219 aa) Residues 318-536	(CHO)-K1 cells	Freund's complete Boost: Freund's in-complete	S.C.	Mice	✓	✓	✓	N/A		[66]
	Truncated S-protein	Insect cells	Protollin	I.N.	Mice	✓	N/A	✓	No significant histopathology findings	Significantly lower virus titers in I.N. route than I.M. due to induction of IgA	[77]
SARS-VLPs											
			Without adjuvant	I.M.	Mice	✓	N/A	✓	No weight loss.		
S-protein Residues 1-1196aa +	Insect cells		Without adjuvant	I.N.	Mice	✓	N/A	✓	Minor weight loss (3-4%)		[59]

TABLE 1: Continued.

Vaccine type	Vaccine compound	Production system	Adjuvant	Route	Animal model	Humoral	Immune response cellular	protective immunity	Side effect	Note	Reference
Influenza	M1 protein Residues 531-568aa		Alum	I.M.		✓	N/A	✓	No weight loss		
	S-protein Residues 1-1196aa	Insect cells	Without adjuvant	I.M.	Mice	✓	N/A	✓	7.5-15% Weight loss		[59]
			Without adjuvant	I.N.		✓	N/A	x			
			Alum	I.M.		✓	N/A	✓	No weight loss		
			AuNPs			✓	N/A	✓	Ruffled fur and body weight loss for all groups. Eosinophilic infiltrations in the lungs of both S-protein and S +AuNP immunized mice		[63]
S-protein Residues 1-1194	Insect cells	TLR agonists	S.C.	Mice	✓	N/A	✓				
Viral vector based	MVA-S Full length	Primary CEF cells	Without adjuvant	I.M. I.N.	Mice	✓ ✓	N/A N/A	✓ ✓	No obvious disease		[91]
	MVA-S Full length	BHK21 cells	Without adjuvant	S.C. and I.P.	Ferrets	✓	N/A	X	Enhanced hepatitis in ferrets after SARS-CoV challenge	No significant effects on the level of SARS-CoV replication in ferrets.	[61]
	MVA-S Full length	BHK21 cells	Without adjuvant	S.C. and I.P.	Ferrets	✓	N/A	X	No enhanced pathology during SARS-CoV infection of liver	Presence of virus in all the clinical specimens.	[60]
	Attenuated parainfluenza virus (BHPV3)-S protein Full length	LLC-MK2 cells	Without adjuvant	I.N.	African green monkeys	✓	N/A	✓	No evidence of immune-mediated enhancement of infection or disease		[87]
	NDV-BC/S Full-length	Embryonated chicken eggs	Without adjuvant	I.N. and I.T.	African green monkeys	✓	✓	✓	No evidence of enhanced clinical disease	Numbers of blood SARS-CoV specific T-cells was low	[90]

TABLE 1: Continued.

Vaccine type	Vaccine compound	Production system	Adjuvant	Route	Animal model	Humoral	Immune response cellular	protective immunity	Side effect	Note	Reference
	NDV-VF/S Full-length					N/A	N/A	X			
	NDV-BC/S1 Residues 1-762										
	AAV-RBD (193-aa)			I.M.	Mice	✓	✓	✓		I.N. vaccination induced much stronger responses than I.M. route but with the same protective immunity effectiveness.	[45]
	Residue 318-510	HEK293T cells	Without adjuvant	I.N.		✓	✓	✓	No antibody-mediated disease enhancement		
	VSV-S Full length	BHK-21 cells	Without adjuvant	I.N.	Mice	✓	N/A	✓	No enhancement of infection		[86]
DNA based	pCI-WPRE-S Full length	Mammalian 293T cells	Without adjuvant	I.M.	Mice	✓	N/A	✓	N/A		[79]
	Plasmid DNA-S Full length	Synthetic Human preferred codons	Without adjuvant	I.M.	Mice	✓	✓	✓	No enhancement of infection	Protection was mediated by a humoral immune mechanism.	[78]

Abbreviations: I.M.: intramuscular; I.N.: intranasal; I.P.: intraperitoneal; I.T.: intrathecal; N/A: not available; X: not induced. *Survival rate is given under the protective immunity column if it was indicated in the reference article.

immune system but despite this shielding effect, T cell immune response to S2 domain that has been reported from fully recovered SARS patients suggests that it can be a candidate antigen for coronavirus vaccine development [39]. In addition, extra caution should be taken in designing vaccines that specifically target the S2 domain as disease severity can be augmented by elevating viral fusion to host cells at the early stage of the infection before activation of prior vaccine-induced immune responses [40, 41].

