REVIEW ARTICLE

# Receptor-binding domain as a target for developing SARS vaccines

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**ABSTRACT**

A decade ago, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) caused a global pandemic with a mortality rate of 10%. Reports of recent outbreaks of a SARS-like disease caused by Middle East respiratory syndrome coronavirus (MERS-CoV) have raised serious concerns of a possible reemergence of SARS-CoV, either by laboratory escape or the presence of a natural reservoir. Therefore, the development of effective and safe SARS vaccines is still needed. Based on our previous studies, we believe that the receptor-binding domain (RBD) in the S1 subunit of the SARS-CoV spike (S) protein is the most important target for developing a SARS vaccine. In particular, RBD of S protein contains the critical neutralizing domain (CND), which is able to induce highly potent neutralizing antibody response and cross-protection against divergent SARS-CoV strains. Furthermore, a RBD-based subunit vaccine is expected to be safer than other vaccines that may induce Th2-type immunopathology. This review will discuss key advances in the development of RBD-based SARS vaccines and the possibility of using a similar strategy to develop vaccines against MERS-CoV.

**KEY WORDS**

Virus; severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV); receptor-binding domain (RBD); spike protein; vaccine

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## Introduction

Severe acute respiratory syndrome (SARS) caused by a novel human coronavirus (SARS-CoV) emerged from Guangdong Province, China, in late 2002. By the end of 2003, it had spread to more than 30 countries, affecting 8,096 people and causing 774 deaths (a case fatality rate of about 10%) (1-3). Although the global SARS pandemic was brought under control in July 2003, reports of sporadic cases in China from late 2003 to early 2004 (4) raised concerns about the reemergence of SARS-CoV through either zoonotic reintroduction or laboratory escape (5,6).

Most recently, a close relative of SARS-CoV, Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), has been identified as the pathogen causing outbreaks of SARS-like illness with a case fatality rate of 55% in the Middle East, Europe and Africa (7-9). These reports have raised concerns over the possibility of a reemergence of SARS-CoV and, hence, call for the development of effective and safe SARS vaccines to combat any future SARS pandemic (10).

## Identification of the receptor-binding domain (RBD) in the SARS-CoV spike protein and its role in viral entry into the target cell

SARS-CoV is a single, nonsegment and positive-stranded RNA virus with envelope. Its genomic RNA consists of 29,736 nucleotides, two thirds of its 5'-encoding nonstructural RNA replicase polyprotein and one third of its 3'-encoding structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (11).

The S protein of SARS-CoV is a type I transmembrane envelope glycoprotein (Env), which plays a significant role in receptor binding, membrane fusion and virus entry. The entry of SARS-CoV is initiated by binding of the S protein to the cellular receptor angiotensin-converting enzyme 2 (ACE2) (12). The virion-ACE2 complex is then translocated to endosomes. Cathepsin L inhibitors could significantly block the entry of SARS-CoV, indicating that S protein is cleaved by endosomal acid proteases (cathepsin L) to activate its fusion activity (13). After the fusion peptide (FP) inserts into the endosomal membrane, the heptad repeat 1 and 2 (HR1 and HR2) domains in the S

