# How the COVID-19 Overcomes the Battle? An Approach to Virus Structure

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Coronaviruses primarily cause zoonotic infections, however in the past few decades several interspecies transmissions have occurred, the last one by SARS-CoV-2, causing COVID-19 pandemic, posing serious threat to global health. The SARS-CoV-2 spike (S) protein plays an important role in viral attachment, fusion and entry. However, other structural and non-structural SARS-CoV-2 proteins are potential influencers in virus pathogenicity. Among these proteins; Orf3, Orf8, and Orf10 show the least homology to SARS-CoV proteins and therefore should be further studied for their abilities to modulate antiviral and inflammatory responses. Here, we discuss how SARS-COV-2 interacts with our immune system.

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Coronaviruses are a large family of viruses infecting many species including humans. The first coronavirus, the prototype murine coronavirus strain JHM was reported in 1949.[1] Since for a long time the only disease caused by human coronaviruses (CoV) was the common cold, this family of viruses remained relatively neglected until several human illnesses causing a range of upper and lower respiratory, enteric, and neurological diseases were identified.[2,3] The first severe disease caused by coronavirus was the Severe Acute Respiratory Syndrome (SARS), a pandemic in China in 2003.[4,5] Next was an outbreak of a severe illness in 2012 in Saudi Arabia called the Middle East Respiratory Syndrome (MERS).[6] The estimated case fatality for these two cases were about 11% and 35%, respectively. Yet another disease, COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was reported in December 2019 in China. SARS-CoV-2 belongs to betacoronaviruses and shows about 79% similarity with SARS-CoV, the origin of the virus is not well known yet however, it shows the highest similarity (~89%) with the horse-shoe bat coronavirus, therefore being thought to have been transmitted from bats to human through an intermediate host.[7-9] The disease very soon turned to a pandemic as of April 23rd 2020, nearly 2,7 million cases were documented, of which about 191 000 were killed. However, there are many mild cases that have gone undiagnosed.[10] In all three cases, there is strong evidence for zoonotic origins and interspecies transmission.[11]

Studies show that similar to SARS-CoV, the SARS-CoV-2 remains active on plastic and stainless steel surfaces for two to three days under the laboratory conditions and it remains infectious for up to 24 hours on cardboard and four hours on copper. Under the same laboratory conditions, the virus is detectable in aerosols for up to three hours, suggesting that people may acquire SARS-CoV-2 through the air and after touching contaminated objects. However, under real-world conditions, factors such as temperature, humidity, ventilation, and the amount of virus deposited affect these results.[12] A study on SARS-CoV has previously

shown that the virus viability is rapidly lost at higher temperatures and higher relative humidity (38°C, and > 95%; respectively).[13] This seems also to be the case for SARS-CoV-2, as recent data shows the decreased number of SARS-CoV-2 cases upon increase in temperature both at local as well as global levels. Higher temperature is suggested to prevent the spread of virus carrying droplets perhaps through faster evaporation.[14] Otherwise, differences in epidemiologic characteristics are perhaps due to other factors such as high viral load in the upper respiratory tract[12] as well as the possibility by which asymptomatic-infected individuals could transmit the coronavirus.[15,16]

Coronaviruses are enveloped, positive-sensed single stranded RNA viruses, with a 26–32 kb length non-segmented genome.[2,3,17] The genome includes 14 open reading frames (ORFs, Figure 1). The first two overlapping ORFs (ORF1a and ORF1b) located at the 5′ end occupy about two third of genome and encode proteins which are auto-proteolytically processed into 16 non-structural proteins (Nsp1 to 16).[11] Nsps play vital roles ranging from RNA replication and transcription of the virus to the inhibition of interferon (IFN) signaling.[18] The function of the Nsps are summarized in Table. Interactions between several of SARS-CoV-2 Nsp proteins and human proteins have also been highlighted in a protein-protein interactions (PPIs) study showing interactions with host DNA replication for Nsp1, vesicle trafficking for Nsp6, 7, 10, 13, 15, cytoskeleton for Nsp1,13 and mitochondrial interactions for Nsp4 and Nsp8.[18] Nsp13 is also reported to interact with key players of the interferon pathway as well as multiple proteins of NF-κB inflammatory response.[18]

