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From SARS-CoV to SARS-CoV-2: safety and broad-spectrum are important for coronavirus vaccine development

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ABSTRACT

The global pandemic of COVID-19 caused by SARS-CoV-2 (also known as 2019-nCoV and HCoV-19) has posed serious threats to public health and economic stability worldwide, thus calling for development of vaccines against SARS-CoV-2 and other emerging and reemerging coronaviruses. Since SARS-CoV-2 and SARS-CoV have high similarity of their genomic sequences and share the same cellular receptor (ACE2), it is essential to learn the lessons and experiences from the development of SARS-CoV vaccines for the development of SARS-CoV-2 vaccines. In this review, we summarized the current knowledge on the advantages and disadvantages of the SARS-CoV vaccine candidates and prospected the strategies for the development of safe, effective and broad-spectrum coronavirus vaccines for prevention of infection by currently circulating SARS-CoV-2 and other emerging and reemerging coronaviruses that may cause future epidemics or pandemics.

In December 2019, the outbreak of an unexplained pneumonia similar to severe acute respiratory syndrome (SARS) in Wuhan, China was reported by the Health Commission of Hubei Province, China. This severe respiratory illness was identified by multiple diagnostic methods as an infection by a novel coronavirus [1–4], which was temporarily denoted as 2019-nCoV by World Health Organization [5], and renamed "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2) by the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) [6]. The new coronavirus-associated pneumonia was designated as coronavirus disease 2019 (COVID-19) by WHO (https://www.who.int/emergencies/diseases/novelcoronavirus-2019 (accessed Feb 18, 2020)).

It has been originally reported that COVID-19 cases are associated with the exposure to a large seafood and animal market in Wuhan, suggesting an animal-to-human transmission. Later on, more epidemiologic data indicate a human-to-human transmission of SARS-CoV-2 [7–11]. As of April 24, 2020, there are 84324 confirmed cases, 4642 death cases in China, and 2,626,334 confirmed cases, 181,938 cases of death in other countries (http://2019ncov.chinacdc.cn/2019-nCoV/global.html). Currently, the mortality rate in China is about 5.5%, however, about 6.9% globally. In China, there are 1303 existing confirmed cases, of which 983 are asymptomatic and vast majority are imported cases right now (http://2019ncov.chinacdc.cn/2019-nCoV/index.html). Therefore, the mortality rate in China should be closer to the real one. Of course, the mortality rate is closely related to the capacity of the local health care system. Hubei province was the most-affected area in China for the outbreak of COVID-19. At that time, local medical resources were extremely scarce; thus the mortality rate in Hubei reached 6.62% while that of the remaining regions of China is only 0.8%.

Different groups estimated that the basic reproductive number (R_0) for SARS-CoV-2 was approximately 2.68 [12], 2.2 [13], and even 3.8–6.47 [14–16]. The U.S. CDC has just adjusted R_0 to 5.7, meaning that each patient has spread infection to another 5.7 individuals on average. While the R_0 of SARS-CoV was estimated to be 2–5 [17], it seems that the infectivity of SARS-CoV-2 is stronger than that of SARS-CoV. SARS was the first new infectious disease identified in the twenty-first century. This acute, and often severe, respiratory illness originated in the Guangdong province of China in November 2002

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[18]. By the end of June 2003, there were 8450 cases and 810 deaths caused by SARS (www.cdc.gov/mmwr/mguide_sars.html). The overall fatality of SARS is about 10% in the general population, but approximately 50% in patients aged 65 years old and older [19]. Although the mortality rate of COVID-19 is not as high as that of SARS in 2002–2003, the number of confirmed cases has markedly surpassed that of SARS. In addition, the incubation period of COVID-19 ranged from 1 to 14 days, with an average of 10 days, and the patients in the incubation period or presymptomatic infected-individuals could potentially transmit the virus to uninfected people, which makes the infectivity of SARS-CoV-2 far exceeds that of SARS-CoV. SARS-CoV-2 is mainly transmitted through droplets and close contact, while aerosol is also a potential mode of transmission. Additionally, people of all ages are susceptible to the novel coronavirus, while older males with comorbidities are most vulnerable and more likely to develop severe and even fatal respiratory diseases [7,20]. The most thorough and strict control measures, including social distancing, limiting the flow of people and transportation, and canceling public activities, have taken effect in China. But the pandemic has spread to more than 200 countries and the best time window for controlling has been missed out. The SARS-CoV-2 seems highly possible to exist for a long time and the world is entering a period of normalized prevention and control. Currently, there is no specific drug for the virus. Although the drugs currently considered effective for COVID-19 are chloroquine and remdesivir, the latter one is a nucleotide analogue pro-drug that can inhibit RNA dependent RNA synthetase (RdRp) of virus [21], the existing data cannot fully prove the efficacy and safety of these drugs for treating SARS-CoV-2 infection. Therefore, it is urgent to develop an effective vaccine to prevent SARS-CoV-2 infection.

