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Coronavirus Spike (S) Protein: A Brief Review on Structure-Function Relationship, Host Receptors, and Role in Cell Infection

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Authors' contributions

This work was carried out in collaboration among all authors. Author LPA designed the study, managed the literature searches and the first draft of the manuscript. Authors LLSP and VG contributed to the literature searches and the first draft of the manuscript. Authors EVP and PMGP contributed to the review and editing writing. Author THN managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Coronaviruses (CoVs) are a broad group of spherical and enveloped viruses that cause diseases in humans and animals. CoVs have become a major threat to public health in the past two decades, exemplified by epidemics of acute respiratory syndromes and, most

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recently, the coronavirus disease 2019 pandemic. The envelope of CoVs contains spike (S) proteins, which are transmembrane proteins with a crown-like shape involved in cell attachment, cell–cell fusion, host tropism, and pathogenesis. The receptors for spike proteins in host cells can be glycans and proteins. This review approaches the structural and functional aspects of the S protein of CoVs. Several issues are presented, including the structure–function relationship, examples of host receptors, S protein-host cell connection, and its role in the entry of the virus into host cells. The S protein is one of the main targets of studies on the evolutionary relationships between CoVs, mapping of cross-host transmission events, changes in virulence, variations in disease severity level, and the development of therapeutic strategies and vaccines.

Keywords: Severe acute respiratory syndrome; coronavirus; envelope proteins; 2019 New Coronavirus Pandemic; spike protein.

ABBREVIATIONS

ACE2: Angiotensin-Converting Enzyme 2; BCoV: Bovine Coronavirus; CEACAM1a: Carcinoembryonic Antigen-related Cell Adhesion Molecule 1a; CoVs: Coronaviruses; COVID-19: Coronavirus Disease 2019; CTD: C-terminal domain; DPP4: Dipeptidyl Peptidase 4; HCoV: Human Coronavirus; HR: Heptad Regions; IBV: Infectious Bronchitis Virus; MERS: Middle East Respiratory Syndrome; MHV: Mouse Hepatitis Virus; NTD: N-terminal domain; ORF: Open Reading Frame; PRCoV: Porcine Respiratory Coronavirus; RNA: Ribonucleic Acid; SARS: Severe Acute Respiratory Syndrome; TGEV: Transmissible Gastroenteritis Virus; α-CoV: alphacoronavirus; β-CoV: betacoronavirus; γ-CoV: gammacoronavirus; δ-CoV: deltacoronavirus.

1. INTRODUCTION

Coronaviruses (CoVs) are a broad group of viruses that have the remarkable ability of being transmitted between humans and animals [1]. For example, the first cases of an acute respiratory infection caused by a novel CoV at the end of 2019 (which would later turn into the current pandemic) is believed to have occurred as a result of exposure to wild animals in a Chinese seafood market [2]. Factors including agricultural intensification and environmental changes allow for the frequent mixing of these viruses and facilitate the crossing of species and genomic recombination [3]. CoVs recognize a variety of cell surface molecules in their host receptors, including proteins, sugars, and heparan sulfate. To infect a new host species, CoVs can adapt to the receptor of the new host either by mutation or recombination [4].

CoVs are spherical and enveloped viruses that contain a single-stranded and positive-sense RNA genome. They belong to the order Nidovirales, family Coronaviridae, and subfamily Orthocoronaviridae, which is divided into four genera: alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus, or α-CoV, β-CoV, γ-CoV, and δ-CoV, respectively [5].

CoVs have been known in veterinary medicine for many decades since some of these viruses cause diseases in animals, including birds, bats, rodents, swine, ruminants, equines, and dogs. Infectious Bronchitis Virus (IBV) causes infectious bronchitis in birds, while Bovine Coronavirus (BCoV) is responsible for enteric and respiratory diseases in ruminants. The Mouse Hepatitis Virus (MHV) causes severe disease in cats, ferrets, and laboratory animals (e.g., mice). Transmissible Gastroenteritis Virus (TGEV) is responsible for acute gastroenteritis in swine, and its derivative Porcine Respiratory Coronavirus (PRCoV) causes a mild respiratory disease in pigs [6]. The CoVs that cause diseases in humans will be commented in the next section.