The 3′ end of genome encodes four main structural proteins: spike (S) protein, nucleocapsid (N) protein, membrane (M) protein, and envelope (E) protein (Figure 1); all of which are required for the structurally complete viral particle (Figure 2).[19] Among these four structural proteins, the ~200 kDa glycoprotein S is essential for tissue tropism, attachment of the virus to the host cell surface receptors, membrane fusion and thereby entry into the host cell. The coronavirus S proteins require protease cleavage between the amino-terminal S1 and the carboxy-terminal S2 domain, activated by receptor binding domain (RBD) located at S1 subunit, which allows S2 conformational change, membrane fusion, virus entry and syncytia formation.[19-21] A variety of host proteases in different cell types and tissues e.g. furin, trypsin, cathepsin, elastase, and transmembrane protease serine 2 (TMPRSS2) activate the S protein of SARS-CoV have been so far identified.[22] It is particularly speculated that cathepsin L, TMPRSS2, and furin-like proteases could participate in cleavage of SARS-CoV-2 S1/S2, thereby allowing S2 to drive fusion of viral and cellular membranes.[23-6] Similar to SARS-CoV S, angiotensin-converting enzyme 2 (ACE2) has been shown to be the functional, high affinity receptor for SARS-CoV-2 S.[21,23,27] However, the SARS-CoV-2 RBD shows significantly higher binding affinity to ACE2 receptor compared to SARS-CoV.[21] In addition, it has been suggested that the modest ACE2 expression in the upper respiratory tract might be a limiting factor for SARS-CoV transmissibility while considering the higher transmissibility of SARS-CoV-2, it is possible that additional cellular attachment-promoting factors such as binding to cellular glycans by the virus S1 domain might ensure the higher transmissibility of SARS-CoV-2.[26,28] It has also been shown that the

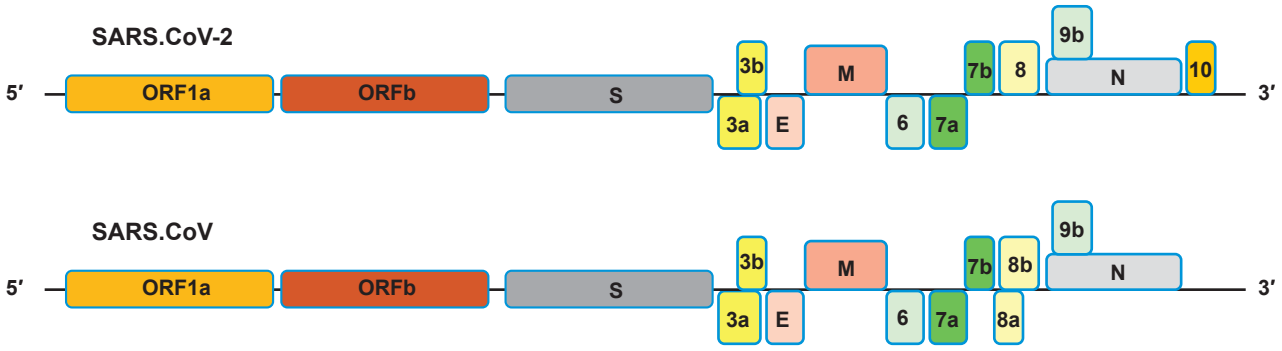


**Figure 1.** Schematic representation of the genome organization of SARS-CoV-2 compared to SARS-CoV.

SARS-CoV-2 Proteins, Sequence Similarities and Functions Partly Based on the SARS-CoV Homolog

| ORF / Protein | Seq. Similarity (%) | SARS-CoV-2 | SARS-CoV |
|---|---|---|---|
| | | Protein Function | |
| ORF1a,b | | | |
| Nsp1 | 91 | Host Cell mRNA Degradation, Inhibiting Interferon Signaling | |
| Nsp2 | 83 | | |
| Nsp3 | 86.5 | Essential Component of the Replication/Transcription Complex<br>Blocking Host Innate Immune Response, Double Membrane Vesicle (DMV) Formation | |
| Nsp4 | 91 | DMV Formation, Viral Replication | |
| Nsp5 | 99 | Main Protease, Inhibiting IFN Signaling | |
| Nsp6 | 95 | Restricting Autophagosome Expansion, DMV Formation | |
| Nsp7 | 100 | Component of Multimeric RNA Polymerase | |
| Nsp8 | 99 | Component of Multimeric RNA Polymerase | |
| Nsp9 | 98 | Replicase, RNA/DNA Binding | |
| Nsp10 | 99 | Viral RNA Proofreading, Methyltransferase | |
| Nsp11 | 92 | | |
| Nsp12 | 98 | RNA Polymerase | |
| Nsp13 | 100 | Helicase, RNA 5′-triphosphatase | |
| Nsp14 | 99 | Exoribonuclease, Modulating Innate Immune Response | |
| Nsp15 | 96 | Endoribonuclease | |
| Nsp16 | 98 | Methyltransferase | |
| S | 87 | Spike Protein, Binds to ACE2 | Spike Protein, Binds to ACE2, Inducing Expression of IL6, IL8, and TNF Through NF-κB Activation in Macrophages |
| ORF3a | 85. | Attacking the Heme on the 1-beta Chain of Hemoglobin | NLRP3 Inflammasome Activation |
| ORF3b | 9.5 | | Interferon Antagonist |
| E | 96 | Envelope Protein | Envelope Protein |
| M | 96 | Membrane Protein | Membrane Protein, Blocks IFN-β Production |
| ORF6 | 86 | | Type I IFN Antagonist |
| ORF7a | 90 | | Inducing Inflammatory Response by Activating NF-κB and the IL-8 Promotor, Inducing Apoptosis |
| ORF7b | 84 | | |
| ORF8 | 45 | Forming Complex with Porphyrin | Activating the NLRP3 Inflammasomes and Triggers Cell Death |
| N | 95 | | Nucleocapsid Phosphoprotein |
| ORF9b | 85 | | Inducing Apoptosis, IFN Antagonist |
| ORF10 | - | Attacking the Heme on the 1-beta Chain of Hemoglobin | |

