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# FOCUS ON RECEPTOR-BINDING DOMAIN OF CORONAVIRUS SPIKE GLYCOPROTEIN AS A POTENTIAL SOURCE OF NEUTRALIZING ANTIBODIES: POSSIBLE IMPLICATIONS FOR DESIGNING OF SUBUNIT VACCINE AGAINST VIRAL INFECTION

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**ABSTRACT**
Human coronaviruses (CoV) are enveloped positive-stranded RNA viruses belonging to the order Nidovirales, and are mostly responsible for upper respiratory and digestive tract infections. The CoV spike glycoprotein (S) is a structural protein that is involved in the entry of virus in the host cells. The S protein contains a conserved Receptor Binding Domain (RBD), which recognises and interacts with the angiotensin-converting enzyme 2 (ACE2) receptors in host cells for entry. In this article, the significance of the specific domain of the surface protein and implications to explore the same for clinical purposes in connection to treatment of viral infection has been elucidated.

**KEYWORDS:** 2019-nCoV; SARS-CoV; spike glycoprotein; Receptor Binding Domain; vaccine.

## 1. BACKGROUND

In December 2019, a new CoV (2019-nCoV) has been detected in the city of Wuhan, and this emerging viral infection was associated with severe human respiratory disease with a ~2–3% fatality rate.[1] The virus was presumed to have initially been transmitted from an animal reservoir to humans possibly via an amplifying host. However human-to-human transmission has been reported, leading to a sustained epidemic spread with>31,000 confirmed human infections, including>640 deaths, reported by the WHO in early February 2020. The estimated effective reproductive number (R) value of ~2.90 (95%: 2.32–3.63) at the beginning of the outbreak raised the possibility of pandemics.[2] This prompted WHO to declare it as a Public Health Emergency of International Concern.

Based on its genome sequence, 2019-nCoV belongs to lineage b of Beta coronavirus, which also includes the SARS-CoV and bat CoV ZXC21, the latter and CoV ZC45 being the closest to 2019-nCoV. 2019-nCoV shares ~76% amino acid sequence identity in the Spike (S)-protein sequence with SARS-CoV and 80% with CoV ZXC21.[3] The coronavirus S-protein is the structural protein responsible for the crown-like shape of the CoV viral particles (Fig. 1), from which the original name “coronavirus” was coined. The ~1200 amino acid long S-protein belongs to class-I viral fusion proteins and contributes to the cell receptor binding, tissue tropism and pathogenesis.[4,5] It contains several conserved domains and motifs (Fig. 2). The trimetric S-protein is processed at the S1/S2 cleavage site by host cell proteases, during infection. Following cleavage, also known as priming, the protein is divided into an N-terminal S1-ectodomain that recognises a cognate cell surface receptor and a C-terminal S2-membrane-anchored protein involved in viral entry. The SARS-CoV S1-protein contains a conserved Receptor Binding Domain (RBD), which recognises the angiotensin-converting enzyme 2 (ACE2).[6] The SARS-CoV binds to both bat and human cells, and the virus can infect both organisms.[7,8] The 18 residues of ACE-2 interact with the RBD (contain 14 amino acids) of SARS-CoV spike protein.[9] The S1 domain interacts with the ACE-2 or DPP-4 receptors of the host (Fig. 3). Anti-ACE-2 antibody blocked viral entry and replication in Vero E6 cells.[6,9] Thus, the coronavirus entry into susceptible cells is a complex process that requires the concerted action of receptor-binding and proteolytic processing of the S protein to promote virus-cell fusion.

## 2. Spike protein of CoV: a potential source of antibodies

As the coronavirus S glycoprotein is surface-exposed and mediates entry into host cells, it is the main target of neutralizing antibodies (Abs) upon infection and the

focus of therapeutic and vaccine design. S trimers are extensively decorated with N-linked glycans that are important for proper folding[10] and for modulating accessibility to host proteases and neutralizing Abs.[11-14] Potent human-neutralizing Abs were previously characterized from rare memory B cells of individuals infected with SARS-CoV[15] or MERS-CoV[16] in complex with SARS-CoV S and MERS-CoV S to provide molecular-level information of the mechanism of competitive inhibition of $S^B$ attachment to the host receptor.[12]