Structurally, the envelope of these viruses contains four main structural proteins (Fig. 1): spike proteins (S), membrane proteins (M), envelope proteins (E), and nucleocapsid proteins (N) [7]. The N protein is a component with a structural function, that is involved in processes related to the viral genome, viral replication, and host cell response to the virus. The M protein plays a role in determining the shape of the cell envelope, and is able to bind to all other proteins. The E protein is involved in the production and maturation of the virus [8]. The S protein, which inspired the name “coronavirus” due to its crown-

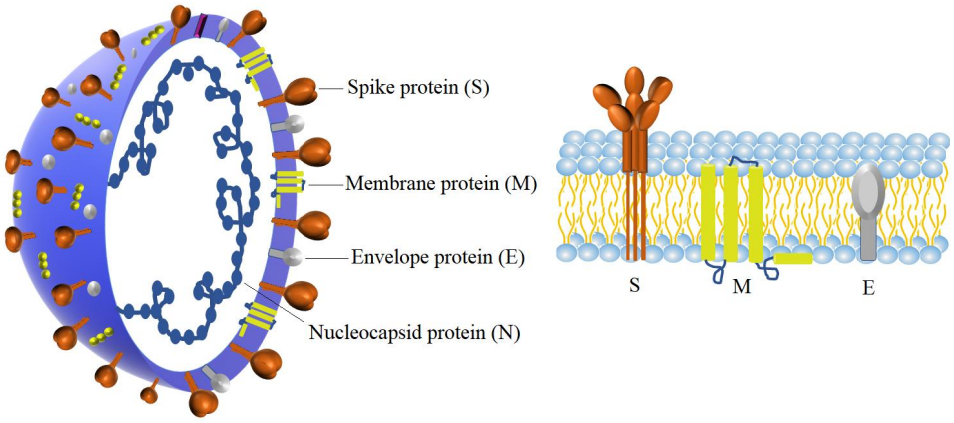

Fig. 1. Schematic representation of the coronaviruses structure showing the four main envelope proteins: Spike protein (S), membrane protein (M), envelope protein (E) and nucleocapsid protein (N). The image also shows a segment of the viral lipid bilayer containing the S, M and E proteins

like (corona) shape, is a transmembrane protein found in the external portion of the virus [9].

In the viral membrane, S proteins exert crucial functions: they bind to cellular receptors, trigger cell attachment, and induce cell-virus fusion. The accomplishment of these three events allows for the release of the viral RNA genome into the host cell and the subsequent start of the viral replication cycle [10]. In addition, the S protein is the primary determinant of the host tropism and pathogenesis of CoVs [11].

Since the S protein is exposed on the surface and mediates entry into host cells, it is the primary target of antibody neutralization during infection. As such, understanding the structure and function of this protein will aid in the development of monoclonal antibody drugs and in the design and development of vaccines [12]. In this context, this review approaches the structural and functional aspects of the S proteins of CoVs. The following issues are presented: structure–function relationship, examples of host receptors, and their interaction with the S protein, and the role in the entry of the virus into host cells.

2. PARTICULARITIES OF CORONA-VIRUSES CAUSING RESPIRATORY SYNDROME IN HUMANS

CoVs have caused major public health problems in recent decades, as exemplified by the epidemics of Severe Acute Respiratory Syndrome (SARS) in 2002, caused by the coronavirus SARS-CoV and originated in China, and of Middle East Respiratory Syndrome (MERS) in 2012, caused by MERS-CoV and originated in Saudi Arabia, which subsequently spread across several countries [13]. As reported in epidemiological studies, SARS affected over 8,000 people, with 774 deaths in 26 countries [14], while MERS-CoV infected 2,494 people and caused 858 deaths in 27 countries [15]. SARS-CoV and MERS-CoV are both β-CoVs [2].