human T lymphocytes are more sensitive to SARS-CoV-2 infection than SARS-CoV indicating that the S protein in SARS-CoV-2 mediates infectivity even in cells expressing low ACE2 levels, which would be one explanation for why the transmission rate of SARS-CoV-2 is so high.[29]

Upon the host-cell and virus ACE2/S protein mediated binding and fusion, and viral entry, the viral genomic RNA is released and translated into viral polymerase proteins. The negative sense RNA then is synthesized and serves as a template to make the genomic positive sense RNA. The viral nucleocapsids (RNA+N protein) are synthesized in the cytoplasm whereas, the other structural proteins; S, M and E proteins are transcribed and translated in the ER and transported to the Golgi. Eventually the mature virion consisting of Nucleocapsid, S, M and E proteins are further assembled in the ER-Golgi intermediate compartment (ERGIC) and then released from the host cell by exocytosis (Figure 2).[30,31]

Although the genetic composition is very similar in both viruses; Orf3, Orf8, and Orf10 at the 3′ end of genome show limited homology. Orf3 of SARS-CoV-2 is significantly different from the SARS-CoV Orf3a, which in SARS-COV induces NLRP3 (NOD-like receptor family pyrin domain-containinig-3) inflammasome activation or Orf3b of SARS-CoV which is an interferon antagonist, therefore, it might be interesting to compare these