In an earlier study, a recombinant S glycoprotein trimeric (triSpike) of SARS-CoV was able to elicit a neutralizing and protective immune response in experimental animals.[13] Vaccinated animals showed no signs of enhanced lung pathology or hepatitis and viral load was undetectable or greatly reduced in lungs following challenge with SARS-CoV. Since full-length S protein contains RBD, other viral functional domains and multiple neutralizing epitopes, it is expected to induce more potent neutralizing antibodies than S1 or RBD alone. However, the non-neutralizing epitopes present in full-length S protein may exhibit the phenomenon of Antibody-dependent enhancement (ADE) of infection (due to enhancing antibodies), as described for HIV and Ebola virus.[17-20] The S proteins from some coronaviruses could also induce enhancing antibodies. For example, immunization of felines with a vaccinia virus vector encoding the S protein of feline infectious peritonitis virus (FIPV) resulted in enhancement of virus replication after virus challenge[21,22] and the epitopes that elicit enhancing antibodies were localized in the S protein.[23] This may indicate the possibility of enhancing antibodies to "suppress" or ''neutralize'' the neutralizing-antibody activity, resulting in reduced neutralizing-antibody titers.

### 3. Immunogenic behaviour of Receptor-binding Domain: Implications for vaccine design

Several studies have previously pointed towards the receptor binding domain (RBD) within S1 as key target for neutralizing antibodies, which may interfere with the receptor binding process, as reported in the case of SARS-CoV.[24,25] A human monoclonal antibody (mAb) against the S1 domain (of S protein) from a non-immune human antibody library was described to block association of S-protein with ACE2.[26] Neutralizing sera from vaccinated mice prevented triSpike binding to soluble human ACE2 receptor indicating that the RBD is accessible to antibodies and suggesting receptor binding inhibition as key mechanism of virus neutralization in triSpike immunized animals.[13]

These findings on SARS-CoV provides possible insight on tailoring the S1 or RBD domain of the S-protein of 2019-nCoV for immunotherapeutic purpose. The RBD of S protein is not only a functional domain mediating virus-receptor binding but may also serve as a critical neutralization determinant of 2019-nCoV, based on previous observations and reports on SARS-Cov. Therefore, the idea of S1 or RBD-based subunit vaccine may be suitably explored in an attempt to prevent the viral infection and associated complications that may lead to fatality. As pointed by researchers in John Hopkins University, a slow rate of mutation of coronavirus is likely to allow a successfully designed vaccine to be effective for long-term.

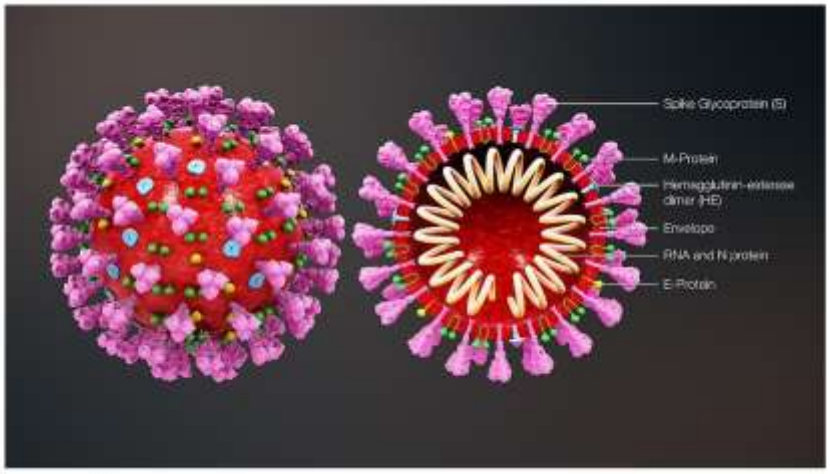


**Figure 1: Structure of coronavirus.**

A cross-sectional model of coronavirus depicting the surface spike glycoprotein (S)

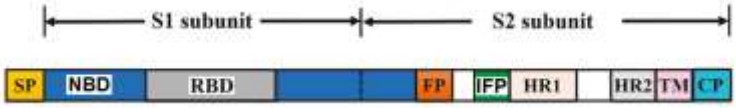


**Fig. 2: Schematic diagram of the human 2019-nCoV S-protein.**

The S protein consists of S1 and S2 domains. There is a signal peptide (SP) located at the N-terminus of the S protein. The S1 domain contains a receptor-binding domain (RBD). The S2 domain contains a cytoplasm domain (CP), a transmembrane domain (TM), and an ectodomain composed of a putative internal fusion peptide (FP) and heptad repeat 1 and 2 (HR1 and HR2) regions.