1. Biological characteristics of SARS-CoV-2

SARS-CoV-2 is an enveloped single-stranded positive-sense RNA virus. Generally, coronavirus falls into four genera-α, β, γ, δ on the basis of their phylogenetic relationships and genomic structures. α and β coronaviruses infect only mammals while the γ and δ coronaviruses mainly infect birds [22–25]. Among them, there are 7 species that can infect human beings, that is, common coronavirus including HCoV-229E and HCoV-NL63 that belong to α coronaviruses, HCoV-OC43 and CoV-HKU1 that belong to β coronaviruses, and three highly pathogenic viruses, SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 that all belong to β coronaviruses [17,26,27]. The novel coronavirus was initially isolated from three patients and the complete genome sequences were obtained. The three SARS-CoV-2 genomes clustered together within the sarbecovirus subgenus, which shows the typical β-coronavirus organization: a 5′ untranslated region (UTR), replicase complex (orf1ab), S gene, E gene, M gene, N gene, 3′ UTR, and several unidentified nonstructural open reading frames [1]. Chinese researchers have found that a gene sequence of coronavirus carried by Chinese rhinolophine is 96% identical with that of SARS-CoV-2, thus rhinolophine is considered as the natural host of SARS-CoV-2 [27,28]. Unfortunately, the intermediate host has not been determined yet, although it is speculated that pangolin may be the intermediate host. Based on the results of gene comparison, it was showed that SARS-CoV-2 was closely related (with 88% identity) to two bat-derived SARS-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, eastern China, and SARS-CoV (about 79% identity), but was more distant from MERS-CoV (about 50% identity) [29]. Furthermore, according to the rich knowledge about SARS-CoV and the newly released sequence of SARS-CoV-2, the receptor-binding domain (RBD) of SARS-CoV-2, including its receptor-binding motif (RBM) that directly contacts angiotensin-converting enzyme 2 (ACE2), is similar to that of SARS-CoV, strongly suggesting that SARS-CoV-2 uses ACE2 as its receptor [30]. Currently, more structural and functional studies confirmed that ACE2 acted as the receptor for SARS-CoV-2 [31,32].

2. The strategies for previous development of SARS-CoV vaccines

The above analysis in gene level revealed that SARS-CoV-2 was very similar to SARS-CoV. Since the outbreak of SARS at the end of 2002, scientists have developed a variety of vaccines against SARS-CoV using their own vaccine platforms [33–37]. However, most vaccines are in preclinical studies, and only a few of them have been reported to under assessment in clinical trials (https://clinicaltrials.gov/ct2/show/nct03615911; https://clinicaltrials.gov/ct2/show/nct0339578). Currently, no vaccine has been approved for the prevention of SARS. Since effective antiviral strategies to control SARS-CoV and SARS-CoV-2 infections are still lacking, vaccination is still regarded as the major approach for preventing potential re-emergence of SARS-CoV, and more importantly, for preventing and controlling the current outbreak of SARS-CoV-2. Very recently, the Coalition for Epidemic Preparedness Innovations (CEPI) has reported that the global vaccine R&D effort in response to the COVID-19 pandemic is unprecedented in terms of scale and speed. As of 8 April 2020, the global COVID-19 vaccine R&D landscape includes 115 vaccine candidates, of which 5 advanced candidates including mRNA-1273 from Moderna, Ad5-nCoV from CanSino Biologicals, INO-4800 from Inovio, and LV-SMENP-DC and pathogen-specific aAPC from Shenzhen Geno-Immune Medical Institute have recently moved into clinical development, and the lead developers are mainly in the United States and China (Fig. 1) [38]. It sounds exciting, but we should always be cautious. Previously, a number of SARS vaccine candidates were suspended by the U.S. FDA because of the safety concern. Here, we summarized the advantages and disadvantages of the vaccine candidates against SARS-CoV to provide experience for the vaccine R&D of SARS-CoV-2 and avoid possible risks as far as possible.