4. What Immune Responses Are Required after Coronavirus Vaccination?

Based on clinical studies, we can understand how the immune system of the patients reacts to coronavirus [42, 43]. In general, the viral infection will be responded with humoral and cellular responses, which will be initiated by the innate immune system followed by the adaptive immune response. The latter consists of B cells that produce antibodies and T cells that kill virus-infected cells and both induce memory responses [44]. Generally, NABs are the key factor for protecting human and animal models from coronavirus infections [17, 28, 45]. Sera of SARS patients indicate the presence of antibodies against SARS-CoV S, M, E, and N proteins, but NAb is only induced by S-protein [46]. Although anti-N antibody is present in SARS patient sera at a high-level and persisted for a long time (30 weeks after infection), recombinant N-protein could not induce a detectable NAb in rabbit to neutralize the SARS-CoV infection [11]. Besides, deficient antibody production against S-protein in SARS-CoV infected patients with fatal outcomes has been reported that emphasizes the crucial role of the S-protein NAb in SARS immunity [47]. Antibodies from the sera of 623 SARS patients were able to neutralize viruses containing S-protein from four different SARS-CoV strains, suggesting the potential cross-reactivity of these antibodies [48]. SARS-CoV-specific NABs peak at four months and after that, gradually decline over time, indicating a reduction of the memory B cells against the virus [46, 49–51].

On the other hand, memory CD8 T cells can persist for at least six years in SARS patients who had recovered from the infection. These T cells are able to recognize and remove the infected cells in the lungs of patients. Whereas the memory B cell response and consequently NABs are short-lived in SARS patients, generating long-lived memory T cell response is important, and it can be a complementary strategy in SARS vaccine design [46]. Interestingly, among the SARS-CoV CD8 T cell epitopes derived from different structural proteins, most peptides belong to S-protein [52]. In a clinical phase I study, a truncated S-protein DNA vaccine produced in bacterial cells induced SARS S-protein specific T cell response in all subjects and NAB responses detected in 80% of the individuals [53]. A DNA S-protein vaccine induced both CD4 and CD8 T cell responses, and S2 fragment encoding amino acid 681-980 elicited specific Cytotoxic T lymphocyte (CTL) response in animal models [41, 54]. In another preclinical study, S-protein specific memory CD8 T cells could protect the mice against viral lethal challenge in the absence of NABs, indicating that protection was elicited by

memory CD8 T cells [55]. Also, dysregulated innate immune response that is a critical factor in the pathogenesis of SARS-CoV can be negated by a potent T cell response [56]. Subsequently, the induction of cellular immune response is a goal for vaccine preparations as it plays an important role in antiviral immunity [53, 55, 57, 58].

Moreover, the studies suggested that mucosal immune responses represented by secretory IgA will also be important in the prevention of SARS-CoV infection [45]. Hence, intranasal (IN) immunization may be a preferable route to generate lung memory T cells and IgA specifically. However, IN immunization that produced local immunity in the upper respiratory tract but not in the lower tract where the virus replicates, performed less lung virus titer reduction than intramuscular (IM) route [59]. So, mucosal immune response localization should be considered while designing IN coronavirus vaccines. Eventually, future vaccines against coronaviruses should emphasize the generation of systematic and mucosal memory T cell responses and induction of long-lived NAb against S-protein for optimal protection and virus clearance in the time of infection. Further, cross-protective immunity needs to be considered in the aim of coronavirus universal vaccine design.

5. In Vivo Protective Immunity of SARS-CoV Spike-Based Vaccine Candidates

In this article, recombinant S-protein-based vaccines, which include full-length or fragment vaccines, DNA-based vaccine, and viral vector-based vaccines that induce protective immunity in animal models have been reviewed (Table 1).

5.1. Protein-Based Vaccine. Full-length S-protein vaccines might be able to induce unwanted immune responses resulting in antibody-mediated disease enhancement (ADE) that can cause inflammatory and liver damage or enhancing infection after being challenged with SARS-CoV in animal models [60–63]. On the other hand, it has been demonstrated that fragments of S-protein, such as truncated S-protein or RBD, have a great potential to be effective vaccine candidates against SARS with no evidence of harmful immune responses [28, 64, 65]. In several studies, Du and colleagues proposed SARS-CoV RBD as a fragment immunogen that can induce high titers of NAb and protective immunity in mice without immunopathological damages after being a challenge with the virus [17, 66, 67]. Administration of SARS-CoV recombinant S polypeptide (amino acids 14 to 762) elicited NAb in mice and protected the animals against upper and lower respiratory infection with SARS-CoV. This protein segment contains the RBD region as well as immunodominant and neutralizing epitopes [31, 68, 69].