A global pandemic of coronavirus disease 2019 (COVID-19), caused by a new β-CoV, SARS-CoV-2, was declared by the World Health Organization (WHO) in March 11, 2020. As of July 06, 2020, the number of COVID-19 cases was 11,527,382, with 535,723 deaths [16]. In Brazil, 1,626,071 cases and 65,556 deaths have been reported by July 6th, with a recognized underreported number of cases. In addition to SARS-CoV, MERS-CoV, and SARS-CoV-2, four endemic coronaviruses (HCoV-NL63, HCoV-229E, HCoV-OC43, HCoVHKU1) cause the common flu, raising the total number of CoVs capable of infecting humans to seven [12].

SARS-CoV is likely to have originated in wild bats, which are considered a reservoir host [1]. Bats are also strongly believed to have been the origin of SARS-CoV-2, while another study has suggested that pangolins are also natural reservoirs of SARS-CoV-2-like viruses [17]. However, the direct precursor sequence of the virus and whether it had an intermediate host or not have yet to be determined [12]. SARS-CoV-2 is known to be 79.6% similar to SARS-CoV and

50% similar to MERS-CoV at the genomic level [18]. In addition, the SARS-CoV-2 genome is 96.2% identical to the bat CoV RaTG13 and 91.02% similar to pangolin CoV [17]. SARS-CoV-2 genomes have shown to be highly homogeneous, with a relatively low mutation rate; however, novel mutations with influence on virulence, for example, have been reported [19].

COVID-19 has spread across multiple continents causing severe and sustained human-to-human transmission [2]. Social distancing measures have been adopted worldwide due to the fact that SARS-CoV-2 can be transmitted between humans through respiratory droplets and direct contact with mucous membranes (eyes, mouth, and nose) [18]. Airborne transmission is also thought to be possible [20]. In addition, the digestive tract is another potential route of transmission [17]. The incubation period for SARS-CoV-2 is estimated to be 3-7 days (ranging 2-14 days), indicating a long transmission period [21]. In addition, it has been reported that asymptomatic patients of COVID-19 effectively transmit the virus during the incubation period [22].

CoVs often have strategies to evade the immune system. SARS-CoV-2 has several complex components that allow it to evade the immune system, contributing to its virulence, leading to greater infectivity and mortality. SARS-CoV-2 apparently exhibits more deleterious effects in people affected by pre-existing conditions and comorbidities, including hypertension, diabetes, and cardiovascular or kidney diseases, which affect the severity of the disease pathogenesis [8]. However, individuals without pre-existing conditions belonging to different age groups have died due to COVID-19.

Clinical signs associated with SARS-CoV-2 are fever, pneumonia, fatigue, myalgia, dry cough, and dyspnea. Less common symptoms include headache, nasal congestion, sore throat, diarrhea, and vomiting. Some patients experience septic shock and acute respiratory distress syndrome, which can progress to respiratory failure and death [23].

3. THE CORONAVIRUSES SPIKE (S) PROTEIN

3.1 General Characteristics

The structural and functional organization of the S protein is similar to that of other viral glycoproteins, including the influenza hemagglutinin and the human immunodeficiency virus envelope protein, which are collectively referred as class I membrane fusion proteins. The presence of an N-terminal surface subunit (S1), which harbors the receptor binding domain, and a C-terminal transmembrane unit (S2), which contains the functional elements required for membrane fusion (Fig. 2A), is a common feature of these proteins [24].

Following the release of the viral genome into the host cell, the translation of the open reading frame (ORF) produces non-structural proteins that form the viral replicase-transcriptase complex. This complex participates in viral genome replication and transcription. The viral RNA encodes several accessory proteins and the four structural proteins S, E, M, and N. In the endoplasmic reticulum-Golgi apparatus intermediate compartment, the S protein monomer is extensively modified via *N*-glycosylation and trimerizes. For some CoVs, the spike protein can also be partially processed by furin and furin-like proteases [25].