2.1. Inactivated vaccines: advantages and disadvantages

It is known that most of the current influenza vaccines are inactivated vaccines, which plays a very important role in

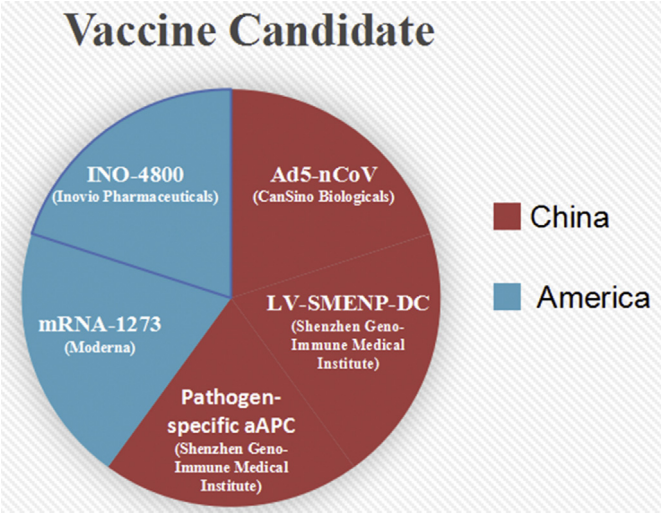

Fig. 1. Distribution of clinical-phage vaccine candidates for COVID-19 and their lead developers.

protecting people from influenza virus infection. Some approaches, such as formaldehyde, UV light, and β-propiolactone have been applied in the preparation of inactivated vaccines against SARS-CoV, which generally induced robust serum-neutralizing antibody (Ab) response in different immunized animals [39–41]. For instance, He Y. et al. showed that high-titer antibodies elicited by the inactivated SARS-CoV in the immunized animals could recognize S protein, especially the receptor-binding domain (RBD) in the S1 subunit, and potently block SARS-CoV entry [42]. Some groups evaluated the safety of the inactivated vaccine for SARS-CoV and the results showed that all vaccines induced serum neutralizing antibody and significant reductions of SARS-CoV after viral challenge. However, challenge of mice given any of the inactivated vaccines led to occurrence of lung lesion based on th2-biased immunopathology with prominent eosinophil infiltration [43–45]. In addition, Fan Luo et al. intramuscularly immunized Chinese rhesus macaques with 2.5 μg beta-propiolactone-inactivated whole SARS-CoV Z-1 vaccine, and boosted the immunization on day 7. Although this study revealed no exacerbation of infection in the immunized macaques following the challenge with SARS-CoV NS-1, the authors suggested that an antibody-dependent enhancement (ADE) assay should be performed in vitro to examine whether the immune sera induced by inactivated vaccine would mediate ADE in some cell lines such as K562, THP-1, and monkey's peripheral blood mononuclear cells [46]. Therefore, we should be cautious in using the inactivated SARS-CoV as a vaccine since it may cause harmful immune and/or inflammatory responses.

2.2. Live-attenuated vaccines: advantages and disadvantages

Historically and contemporarily, live-attenuated vaccines have been used successfully in controlling measles, mumps, rubella, polio, yellow fever, and chickenpox infections and outbreaks [47,48]. Some researchers constructed recombinant forms of the full-length SARS-CoV S protein or RBD of S protein that was encoded into the highly attenuated modified vaccinia virus Ankara (MVA) [49], recombinant adeno-associated virus (rAAV) [50], or an attenuated parainfluenza virus (BHPIV3), respectively [51,52]. BALB/c mice were inoculated intranasally or intramuscularly with MVA/S and neutralizing antibodies were induced. Moreover, MVA/S administered by either route elicited protective immunity, as shown by reduced titers of SARS-CoV in the upper and lower respiratory tracts of the mice after viral challenge. Du L.et al. evaluated the immune protection of a rAAV encoding RBD vaccine in mouse model by intranasal inoculation, which induced strong mucosal immune responses and provided long-term protection against SARS-CoV infection [53]. Other groups reported that immunization of monkeys via the respiratory tract with BHPIV3/SARS-S induced the production of SARS-CoV-neutralizing serum antibodies. After the challenge with SARS-CoV, all monkeys inoculated with BHPIV3/SARS-S did not shed any SARS-CoV while monkeys in control group shed SARS-CoV for 5–8 days [49,52]. Weingartl H et al. immunized ferrets with rMVA-S and the immunized ferrets developed a more rapid and vigorous neutralizing antibody response compared with control animals; however, after challenge with SARS-CoV, they also exhibited stronger inflammatory responses in liver tissue than control animals, suggesting that vaccination with rMVA-S is associated with enhanced hepatitis [54], which is not seen in the above infection model. It is suggested that we should not only use mice or monkeys as animal model, but also use ferrets as the infection model, because of its sensitivity to SARS-CoV. Thus, sometimes we do not observe the pathological damage caused by vaccine immunization, which may be that the animal model used is not representative enough.