To obtain highly expressed RBD economically and conveniently with biological functions, 193 amino acids of RBD (RBD-193) residues 318–510, have been expressed in different systems: mammalian cells 293T (RBD193-293T) and chinese hamster ovary K1 (RBD193-CHO-K1), insect cells Sf9 (RBD193-Sf9) and, *E. coli* (RBD193- Ec) [28, 70]. All RBD expressed proteins except RBD193-CHO-K1 induced complete protection against viral challenge in immunized mice.

Virus replication was detected in two of five mice which were vaccinated with RBD193-CHO-K1. Interestingly, by adding 26 amino acids at the C terminal of RBD193 while expressed in the same CHO-K1 cell line (RBD219-CHO-K1), full protection against SARS-CoV challenge was observed due to high titers of RBD-specific antibody production. The extension may affect RBD structure into more immunogenic or more stable conformation, which could cause the induction of more potent protective antibodies in animal models compared to RBD193-CHO-K1. Cellular immune responses were also detected in RBD193-CHO-K1 and RBD219-CHO-K1 vaccinated mice [28, 70]. Although the mammalian expressed RBD elicited stronger NAb responses than those expressed in insect and *E. coli*, Du and colleagues suggested any immunogens that elicited the serum NAb titers of >1:500 in vaccinated mice would be effective enough for protection. So RBD193-Sf9 and RBD193-Ec were also considered as effective vaccines against viral challenge in mice with mean value >1:700 for the serum NAb titers [28]. In general, insect and *E. coli* expression systems have lower production costs with a high productivity rate compared to the mammalian cell expression system, which make them more feasible for mass vaccine production [71, 72].

As the subunit vaccine may represent low immune responses, adjuvants or immunopotentiator can be used to increase immunogenicity by helping better presenting of immunogens to immune cells. The recombinant virus-like particles (VLP) have been shown to be an effective immunopotentiator and delivery system for foreign antigens in vaccine development. The repetitive antigen pattern on their surface can cause a stronger and broader induction of immune responses to the foreign antigen that is incorporated into the VLP [73, 74]. Liu et al. developed SARS S-protein VLP vaccine candidate with similar morphology and size to the SARS-CoV, and the IM immunization of the vaccine protected 100% of vaccinated mice from death and significantly reduced lung virus titer [59]. It is also important to concern about structural changes of immunogens that may reduce the immunogenicity after adjuvant implications in vaccine development. In one study, gold nanoparticles (AuNPs) containing S-protein showed weak protective ability against the SARS-CoV challenge compared to the control group because of the low induction of virus-specific IgG and NAb, which may be related to S-protein structural changes upon binding to AuNPs [63].

In the case of respiratory infections like SARS, both serum and lung immune responses are important, and it has been shown that relying on a systemic response may not be enough to reduce SARS-CoV infection in host [55, 75]. Mucosal immunization offers several advantages over other routes of antigen delivery, including convenience, cost-effectiveness, and induction of both local and systemic immune responses [64, 76]. One study indicated that only IN immunization of mice by truncated S-protein with protollin adjuvant induced antigen-specific IgA responses in lung lavage fluid while both IN and IM administrations of the vaccine elicited comparable systemic responses. Serum collected IgG was able to possess strong protective activity against SARS-CoV, but the virus titer in the lung of mice was much

lower for the IN vaccine compared to the IM route, and there was a qualitative correlation between the level of IgA and virus titer in the lung of animals. The fact that IM immunization developed a high level of serum antibodies but not detectable mucosal IgA with higher viral lung titer than IN route strongly indicates the essential role of the mucosal responses in SARS immunity [77].