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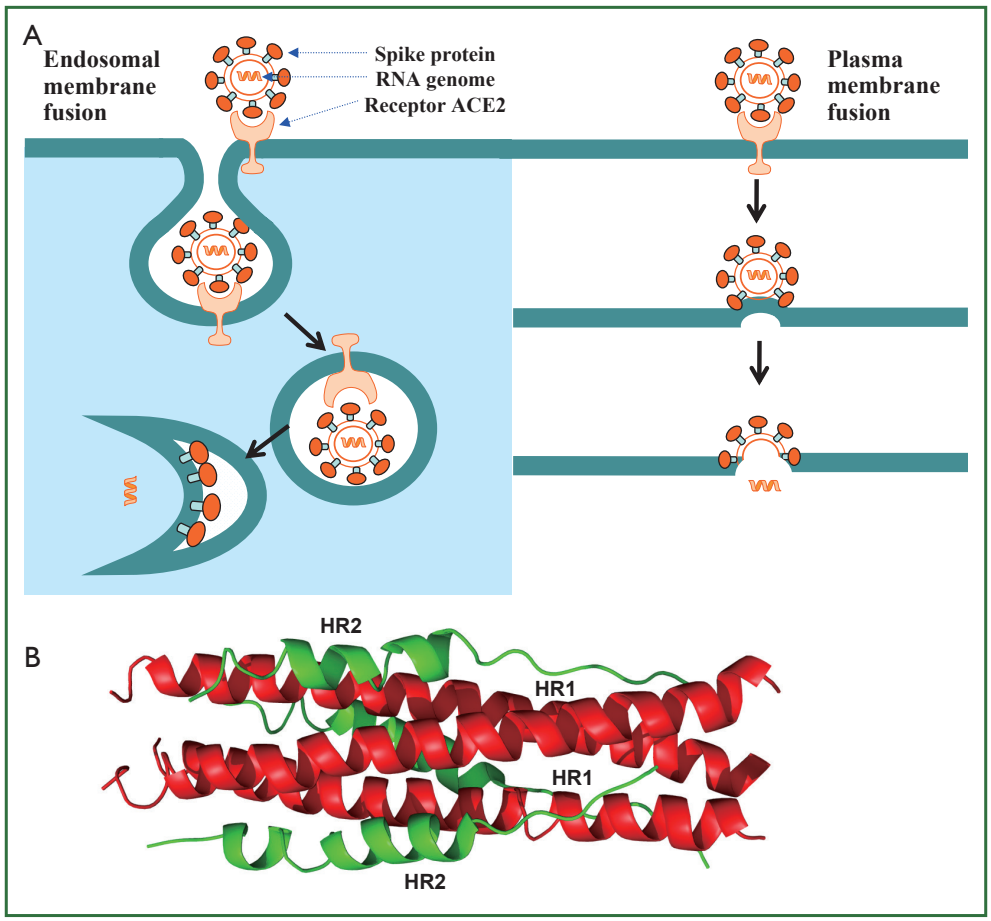


**Figure 1.** The models of SARS-CoV entry into the target cell. A. SARS-CoV enters into the target cell mainly through endosomal membrane fusion (left side) and alternatively via plasma membrane fusion (right side) (16); B. Fusion core structure formed by the HR1 and HR2 domains in the SARS-CoV S protein; The fusion core is a six-helix bundle (6-HB) with three HR2 α-helices packed in an oblique antiparallel manner against the hydrophobic grooves on the surface of the central HR1 trimer (17).

protein interact with each other to form a six-helix bundle (6-HB) core, which brings the viral envelope and the cellular plasma membrane into close proximity for fusion. The viral RNA genome is then released into the cytoplasm (14,15) (Figure 1). Alternatively, SARS-CoV may also enter the target cell through plasma membrane fusion in a manner similar to HIV. After the S1 subunit of SARS-CoV S protein binds to ACE2, the S2 subunit changes conformation by inserting the fusion peptide into the plasma membrane. The HR2 domain interacts with the HR1 trimer to form 6-HB core, leading to the fusion between the viral envelope and the cellular plasma membrane (16).

Subsequently, the genomic RNA genome serves as a template for synthesizing full-length and subgenomic-length negative-strand RNAs, which then serve as template for synthesis of mRNA. Viral proteins are translated and then transported to the lumen of the ER-Golgi intermediate compartment (ERGIC) (18). From genomic RNA and N protein in the cytoplasm, viral nucleocapsids are assembled. Through exocytosis, virions are then released from the cell (Figure 2).