The S protein is a large transmembrane protein with an estimated molecular mass of up to 220 kDa. Spike possesses between 21 and 35 *N*-glycosylation sites in each monomer. This large amount of glycosylation protects most of the spike protein surface, preventing attack by proteases, for example [26]. The size of the spike protein varies greatly between CoVs species, ranging from ca. 1100 to 1600 residues in length. Similar to other class I membrane fusion proteins, the spike protein contains an α-helical coiled-coil structure [5].

The S protein consists of three segments: an ectodomain, a single-pass membrane anchor, and a short endodomain. The ectodomain of all CoVs spike proteins share the same organization in the two functionally distinct subunits mentioned before: S1, which is involved in receptor recognition, and S2, which facilitates membrane fusion and anchors the spike protein into the viral membrane. The S1 subunit diverges in sequence even among virus species of the same genus, while the S2 subunit is the most conserved region of the protein. A notable distinction between the spike proteins of different coronaviruses is the fact that they can be cleaved or not during the assembly and exocytosis of the virions. In most α- and β-CoVs, with few exceptions, the virions harbor an uncleaved spike protein, whereas in some β- and

all γ-CoVs, the protein is cleaved between the S1 and S2 domains [5].

The amino acid sequence of the S protein in SARS-CoV-2 is 76.47% identical to that of SARS-CoV, with the same structural conformation and electrostatic properties in the interaction interface [27]. Coutard et al. reported a furin-like cleavage site in the S protein of SARS-CoV-2, which is absent in other lineages of β-CoVs. RNA viruses mutate with high frequency, but differences in S proteins in the emergent SARS-CoV-2 variants are much less frequent [28]. A study performed in late February and early March 2020 found that the amino acid residue sequences of S proteins in SARS-CoV-2 isolates from different countries remained identical, if not very similar [26]. The author reported that, with respect to the original Wuhan isolate, phenylalanine (F) was replaced by cysteine (C) as residue 797 in a Swedish isolate, while alanine (A) was replaced by valine (V) as residue 990 in an Indian isolate.

3.2 The S1 Subunit

The S1 subunit contains two independent domains, namely, an N-terminal domain (NTD) and a C-terminal domain (CTD), both able to function as the receptor-binding domain of CoVs [29]. The NTD usually binds carbohydrate moieties, while the CTD binds proteinaceous receptors. The NTD appears to have been deleted from the S protein of HCoV-229E and PRCoV, which suggest that this domain is dispensable for some CoVs. PRCoV is a naturally occurring variant of TGEV, and the deletion of the NTD is linked to the shift of tissue tropism from the enteric (TGEV) to the respiratory tract (PRCoV) [30]. MERS-CoV utilizes the CTD to bind the dipeptidyl peptidase 4 (DPP4) at the membrane of target cells [25]. Besides, studies have revealed that the CTD of SARS-CoV and SARS-CoV-2 recognizes the angiotensin-converting enzyme 2 (ACE2), abundantly detected on the membrane of lung and small intestine cells [2].

The spike protein in CoVs is thought to have evolved from a more basic structure in which receptor recognition was confined to the CTD domain of S1 [31]. There is evidence supporting the hypothesis that the NTD was acquired at a later point in the evolutionary history of CoVs. Due to structural similarities between the NTD of S proteins and some host cell proteins, it has been suggested that this domain emerged from the acquisition of a host sugar-binding galectin-like domain by an ancestral coronavirus [32]. This acquisition may have resulted in a great extension of CoV host range, resulting in an increase in CoV diversity [33].

The S1 subunit of β-CoVs displays a multidomain architecture organized in four distinct domains (A–D). Domains A and B serve as receptor-binding domains. The core structure of domain A displays a galectin-like β-sandwich fold, whereas domain B contains a structurally conserved core subdomain of antiparallel β-sheets [34]. Domain B is decorated with an extended loop that differs greatly in size and structure between species of β-CoVs. Domains C and D consist of discontinuous parts of the primary protein sequence and form β-sheet-rich structures directly adjacent to the S2 core [4].