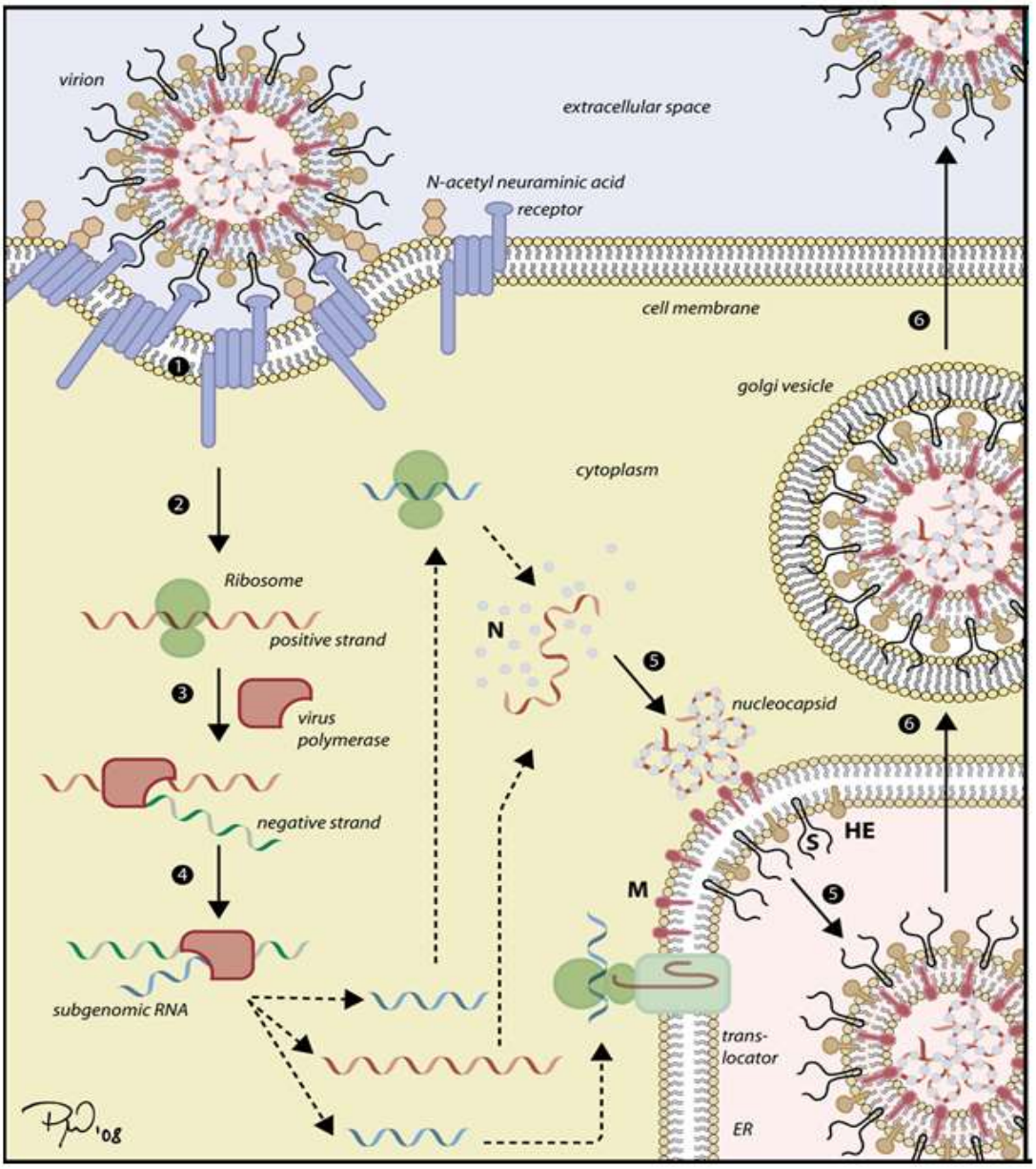


**Fig. 3: The life cycle of coronavirus in host cells.**

The S proteins of coronavirus binds to cellular receptor angiotensin-converting enzyme 2 (ACE2) which is followed by entry of the viral RNA genome into the host cell and translation of structural and non-structural proteins follows. This is followed by assembly and budding into the lumen of the ERGIC (Endoplasmic Reticulum Golgi Intermediate Compartment). Virions are then released from the infected cell through exocytosis.

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