The SARS-CoV S protein consists of S1 surface subunit, which is responsible for receptor-binding, and S2 transmembrane subunit, which mediates membrane fusion. A fragment spanning the residues 318-510 in S1 subunit is the minimal RBD (19,20). The RBD contains a loop region (residues 424-494), termed receptor-binding motif (RBM) (Figure 3A), which makes complete contact with the receptor ACE2. Interestingly, the RBM region is tyrosine-rich. Six out of 14 residues of RBM that are in direct contact with ACE2 are tyrosines. Two residues in RBM, Asn479 and Thr487, determine SARS disease progression and SARS-CoV tropism (22,23). Substitutions of these two residues may change the animal-to-human or human-to-human transmissibility of the virus (Figure 3B) (21). The multiple cysteine residues in the RBD region are important for maintaining the functional conformation of the RBDs of SARS-CoV (21) and MERS-CoV (24).

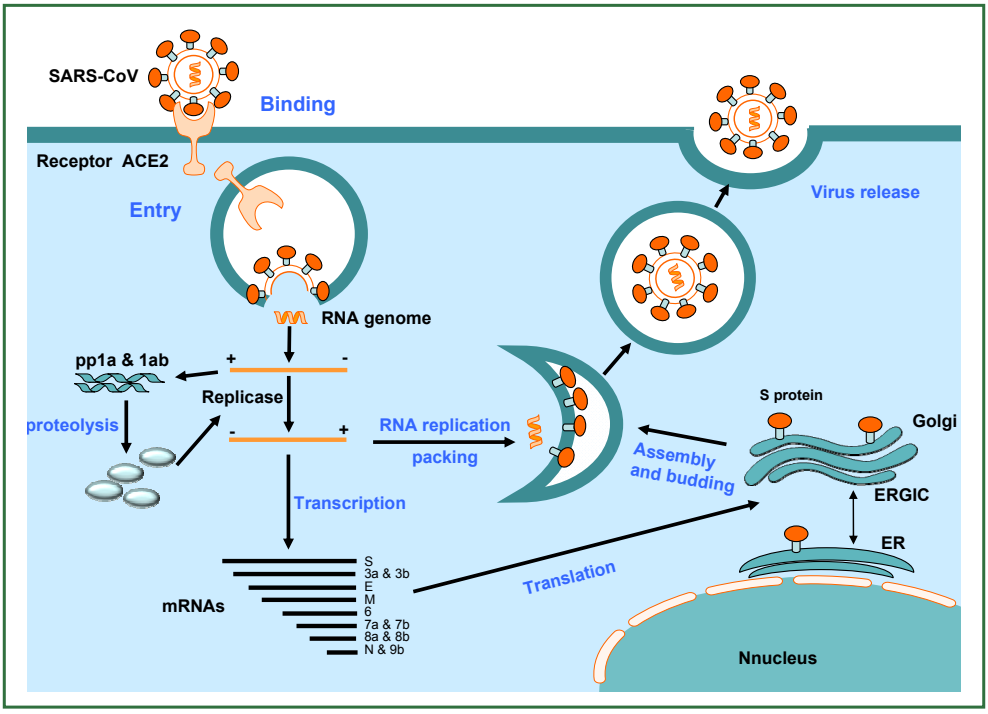


**Figure 2.** The life cycle of SARS-CoV. SARS-CoV starts its life cycle from the binding of the virion, via its S protein, to the receptor ACE2 on the target cell (12), and the virion is taken in by endocytosis. Its S protein changes conformation in order to facilitate endosomal membrane fusion and release of RNA genome into the target cell. Upon transcription and translation, the viral structural and nonstructural proteins and genomic RNA are then assembled into virions, which are subsequently transported via vesicles and released out of the target cell (5).