3.3 The S2 Subunit

The S2 subunit contains the key segments that facilitate virus-cell fusion. Among them, there is a fusion peptide, two heptad repeat regions (HR1 and HR2), and transmembrane domains, which are well conserved among CoVs species. The fusion peptide appears to be located adjacent to one of the two cleavage sites; this region contains a remarkably conserved motif, I-E-D-L-L-F, which is present in the S proteins of all CoV genera. HR1 and HR2 are composed of patterns of a seven amino acid chain that forms α-helical structures. The HRs participate in the formation of a coiled-coil structure during membrane fusion. The transmembrane domain is found in the C-terminal end of the S2 subunit [34].

4. INTERACTION OF THE S PROTEIN WITH HOST CELL RECEPTORS

Table 1 summarizes the different CoVs and the receptors for their spike proteins. In order to engage a host cell receptor, the receptor-binding domain of the S1 subunit undergoes hinge-like conformational movements. There are two states of the S1 subunit, namely, the “down” and “up” conformations: The down conformation represents an inaccessible state of the receptor-binding domain, whereas the up conformation corresponds to an accessible state. The binding of the S1 subunit of the β-CoVs to a host cell receptor destabilizes the trimeric structure, leading to shedding of the S1 subunit and the transition of the S2 subunit into a highly stable post-fusion conformation [12].

Table 1. List of some important coronaviruses and the receptors of their spike (S) proteins.

Virus	Genus	Disease	Spike receptor
BCoV	*beta*	Respiratory and enteric infections	O-acetylated sialic acids
HCoV-NL63	*alpha*	Mild respiratory tract infections	ACE2
HCoV-OC43	*beta*	Mild respiratory tract infections	O-acetylated sialic acids
IBV	*gamma*	Respiratory and urogenital tract infections	Sialic acids
MERS-CoV	*beta*	Severe acute respiratory syndrome	DPP4
MHV	*beta*	Respiratory and enteric infections	CEACAM1a
PRCoV	*alpha*	Respiratory and enteric infections	APN
SARS-CoV	*beta*	Severe acute respiratory syndrome	ACE2
SARS-CoV-2	*beta*	Severe acute respiratory syndrome (COVID-19)	ACE2
TGEV	*alpha*	Respiratory tract infection	sialic acids

Abbreviations: ACE2: angiotensin-converting enzyme 2; APN: aminopeptidase N; DPP4: dipeptidyl peptidase 4; CEACAM1a: carcinoembryonic antigen-related cell adhesion molecule 1a

Upon binding to the receptor, the spike protein is cleaved by nearby host proteases (Fig. 2B) and releases a signal peptide to facilitates virus entry into host cells. The fusion peptide of the S2 subunit is activated through proteolytic cleavage at a site common to all coronaviruses and is inserted into the target cell membrane. At this stage, the S2 subunit is connected with the viral membrane via its transmembrane domain and with the cellular membrane via the fusion peptide (Fig. 2C). Subsequently, the HR1 and HR2 regions within the S2 subunit fold back onto each other (Fig. 2D), forming a six-helix-bundle structure. As a consequence, the S2 subunit collapses and the fusion of viral and cellular membranes occurs [32].

In the S proteins of α- and β-CoVs, the receptor-binding domain core is structurally conserved, while the receptor-binding motifs, which determine receptor specificity, can vary extensively. For instance, the CTDs of the α-CoVs PRCoV and HCoV-NL63 have similar core structures, suggesting a common evolutionary origin, but diverged in their motifs recruiting different receptors (aminopeptidase N and ACE2, respectively). A similar situation is observed in the CTDs of SARS-CoV and MERS-CoV that bind ACE2 and DPP4, respectively. Conversely, the CTDs of the HCoV-NL63 and SARS-CoV both recognize ACE2, but via distinct molecular interactions (ACE2 recognition via three versus one receptor-binding motif, respectively), which suggests a convergent evolution pathway for these viruses in recruiting the ACE2 receptor [5,31]. The well-conserved nature of DPP4 (detected on lung and kidney cells) across a wide variety of species (e.g. bats, dromedaries, and humans) is associated with the zoonotic capabilities of MERS-CoV [35].