Notably, live-attenuated vaccines are associated with the risk of reversion by either mutation- or recombination–driven processes, which can cause dangerous outbreaks in unvaccinated populations, including animals [48]. Several groups have tried to develop novel strategies in controlling fidelity and attenuating RNA virus pathogenesis. In the case of coronavirus, some mutants have been identified to prevent reversion to repaired virulence [55,56]. Rachel L. Graham et al. provided a proof that rewiring the transcription regulatory networks (TRNs) limited reversion in an live-attenuated coronavirus vaccine candidate which are effective against SARS-CoV [56]. Some SARS-CoV vaccine candidates were tested in clinical trials, showing that they could elicit antibody responses and are safe [57,58], although their long-term safety profiles have not been reported.

2.3. Vaccines based on full-length S protein: advantages and disadvantages

Envelope S protein is the most important surface protein of coronavirus (Fig. 2), which is related to the viral infectivity. The S protein mediates receptor binding and membrane fusion [59,60] and is crucial for determining host tropism and transmission capacity [61,62]. Generally, the S protein of coronavirus is functionally divided into the S1 subunit, responsible for receptor binding, and the S2 subunit, responsible for cell membrane fusion [63–66]. By aligning SARS-CoV-2 S protein sequence with those of SARS-CoV and several bat-SL-CoVs, we predicted that the cleavage site for generating S1 and S2 subunits is located at R694/S695 [67]. In addition, it has been reported that S protein has strong immunogenicity and can induce high titer neutralizing antibody [63]. RBD of SARS-CoV S1 is located in S318-510 and the key RBM is S425-494, of which R453 is critical for the complex formation [68]. Notably, N479 and T487 of the RBD are important for the high–affinity association of S protein with ACE2 [69–72]. Residues 442, 472 and 480 also contribute to receptor recognition and host range of SARS-CoV, although not as much as residues 479 and 487 [25,73,74]. Besides, a point mutation at R441 or D454 of the RBD of SARS-CoV disrupts the antigenic structure and binding activity of RBD to ACE2 [75,76]. By comparing the amino acid sequence of S protein between SARS-CoV-2 and SARS CoV, it was found that the similarities of primary sequence between SARS-CoV-2-S and SARS-CoV-S (isolated from human, civet or bat) are around 76%–78% for the whole protein, around 73%–76% for the RBD, and 50%–53% for the RBM [7,30].

In S1 subunit, SARS-CoV-2 and SARS-CoV shared around 50 conserved amino acids, and the three-dimensional structure of SARS-CoV-2 RBD was composed of a core and an external subdomain, which was more similar to that of SARS-CoV [29]. We aligned the sequence of S protein between SARS-CoV (NCBI Reference Sequence: NC_004718.3) and SARS-CoV-2 (NCBI Reference Sequence: NC_045512.2). As shown in Fig. 3A, the five key residues responsible for the binding of the SARS-CoV RBD (residues 442, 472, 479, 480 and 487; SARS-CoV numbering) to the ACE2 receptor were variable in the SARS-CoV-2 RBD (residues 455, 485, 493, 494 and 501; SARS-CoV-2 numbering). Although the five critical residues in SARS-CoV-2 RBM were different with that of SARS-CoV, residue Gln493 provide favorable interactions with human ACE2, consistent with SARS-CoV-2's capacity for human cell infection. Other critical residues in SARS-CoV-2 RBM (particularly Asn501) are compatible with, but not ideal for, binding human ACE2, suggesting that SARS-CoV-2 has acquired capacity for human-to-human transmission. Phylogenetic analysis indicates that SARS-CoV-2 also potentially recognizes ACE2 from a diversity of animal species (except for mice and rats), implicating that these animal species may act as intermediate hosts or animal models for SARS-

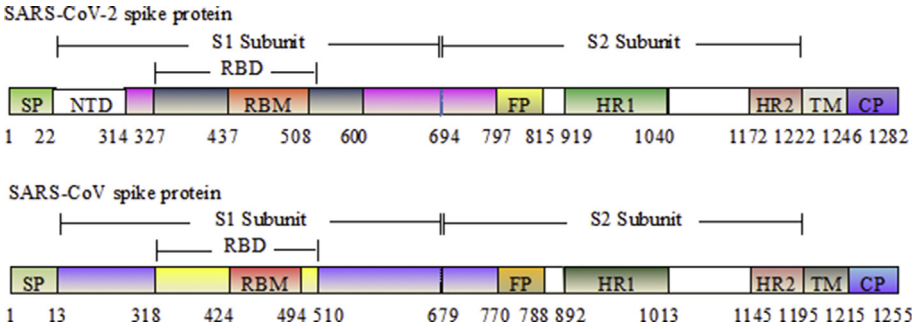

Fig. 2. Schematic of spike protein structure of SARS-CoV-2 and SARS-CoV. SP, signal peptide; NTD: N-terminal domain; RBD, receptor-binding domain; RBM, receptor-binding motif; FP, fusion peptide; HR, heptad repeat; TM, transmembrane domain; CP, cytoplasm domain.