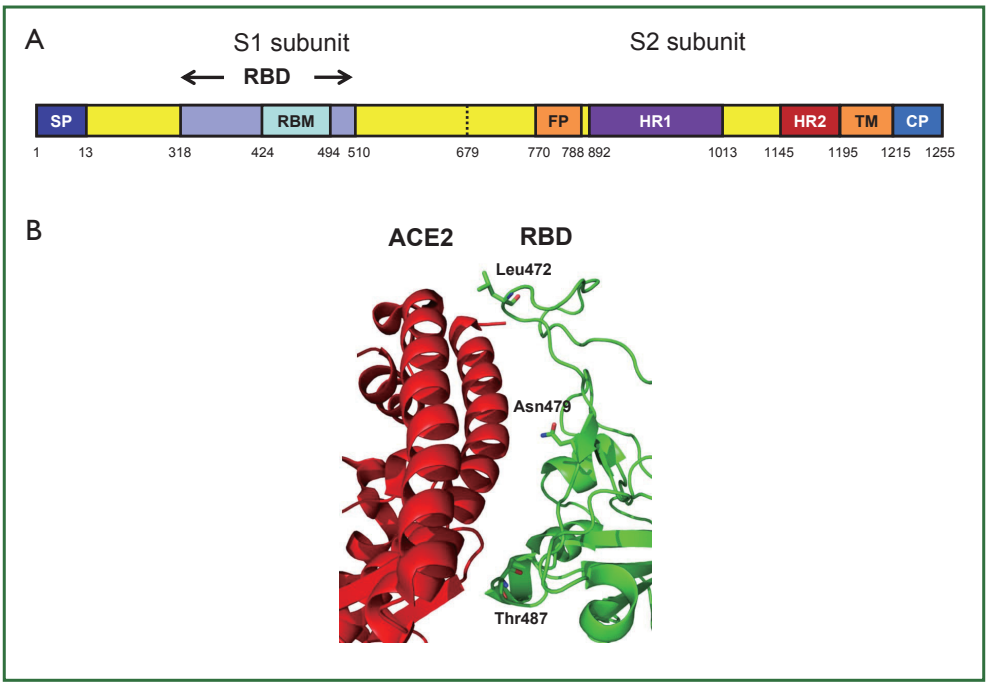


**Figure 3.** The structure and function of the SARS-CoV S protein. A. The functional domains in the SARS-CoV S protein; SP, signal peptide; RBD, receptor-binding domain; RBM, receptor-binding motif; FP, fusion peptide; HR, heptad repeat; TM, transmembrane domain; CP, cytoplasm domain. The residue numbers of each region represent their positions in the S protein of SARS-CoV. B. Interaction between the SARS-CoV S-RBD (green) and ACE2 (red) as shown by the crystal structure of the RBD/ACE2 complex (12,20,21).