As mentioned previously, the NTD appears to mainly bind glycans. The NTDs of the S proteins from TGEV and of the γ-CoV IBV bind to sialic acids, while the NTDs of β-CoVs, including BCoV and HCoV-OC43, have been shown to bind to O-acetylated sialic acids. Only the NTD of MHV is known to interact with a protein receptor, namely the murine carcinoembryonic antigen-related cell adhesion molecule 1a (CEACAM1a) [36]. The S1 subunit of the SARS-CoV-2 spike protein has been reported to bind to heparin, and therefore shows potential in the development of therapeutic drugs using heparin and tailor-made glycosaminoglycans-based antivirals [37].

4.1 Binding of SARS-CoVs Spike Proteins to ACE2

As mentioned above, SARS-CoV most likely developed from SARS-CoV-like viruses in bats. In these animals, viral entry does not occur via binding to ACE2, and the receptor is unknown. However, the replacement of the amino acid sequence of S protein found between residues 323 and 505 with the corresponding sequence of SARS-CoV was sufficient to allow for binding to human ACE2 as a receptor [5,31]. SARS-CoV also has a single intact open reading frame on gene 8, which is another sign of its origin in bats [38].

In SARS-CoV, the residues at positions 442, 472, 479, 487, and 491 in the S protein have been observed at the receptor complex interface with ACE2. However, four of these five critical residues are not preserved in the SARS-CoV-2 spike protein, except Tyr491. Consequently, the binding free energy needed for the binding of the SARS-CoV-2 spike protein to ACE2 increased by 28 kcal/mol compared to that of the SARS-CoV