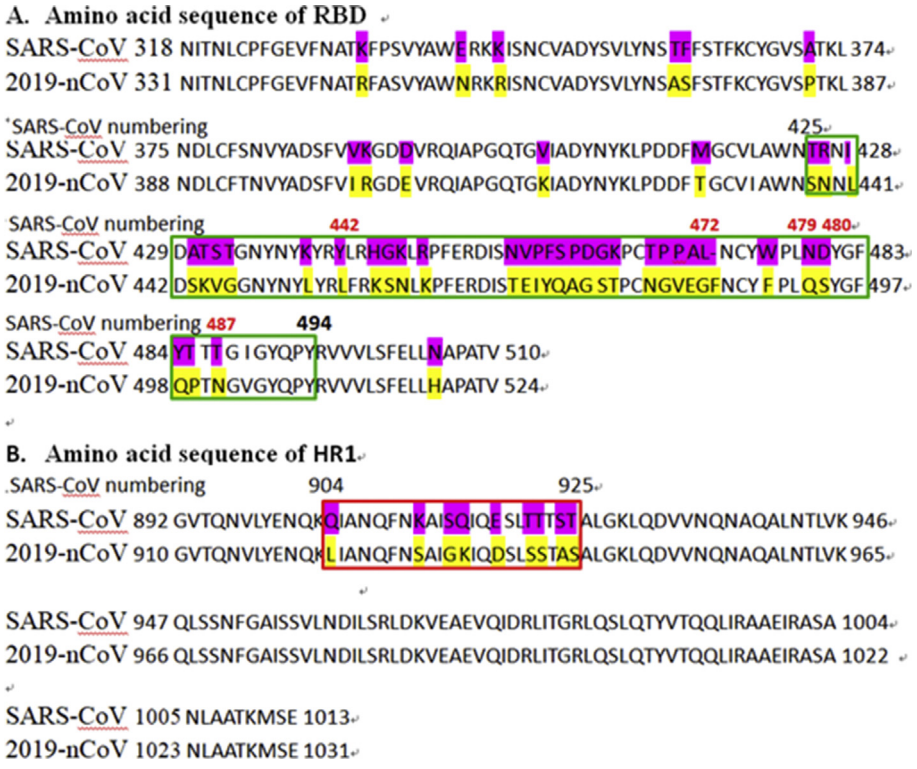

Fig. 3. Specific amino acid variations among the S proteins of SARS-CoV and SARS-CoV-2. Letters in magenta and yellow represent different amino acids in the corresponding sequences of SARS-CoV and SARS-CoV-2, respectively. A. Sequence alignment of SARS-CoV and SARS-CoV-2 RBDs. The red number indicates the core amino acids in RBD when it binds to receptor ACE2. The green frame is the amino acid sequence of RBM. B. Sequence alignment of SARS-CoV and SARS-CoV-2 HR1. The red frame is the location where the variable amino acid residues between SARS-CoV and SARS-CoV-2 HR1.

CoV-2 infections [30]. S2 subunit of coronavirus is divided into two domains, HR1 and HR2. We confirmed that SARS-CoV-2 HR1 and HR2 regions are able to interact with each other to form six helix bundle (6-HB), which brings the viral and cellular membranes in close proximity for fusion [64,77]. As shown in Fig. 3B, several amino acid residues in HR1 of SARS-CoV-2, which are concentrated from 922 to 943 were variable with SARS-CoV (residues 904–925), while HR2 is completely identical between SARS-CoV-2 and SARS-CoV (not shown), suggesting that SARS-CoV-2 S2, especially HR2 is a conserved target for pan-coronavirus vaccine. Together, these results suggest that S protein plays an important role in the infection and immunogenicity of coronavirus which promotes some research teams to prepare a vaccine based on full-length S protein of SARS-CoV-2.