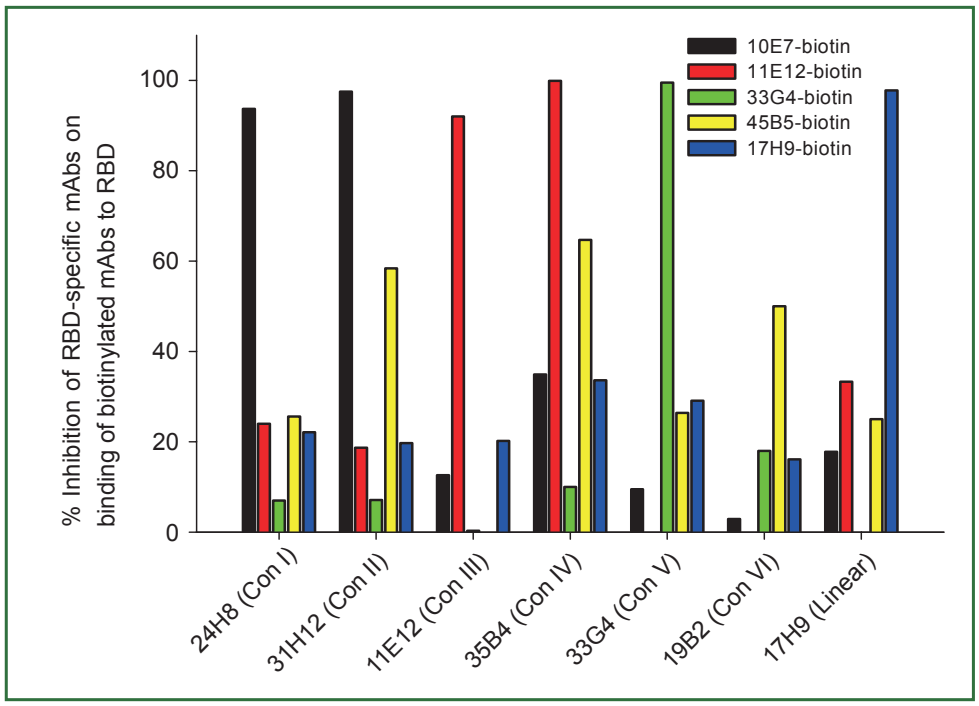


**Figure 4.** Epitope specificity of the RBD-specific mAbs determined by binding competition assays. The mAbs that can recognize different conformation-dependent epitopes in RBD were characterized by binding competition assays using biotin-labeled mAbs as probes. For example, mAbs 10E7 and 45B5 were biotinylated, and the inhibitory activity of the 25 conformation-specific mAbs on the binding of 10E7-biotin and 45B5-biotin to RBD was measured. Both 24H8 and 31H12 could effectively block 10E7-biotin binding to RBD, while 31H12 could also inhibit 45B5-biotin binding to RBD. These results suggest that mAbs 24H8 and 10E7 share the same epitope (Conf I), while 31H12 could bind an epitope (Conf II) which could be recognized by both 10E7 and 45B5. In this way, the 25 conformation-specific mAbs were divided into six distinct competition groups (designated as Conf I-VI) (27).

## RBD contains the critical neutralizing domain (CND) that induces potent and broad neutralizing antibodies

Our previous studies have demonstrated that the antisera isolated from SARS patients and from animals immunized with inactivated SARS-CoV vaccine could react significantly with the RBD of the SARS-CoV S protein, indicating that the RBD possesses potent neutralizing activity (25). Depletion of RBD-specific antibodies from patient or rabbit immune sera by immunoadsorption resulted in significant reduction of the serum-mediated neutralizing activity (26). Antibodies purified from the antisera against SARS-CoV significantly inhibited RBD binding to ACE2, and the affinity-purified anti-RBD antibodies exhibited a relatively higher potency in neutralizing infectivity (26,27). All these results suggest that the RBD of S protein contains a CND and that RBD may therefore be used as an immunogen to induce neutralizing antibodies against SARS-CoV.

We previously found that a single amino acid substitution in the RBD, such as R441A, was able to abolish the immunogenicity of RBD to induce neutralizing antibodies in immunized mice and rabbits and that RBD bearing R441A mutation could not bind to the soluble and cell-associated ACE2, suggesting that some critical residues in the RBM of the RBD are also important residues in the CND. However, as demonstrated by Ye *et al.*, the mutation of R453A in RBM abolished viral entry, but retained the capacity for inducing neutralizing antibodies, suggesting that some residues in CND may not participate in the RBD-receptor interaction (28).

A panel of 27 RBD-specific monoclonal antibodies (mAbs) was isolated from mice immunized with RBD conjugated with IgG Fc (RBD-Fc). Among these, mAbs 4D5 and 17H9 could recognize linear epitopes of RBD, but they showed no neutralizing activity. Using a binding competition assay, the remaining 25 RBD-specific mAbs could be divided into six distinct groups based on the conformation of the epitopes in RBD that they recognized (i.e., Conf I-VI) (Figure 4). We found that only the mAbs recognizing Conf IV and V could efficiently block RBD binding to ACE2, suggesting that the residues in their epitopes are also involved in RBD-ACE2 interaction. The mAbs that recognized Conf I and II did not significantly affect RBD binding with ACE2. Still, they possessed potent neutralizing

activities, indicating that these mAbs could inhibit SARS-CoV infection without interfering in RBD-ACE2 interaction (27). These findings suggest that the RBD of SARS-CoV S protein contains multiple conformational epitopes responsible for eliciting potent neutralizing responses and can therefore serve as a target for development of SARS vaccines.