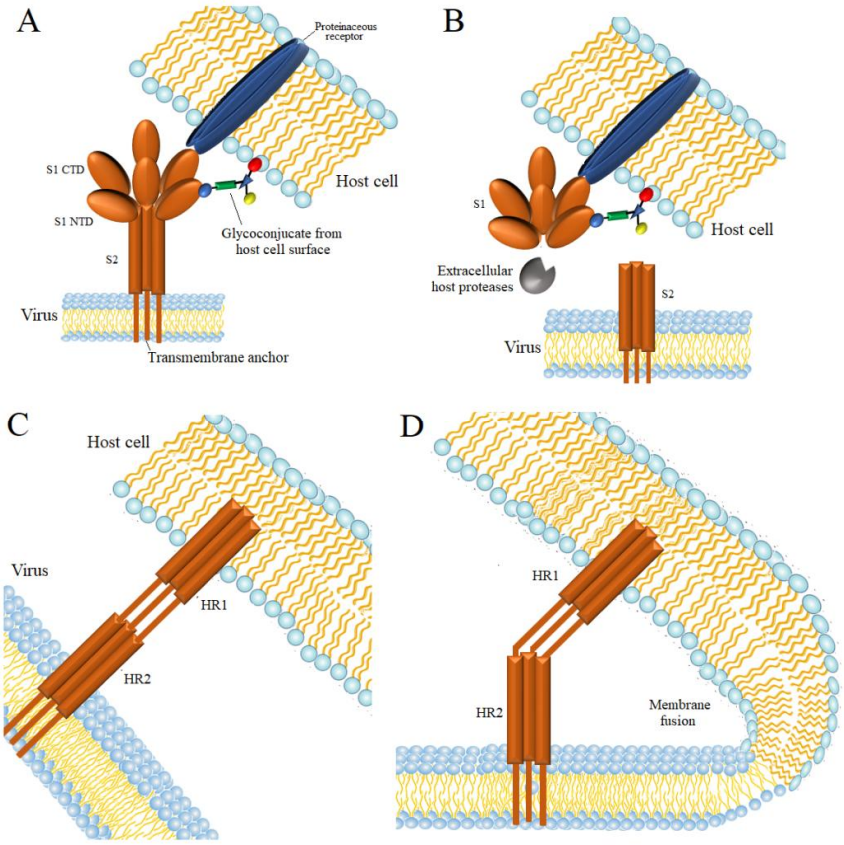

Fig. 2. The roles of spike protein: binding to cellular receptors, cell-virus attachment, and induce cell-virus fusion. (A) Scheme of the spike protein structure showing its N-terminal surface subunit (S1) and the C-terminal transmembrane unit (S2). The interaction with the cell host surface is also represented. The N-terminal (NTD) and C-terminal (CTD) S1 domains are able to interact with a carbohydrate moiety and a proteinaceous receptor, respectively, at the cell surface. (B) The binding of the S1 subunit to a host cell receptor is followed by cleavage of the S1 subunit by host proteases. (C) Next, the S2 subunit binds to cellular membrane through its fusion peptide and (D) the HR1 and HR2 regions within the S2 subunit fold over themselves to promote fusion between viral and cell membranes, forming a six-helix-bundle

protein [27]. The affinity between ACE2 and SARS-CoV-2 spike ectodomain is ca. 10- to 20-fold higher than that between ACE2 and SARS-CoV domain. This may explain the rapid development and strong ability of human-to-human transmission in COVID-19 [39].

The interaction between SARS-CoV-2 S and ACE2 is similar to that between SARS-CoV and ACE2, indicating that they use the same mechanism of entry into host cells [39,40]. While the respiratory system is a primary target of SARS-CoV-2, bioinformatics analysis of the lungs, esophagus, duodenum, ileum, and colon has revealed that the digestive system is also a potential route of entry for COVID-19, since ACE2 is highly expressed in lung alveolar type 2 cells, esophagus upper and stratified epithelial cells, and absorptive enterocytes from the ileum and colon [17].

5. CONCLUSIONS

The S protein in CoVs is one of the main topics in CoV research since it plays a central role in the adhesion and entry of the virus into host cells. In addition, the S protein has a high level of conservation among different CoVs; therefore, its sequence variations can be used to determine evolutionary relationships among the viruses. Studies on S protein could also be used to improve our understanding of changes in virulence and tissue tropism in CoVs, as well as relationships between different hosts and

differences in disease severity. The COVID-19 pandemic has started a race for the development of treatments and vaccines, as well as for the acquisition of information on the origin and evolution of SARS-CoV-2. In this scenario, the S protein represents a good target for the development of vaccines and antiviral drugs, such as monoclonal antibodies and glycosaminoglycan-based drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Banerjee A, Kulcsar K, Misra V et al. Bats and Coronaviruses. Viruses. 2019;11: 41.
2. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med. 2020;382:1199.
3. Jones BA, Grace D, Kock R et al. Zoonosis emergence linked to agricultural intensification and environmental change. Proc Natl Acad Sci USA. 2013;21:8399–8340.
4. Reguera J, Santiago C, Mudgal G, et al. Structural bases of coronavirus attachment to host aminopeptidase N and its inhibition by neutralizing antibodies. PLoS Pathog. 2012;8:e1002859.
5. Belouzard S, Millet JK, Licitra BN et al. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses. 2012;4:1011–1033.
6. Decaro N, Lorusso A. Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. Vet Microbiol. 2020;244:108693.
7. Jiang S, Hillyer C, Du L. Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. Trends Immunol. 2020;41:355–359.
8. Astuti IY. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab Syndr. 2020; 14:407-412.
9. Li F. Receptor recognition and cross-species infections of SARS coronavirus. Antivir Res. 2013;100:246–254.
10. Tortorici MA, Walls AC, Lang Y, et al. Structural basis for human coronavirus attachment to sialic acid receptors. Nat Struct Mol Biol. 2019;26:481-489.
11. Chang HH, Chen PK, Lin GL, et al. Cell adhesion as a novel approach to determining the cellular binding motif on the severe acute respiratory syndrome coronavirus spike protein. J Virol Methods. 2014;201:1–6.
12. Li H, Liu S, Yu X, et al. Coronavirus disease 2019 (COVID-19): Current status and future perspectives. Int J Antimicrob Agents. 2020;20:54.
13. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology. 2018;23:130–137.
14. WHO. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. WHO; 2004. Available:https://www.who.int/csr/sars/country/table2004_04_21/en/
Accessed May 23, 2020.
15. WHO. Middle East respiratory syndrome coronavirus (MERS-CoV). WH; 2019.
Availablr:https://www.who.int/emergencies/mers-cov/en/
(Accessed May 23, 2020).
16. WHO. Coronavirus disease (COVID-19) pandemic. WHO; 2020.
Available:https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
(Accessed July 06, 2020).
17. Zhang T, Wu Q, Zhang Z. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. Curr Biol. 2020;30:1346–1351.
18. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet. 2020; 395:565–574.
19. Jia Y, Shen G, Zhang Y, et al. Analysis of the mutation dynamics of SARS-CoV-2 reveals the spread history and emergence of RBD mutant with lower ACE2 binding affinity. BioRxiv. 2020;034942.
20. Morawska L, Cao J. Airborne transmission of SARS-CoV-2: The world should face the reality. Environ Int. 2020;139:105730.
21. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel

coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20–28 January. Euro Surveill. 2020;25:2000062.
22. Rothe C, Schunk M, Sothmann P et al. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N Engl J Med. 2020;382:970–971.
23. Guan WJ, Ni ZY, Hu Y et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382: 1708–1720.
24. Yuan Y, Cao D, Zhang Y, et al. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat Commun. 2017;8:15092.
25. Tang T, Bidona M, Jaimes JA et al. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. Antivir Res. 2020;178: 104792.
26. Robson B. COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and analysis of a proposed achilles' heel conserved region to minimize probability of escape mutations and drug resistance. Comput Biol Med. 2020;11: 103749.
27. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci. 2020;12:1-5.
28. Coutard B, Valle C, de Lamballerie X et al. The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. Antiviral Res. 2020;176: 104742.
29. Jiaming L, Yanfeng Y, Yao D, et al. The recombinant N-terminal domain of spike proteins is a potential vaccine against Middle East respiratory syndrome coronavirus (MERS-CoV) infection. Vaccine. 2017;35:10–18.
30. Schultze B, Krempl C, Ballesteros ML et al. Transmissible gastroenteritis coronavirus, but not the related porcine respiratory coronavirus, has a sialic acid (N-glycolylneuraminic acid) binding activity. J Virol. 1996;70:5634–5637.
31. Li F. Receptor recognition mechanisms of coronaviruses: A decade of structural studies. J Virol. 2015;89:1954-64.
32. Simmons G, Zmora P, Gierer S et al. Proteolytic activation of the SARS-coronavirus spike protein: Cutting enzymes at the cutting edge of antiviral research. Antivir Res. 2013;100:605–614.
33. Walls AC, Xiong X, Park YJ et al. Unexpected receptor functional mimicry elucidates activation of coronavirus fusion. Cell. 2019;176:1026-1039.
34. Kirchdoerfer RN, Cottrell CA, Wang N et al. Pre-fusion structure of a human coronavirus spike protein. Nature. 2016; 531:118–121.
35. van Doremalen N, Miazgowicz KL, Milne-Price S et al. Host species restriction of Middle East respiratory syndrome coronavirus through its receptor, dipeptidyl peptidase 4. J Virol. 2014;88:9220–9232.
36. Peng G, Xu L, Lin YL et al. Crystal structure of bovine coronavirus spike protein lectin domain. J Biol Chem. 2012; 287:41931.
37. Mycroft-West C, Su D, Elli S et al. The 2019 coronavirus (SARS-CoV-2) surface protein (Spike) S1 Receptor Binding Domain undergoes conformational change upon heparin binding. BioRxiv; 2020.
38. Wan S, Yi Q, Fan S, et al. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). MedRxiv; 2020.
39. Wrapp D, Wang N, Corbett KS et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020; 367:1260–1263.
40. Yang TJ, Chang YC, Ko TP et al. Cryo-EM analysis of a feline coronavirus spike protein reveals a unique structure and camouflaging glycans. Proc Natl Acad Sci USA. 2020;117:1438–1446.

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