A number of vaccine candidates based on the full-length S protein of SARS-CoV have been reported in succession and generally could induce high level of immune response and potent protective immunity [35,78–80]. For example, the expression of full-length S protein and its trimer of urbani strain by recombinant baculovirus can induce adequate neutralizing antibody against human and palm civet SARS pseudoviruses that bears S proteins of homologous or heterologous SARS-CoV variants (such as Tor2, GD03T13, and SZ3 strains) in vaccinated mice [81]. These reports suggested that the full-length S protein is highly immunogenic and able to induce protection against SARS-CoV challenge. Besides, the neutralizing antibodies alone may be able to suppress virus proliferation. However, the risk in safety and final protective effect of the full-length S protein-based vaccine should not be underestimated. Some groups reported that although full-length S protein of SARS-CoV elicited a neutralizing antibody response, it also induced harmful immune responses including triggered infection of human B cells or other immune cells by SARS-CoV, and enhanced

SARS-CoV infection with anti-SARS-CoV-S immune-serum after challenge with homologous SARS-CoV [82–84]. Last year, Liu L. et al. identified that anti-S protein IgG, in productively infected lungs, caused severe acute lung injury by skewing inflammation-resolving response in SARS-CoV macaque models. Subsequently, these antibodies were injected into healthy macaques that were then challenged with SARS-CoV. Interestingly, the macaques receiving the antibodies showed more severe lung injury than the control group, indicating that Antibody induced by SARS S protein vaccine candidate may cause antibody dependent enhancement (ADE) [85]. Thus, it is speculated that the S protein of SARS-CoV contains some epitopes, which may induce humoral or cellular immune responses that exacerbate the pathology in some coronavirus infections [86]. Protein Science, an American biotechnology company, once spent tens of millions to make a full-length S-protein vaccine of SARS-CoV. However, the vaccine development was suspended because of the safety problems in the immunized animals. Exactly, Liu G. et al. found that peptide S597-603 of S protein induced antibodies that enhanced infection both in vitro and in non-human primates [87]. Perhaps, S protein-based vaccines against SARS-CoV could be engineered to avoid ADE via elimination of the S597-603 epitope.

2.4. RBD-based subunit vaccine: advantages and disadvantages

Full length S protein has been truncated into RBD as a vaccine candidate. RBD of SARS-CoV-S contains major antigenic epitopes that can induce not only neutralizing antibodies but also $CD8^+$ T cell-responses [43,63,88–91]. He Y.et al. identified the neutralizing epitopes on the RBD of SARS-CoV-S by a panel of 27 mAbs isolated from the immunized mice. Six structure-dependent epitopes and two adjacent linear epitopes were found, suggesting that the epitopes capable of inducing highly potent neutralizing Ab responses are mainly conformational epitopes [92]. In addition, the neutralizing epitopes in MERS-CoV RBD was confirmed to be mainly conformational as well [93,94]. The modifications in amino acid residues of MERS-CoV-S-RBD based on the structure design could improve its protection against MERS-CoV infection [94], enlightening that S-RBD or modified S-RBD of SARS-CoV-2 is promising for the development of vaccine against SARS-CoV-2.

We have compared the immunogenicity and immune protection of RBD vaccine with full-length S protein. The results suggested that the RBD, from either SARS-CoV or MERS-CoV, had induced Ab responses with higher titer, more potent neutralizing activity and provided longer-term protection in vivo against SARS-CoV infection compared with that induced by full-length S protein. More importantly, SARS-CoV RBD did not cause immune damage in the animal model while full-length S protein could do [51,95–97]. It is speculated that there are some super-antigens in the region beyond the RBD of the full-length S protein. In this way, after the full-length S protein enters the animal model, it will induce abundant non-specific antibodies and immune cell activation, subsequently eliciting a large amount of cytokines, which will lead to severe immune damage, just like the pathogenic course of SARS-CoV. But why the antibody titer induced by full-length S protein is not as high as that induced by RBD. It is speculated that the antigen competition might result in a lower level of neutralizing antibody induced by full-length S protein than that induced by RBD. Therefore, RBD-based subunit vaccine may be the ideal and safer alternative for the development of coronavirus vaccine.

Interestingly, some groups also found that the recombinant RBDs derived from the S protein of Tor2, GD03, and SZ3, the representative strains of human 2002–2003 and 2003–2004 SARS-CoV and palm civet SARS-CoV, respectively, induced high titers of cross-neutralizing Abs against pseudoviruses expressing S proteins of Tor2, GD03, and SZ3 [98], suggesting that the major neutralizing epitopes of SARS-CoV are maintained in the process of cross species transmission, thus RBD-based vaccines could induce extensive protection against human and animal SARS-CoV variants. Probably, the RBD of S protein may serve as one of the most effective and safest vaccines for the prevention of SARS.

Since both SARS-CoV-2 and SARS-CoV recognize ACE2 on the cell surface as the receptor, can RBD-based SARS-CoV-specific vaccines cross protect individuals from SARS-CoV-2 infection? Unfortunately, Ying T. et al. showed that the most potent SARS-CoV-specific neutralizing antibodies (e.g., m396, CR3014) that target the ACE2 binding site of SARS-CoV failed to bind SARS-CoV-2 S protein, indicating that the different amino acids in RBD between SARS-CoV and SARS-CoV-2 has an important effect on the cross-reactivity of neutralizing antibodies [99].