## RBD-based SARS vaccines

Although the inactivated virus-based, DNA-based and viral vector-based vaccine candidates could induce effective neutralizing antibody responses, their safety is a major concern for further development. A double-inactivated SARS-CoV vaccine was proven to elicit eosinophilic and immunoenhancing pathology (29). The SARS-CoV nucleocapsid protein (NP) in the inactivated vaccine may be responsible for this unwanted property (30,31). The full-length S protein may also not be safe. For example, the vaccine candidates containing recombinant S protein are able to cause Th2-mediated immunopathology (32) or some immune enhancement in vaccinated animals (33).

Based on our previous studies, we believe that a RBD-based SARS vaccine is the most effective and safest. First, we have demonstrated that the recombinant RBD expressed in mammalian cells linked to human IgG Fc (RBD-Fc) could induce highly potent neutralizing antibodies in vaccinated mice and rabbits (34). Second, the recombinant RBD without Fc tag expressed in mammalian (293T) cells, insect (Sf9) cells, and *E. coli*, respectively, could induce highly potent neutralizing antibody responses and complete protective immunity against SARS-CoV challenge in mice. Third, a 219-mer (residues 318-536) RBD protein expressed in Chinese hamster ovary (CHO)-K1 cells (RBD219-CHO) and a 193-mer (residues 318-510) RBD stably expressed in CHO cells (RBD193-CHO) could induce strong humoral and cellular immune responses and protection in all vaccinated mice (35,36). Fourth, a recombinant adeno-associated virus (rAAV)-based RBD (RBD-rAAV) vaccine could induce humoral immune response with neutralizing activity in intramuscular (i.m.)-vaccinated BALB/c mice (37). The intranasal (i.n.) application of RBD-rAAV vaccine could induce more potent SARS-CoV-specific systemic and mucosal immune responses than i.m. administration (38). Fifth, priming with RBD-rAAV vaccine and boosting with RBD-specific peptides for T cell epitopes significantly elevated anti-SARS-CoV humoral and cellular immune responses (39). Sixth, RBD-based SARS vaccine could induce high titer of S-specific antibodies with long-term neutralizing activity and long-term protective immunity in an animal model (40). All these results indicate that RBD-based vaccines have good potential to be further developed as an effective and safe vaccine for preventing SARS-CoV infection and combating the recurrence of SARS pandemic in the future.

## Conclusions and prospect

Considering the recent outbreaks of SARS-like disease caused by the newly emerged MERS-CoV and the potential of future recurrence of SARS, development of effective and safe vaccines against SARS-CoV remains a high priority. Our previous studies have demonstrated that the RBD in the S1 subunit of the SARS-CoV S protein contains the CND that can induce highly potent humoral and cellular immune responses, particularly cross-neutralizing antibodies and strong protective immunity. Therefore, RBD-based vaccines show considerable promise for further development as a highly effective SARS vaccine. Furthermore, this strategy could also be employed for the development of vaccines against other emerging infectious diseases caused by enveloped viruses with class I membrane fusion proteins, such as avian influenza A(H7N9) virus (41-43) and MERS-CoV (7,24).

Recently, Chan *et al.* (44) have demonstrated that sera collected from convalescent SARS patients may contain cross-reactive antibodies against MERS-CoV detected by both immunofluorescent and neutralizing antibody tests. Based on bioinformatics analysis, they anticipated that the B-cell epitope that elicited cross-reactive antibodies may be located in the S2 subunit HR2 domain of MERS-CoV. Most recently, we have shown that the mAbs specific for the RBD of SARS-CoV S protein exhibited no cross-reactive or cross-neutralizing activity against MERS-CoV, suggesting that the RBDs of SARS-CoV and MERS-CoV S proteins may not contain the epitopes for inducing cross-reactive antibody responses (45). Therefore, the design and development of a RBD-based vaccine against MERS-CoV will need to follow an experimental path similar to that of our RBD-based SARS vaccine.

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*Disclosure*: The authors declare no conflict of interest.

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