5.2. DNA and Viral Vector-Based Vaccines. Numbers of DNA and viral vectored vaccines against SARS-CoV have been explored. A DNA vaccine that encodes full-length SARS S-protein induced NAb and T cell responses and stimulated protective immunity [78]. Also, S-protein-based DNA vaccine encoding posttranscriptional enhancer (pCI-WPRE-S) not only improved immunogenicity in mice but also lessen DNA vaccine amount in vaccination. 10 μg of the vaccine elicited NAb response equivalent to 25 μg of Yang et al. codon-optimized vector [78, 79]. The vaccine even at a dose as low as 2 μg protects immunized animal against challenge infection [79]. This modification of DNA vaccine can minimize the risk of autoimmune responses or integration of the foreign DNA into the host genome [80]. The DNA S-protein vaccine was able to elicit SARS-CoV-specific CD4 T cell responses in all tested individuals and CD8 T cell responses in 20% of subjects. Also, in prior clinical trials of DNA vaccines against HIV, Ebola, and West Nile virus, vaccine-specific CD4 T cell responses were detected in nearly all subjects, while the frequency of measurable CD8 T cell responses varied from 7% to 64% [53, 81–83]. This aspect of DNA vaccine-induced immunity will require additional considerations while designing a DNA vaccine for coronaviruses.

The live viral vectored vaccines do not involve the complete pathogen, and they are qualified to induce mucosal humoral immune responses that may not be easily happened by DNA or protein vaccines [84, 85]. A vesicular stomatitis virus (VSV) and an attenuated version of the human parainfluenza virus, a common respiratory pathogen in humans, both expressing the SARS-CoV S protein, were protective in animal models against SARS-CoV [86, 87]. However, any viral vector vaccine based on common pathogens in the population would bring the concern regarding significant existence of prior immunity, which may restrict the replication of the viral vector in immunized models and decline the immunogenicity [88, 89]. Newcastle disease virus (NDV) is another viral vector that is antigenically distinct from a common human pathogen, and its natural host is birds. Remarkably, inoculation of the respiratory tract of African green monkeys (AGM) with recombinant NDV encoding SARS S-protein was protective enough against a high challenge dose of SARS-CoV. Vaccination with two constructs of NDV (NDV-VF/S or NDV-BC/S) resulted in a titer of serum NAb that was equaled or exceeded to parainfluenza construct immunization results [87, 90]. Mice immunized with modified vaccinia virus Ankara that contains S-protein (MVA-S) developed NAb and exhibited little or no replication of SARS CoV in the upper and lower respiratory tracts with no obvious disease after inoculation [91]. However, NAb responses in ferret against MVA-S were not protective in animals, but produce strong ADE effects [60, 61].

As S-protein DNA-based vaccine, MVA-S, and a live SARS-CoV demonstrated significant protective immunity in mice, different replication kinetics for SARS-CoV in mice and ferret can be the most likely cause for the difference in protective efficacy in these two animals [61, 78, 91, 92]. Thus, extra caution should be taken in the proposed human trials of SARS vaccines due to the potential liver damage from immunization and virus infection.

The IN vaccination with adeno-associated virus (AAV) encoding SARS-RBD induced strong systemic humoral (IgG) and pulmonary humoral (IgA) responses with neutralizing activity as compared to IM vaccination. The immunization also protects BALB/c mice against SARS-CoV infection. Surprisingly, AAV-RBD vaccination induced stronger CTL responses both in the lung and the spleen, and no sign of ADE in the animals was observed. Besides low systematic antibody responses in IN vaccination compared to the IM route, higher protection against virus challenge was achieved. The protective efficacy of AAV-RBD vaccination against SARS-CoV infection is correlated with the antibodies level, especially lung IgA [45]. Therefore, compared with the IM route, IN vaccination may fulfill multiple criteria for an effective and safe SARS-CoV vaccine.

6. Conclusion

The development of effective SARS-CoV-2 vaccine is crucial in aid of our public health preparedness against the current COVID-19 pandemic. Improved understanding of the protective immunity of SARS S-protein vaccine candidates would provide the immunological evidence base for future vaccine production. Moreover, this review reveals that despite the evidence of cross-reactivity of spike protein antibodies from SARS patients, there is a profound gap for immune response cross-protective evaluation against different strains of the virus. Now, it is time to direct research toward universal vaccine development by focusing on conserved immunogens that elicit potential cross-protective immunity to reduce the global threat of SARS-CoV-2 and any other pathogenic coronaviruses. Such studies would include preclinical experiments and also early phase clinical trials to assess the vaccine's immunogenicity. On the other hand, the previous vaccine studies of coronavirus have shown that immunization could often lead to adverse effect, such as ADE in animal models. Thus, it is important to clearly understand the potential risks of coronavirus vaccines and extra caution must be taken while testing the designed vaccines in human trials. In general, optimization of the vaccination regimen and evaluation of different vaccine strategies may be helpful to improve vaccine safety.

Conflicts of Interest

There is no conflict of interest.

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