2.5. Vaccines based on S2 subunit: advantages and disadvantages

SARS-CoV S2 subunit which is responsible for fusion between virus and target cell membranes is expected to be a vaccine candidate against SARS-CoV [66,100,101]. Several groups showed that S2 of SARS-CoV and/or MERS-CoV S protein could induce neutralizing antibodies although their neutralizing potency is generally lower than that of antibodies specific for RBD [102–104]. However, Guo et al. investigated the immune responses against the recombinant S2 fragment (residues 681–980) in BALB/c mice, and found that the S2 fragment could induce specific cellular immune response and a high level of total IgG but little neutralizing antibodies against infection by SARS-CoV [105]. Additionally, Zeng F et al. purified the total S1- and S2-specific IgG from mice immunized with recombinant S1 or S2 proteins, respectively, and found that anti-S1 and anti-S2 IgGs were able to abolish the binding between S protein and its cellular receptor(s), although anti-S1 IgG showed a significantly higher blocking efficiency [106]. As shown in Fig. 2B, the amino acid sequences of HR1 and HR2 are highly conserved and homologous between SARS-CoV and SARS-CoV-2. In fact, these domains are also highly conserved in other coronaviruses [104], thus the S2 subunit has potential to be used as a target for the development of pan-CoV vaccine against divergent virus strains. Altogether, these results suggested that the S2 domain of SARS-CoV S protein, as a vaccine candidate, may be able to prevent the infection of SARS-CoV.

SARS-CoV can also bind to host cells through alternative receptors, such as DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) and/or LSIGN (liver/lymph node-SIGN) [107,108]. Seven asparagine-linked glycosylation sites in the S protein, including residues at positions 109, 118, 119, 158, 227, 589 and 699, are crucial for DC-SIGN-or L-SIGN-mediated virus entry [109]. Notably, a SARS-CoV-specific human monoclonal antibody, CR3022, could bind potently with SARS-CoV-2 RBD, however, the epitope of CR3022 does not overlap with the ACE2 binding site within SARS-CoV-2 RBD [99], suggesting that SARS-CoV-2 may also have other receptor binding sites except for ACE2, which should be noted in our vaccine design against SARS-CoV-2. Besides, optimization of sequences, components, or immunization routes, inclusion of appropriate adjuvants, or application of combinational immunization approaches are usually required for preparing an effective vaccine [110,111].

All these above results indicated that the modification of the SARS-CoV S protein, specifically, by retaining the conserved amino acids, especially the conserved sequence in the RBD region, and removing the possible epitopes that may cause immune damage may be a good strategy to prepare a broad-spectrum vaccine against SARS-CoV and SARS-CoV-related virus.

3. Implication for development of safe and broad-spectrum vaccines against SARS-CoV-2 and other human coronaviruses

Very recently, many research teams have launched the development of vaccines against SARS-CoV-2. The safety is a crucial issue that we need to be aware of when making vaccines. Meanwhile, there is another issue needs attention. The human and civet isolates of SARS-CoV nestle phylogenetically within the spectrum of SARS-CoV-like (SL-CoV) strains isolated from the bats, and the civets may have served as intermediate amplification hosts that enable SARS-CoV to cross species for animal-to-human transmission [70,112–115]. Additionally, RNA virus might well mutate in ways that would make previously effective vaccines useless. Thus, development of a safe and effective vaccine against both human and animal SARS-CoV strains is highly important for preventing future SARS outbreaks. On the other hand, given the high prevalence and wide distribution of coronaviruses, increasing human–animal interface activities, the large genetic diversity and frequent recombination of CoV genomes, novel coronaviruses are likely to emerge periodically in humans due to frequent cross-species infections and occasional spillover events. A broad-spectrum SARS vaccine that is effective against all the coronaviruses using ACE2 as their receptor can be applied immediately when we face the recurrent threat of coronavirus. Particularly, taken SARS-CoV-2 as an example, the virus might disappear before the vaccine is developed. Therefore, development of safe and broad-spectrum vaccines against SARS-CoV-2 and other SARS-CoV-related virus is of great significance for protecting human from CoV infection. Based on the above analysis of the advantages and disadvantages of various vaccines against SARS-CoV, the modified S protein is expected to be a candidate for a cross protective vaccine against SARS-CoV and SARS-CoV-related virus. Notably, we have recently demonstrated that a vaccine based on RBD of S protein against SARS-CoV could effectively cross neutralize SARS-CoV-2. Moreover, several scientists have tried to design a vaccine against all β-coronavirus, especially these ACE2-associated ones.

In addition to antigens, adjuvants can enhance the immune response and reduce the amount of antigen required for each dose of vaccine, so that more doses of vaccine can be produced and provided to more people. The choice of adjuvants also affects the efficacy of respiratory virus vaccines and adjuvant has also been applied in the R&D of SARS-CoV-2 vaccine. Lung is a complex organ, which is rich in immune cells, such as macrophages. The pulmonary epithelial cells (AECs) also have the characteristics of some immune cells, such as secreting a large number of pulmonary surfactant A, D (PS-A, PS-D). The PS layer forms a strong barrier to prevent nanoparticles and hydrophilic molecules from accessing them, so as to protect the AECs from pathological damage [116]. Type I interferons (IFN-Is) are the chief immune mediators to protect the body from viral infections, and can be secreted by AECs as well as immune cells following viral infection. Therefore, the activation of stimulator of interferon genes (STING) in these two cell types can effectively enhance the production of IFN-I, and recapitulate the immune responses provoked by viral infection [117]. However, it is still a great challenge to delivery STNG agonists into the cytosol of AECs without destroying the integrity of PS layer. Recently, two research teams from China and the United States cooperated to ingeniously use PS-biomimetic liposomes (PS-GAMP) to encapsulated 2′,3′-cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), a natural and effective STING agonist [118,119]. Because of its resemblance to PS, PS-GAMP disguises “self” and escapes from immune surveillance following intranasal immunization. Together with SP-A and SP-D, PS-GAMP enters the alveolar macrophages (AMs), from which PS-GAMP fluxes into AECs through gap junctions between AMs and AECs. Subsequently, the STING pathway in AMs and AECs was activated without breaching PS and alveolar epithelial barriers. Through this mechanism, the adjuvant PS-GAMP combined with inactivated H1N1 vaccine generated broad-spectrum cross protection not only effectively against H1N1, but also against heterosubtypic H3N2, H5N1 and H7N9 virus infection within two days after a single immunization. This cross protection lasted for at least 6 months and induced the persistence of lung $CD8^+$ TRM cells in vivo. It is also showed that PS-GAMP could enhance the recruitment and differentiation of $CD11b^+$ dendritic cells (DC) and $CD8^+$ T cells, as well as the humoral response [120]. Thus, PS-GAMP strategy is a promising “universal” mucosal adjuvant for developing broad-spectrum vaccines against betacoronavirus lineage B (SARS-CoV, SARS-CoV-2, SARSr-CoVs).

4. Conclusion and prospects

There was a significantly harmful immune response after immunizing animals with inactivated vaccine against SARS-CoV following challenge with the homotypic virus, although no obvious safety problem was observed in animal model after immunization. In addition, the inactivated vaccine against one serotype of dengue virus (DENV) can enhance individual infection with other serotypes [121]. These observations suggested that harmful response after immunization with inactivated vaccine against SARS-CoV-2 might also happen. Even if the inactivated SARS-CoV-2 vaccine does not elicit serious harmful immune response, it may enhance the infection of the mutated and/or another novel coronavirus by ADE. The subunit vaccine based on RBD can avoid ADE. However, RBM cannot serve as a universal vaccine candidate because of its large variation among different virus strains. The biggest advantage of mRNA and DNA vaccines or vector vaccines that is currently under clinical trials in the United States is that they do not need adjuvants. However, there are still potential safety risks of these mRNA or DNA vaccines. Therefore, the modified RBD combined with T cell epitopes and effective mucosal adjuvants will be the best choice for the preparation of a broad-spectrum and safe coronavirus vaccine. Of course, it should be noted that the choice of vaccine depends on the mortality rate of the disease. For infectious diseases with high mortality, such as EBOV, the effectiveness of vaccines is more important than safety. However, for low mortality disease (such as COVID-19), the safety of vaccines is more important than effectiveness. Additional safety concerns relate to effectiveness and safety against antigenic variants of SARS-CoV and for safety of vaccinated persons exposed to other coronaviruses.

In a word, a safe and effective vaccine for COVID-19 is urgently needed. However, regulators need to evaluate its safety through a series of virus strains and more than one animal model before allowing the COVID-19 vaccines to be used in humans. Simultaneously, regulators should also see strong preclinical evidence that vaccines can prevent infection and we should speed up the R&D progress on the premise of ensuring the safety and effectiveness of the vaccine [122]. In addition, the vaccine preparation and adjuvant selection for SARS-CoV-2 in the future should attach great importance to its broad-spectrum cross protection, so that if there is similar virus diseases emerged in humans or animals, the vaccine can be applied immediately to exert its protective effect.

Declaration of Competing Interest

The authors declare no conflict of interest.

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