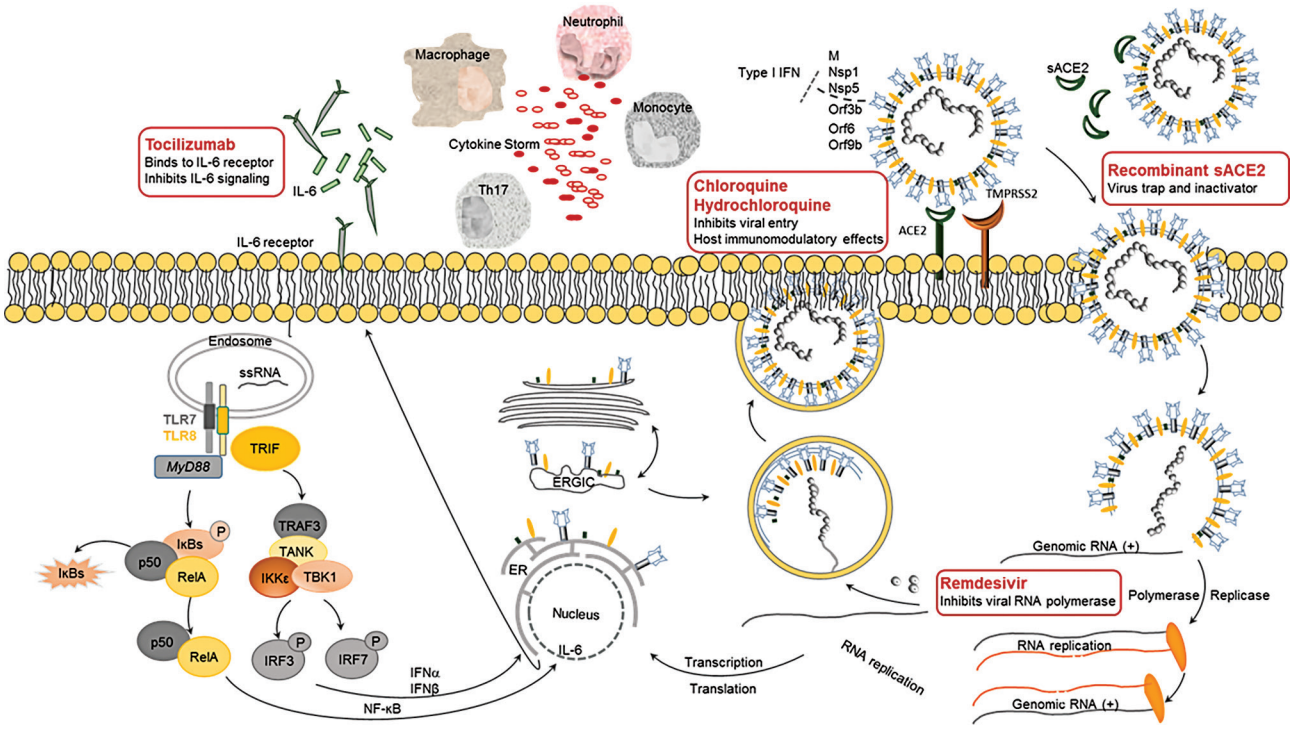


**Figure 2.** Schematic representation of viral entry to the host cell, processing within the cell and virus-induced immune response. Some potential drugs and their targets are also presented.

two proteins for their abilities to modulate antiviral and proinflammatory response.[32] So far, data shows that SARS-CoV-2 ORF3b has a restricted expression perhaps suggesting that it is not a bona fide protein coding gene, while Orf3a seems to interact with cellular vesicle trafficking and may modify endomembrane compartments to favor virus replication (Table).[18] Orf8 seems to be intact in SARS-CoV-2, while SARS-CoV encodes Orf8a and Orf8b. It has been shown that the Orf8b in SARS-CoV forms insoluble intracellular aggregates, which can trigger ER stress, mitochondrial dysfunction, lysosomal damage, autophagy, and cell death in epithelial cells. In macrophages, Orf8b also activates the NLRP3 inflammasomes and triggers cell death.[33] Therefore, it will be clinically important to elucidate the biological function of Orf8 in SARS-CoV-2. The Orf10 of SARS-CoV-2 is reported to interact with members of Cullin 2 RING E3 ligase complex, which plays role in targeting cellular proteins for ubiquitination dependent protein turn-over by 26s proteasome, suggesting that Orf10 may bind to the complex and hijack it for ubiquitination and degradation of restriction factors, a target that might be interesting for drug inhibition.[18] Data also indicates that Orf8 and surface glycoprotein of SARS-COV-2 can bind to Porphyrin and form a complex; while Orf1a,b, Orf10, and Orf3a can attack the heme on hemoglobin 1-beta chain to dissociate the iron from the Porphyrin, and this in turn will lead to less hemoglobin to carry oxygen and carbon dioxide.[34]

The immunological response of SARS-CoV-2 is not yet very well understood but considering the fact that SARS-CoV-2 uses the same ACE2 receptor for entering cells as SARS-CoV, it is likely that alveolar epithelial cells, vascular endothelial cells, macrophages, monocytes and lymphocytes serve as target for SARS-CoV-2 as well.[35] Upon ACE2 dependent SARS-CoV-2 cells infection, the viral genomic RNA or its intermediate products during replication are recognized by TLR7 and TLR8.[36,37] This recognition initiates activation of downstream signaling cascades such as NF-κB, leading to expression of α-IFN, TNF-α (Tumor Necrosis Factor alfa), and the secretion of interleukins (IL-12, IL-6, and IL-1; Figure 2). The innate-adaptive cross talk against viral infections depends greatly on interferon responses. This results in the formation of $CD8^+$ specific cytotoxic T-cells, which leads to the formation of antigen-specific B-cells and antibody production in a $CD4^+$ helper T-cell dependent

manner.[38] For SARS-CoV, the response to viral infection through IFN production is suppressed. Based on genomic sequence similarities, it is speculated that SARS-CoV-2 might use the same strategy to modulate the host innate immune response. Dampening of anti-viral IFN responses results in uncontrolled viral replication, blocks the maturation of dendritic cells and makes the immune system unable to mount efficient adaptive immune response.[39] Interleukin 6 plays also a very delicate and critical role in appropriate adaptive immune response. Indeed, abnormal concentration of IL-6 blocks CD8+ cytotoxic T cells and by inducing suppressor of chemokine signaling-3 (SOCS-3) and PD-1 can paralyze adaptive response to viral infections.[40] Herold *et al.* have shown plasma level of IL-6 higher than 80pg/ml in their patients was significantly associated with respiratory failure and intubation with an AUC of 0.98.[41] IL-6 may also deviate the immune system toward TH17 pathway. Consequently, the influx of neutrophils and monocytes/macrophages leads to overproduction of pro-inflammatory cytokines, the so called cytokine storms (Figure 2), which might be the reason of lung immunopathology.[36,37] Moreover, more recently clinical manifestation of COVID-19 patients orient us also toward diffuse endothelial dysfunction that leads to pulmonary thrombi, severe cardiovascular involvements and antiphospholipid antibody production.[42,43] Magro and co-workers demonstrated the role of alternative and lectin pathway of complement in pathogenesis of diffuse endothelial injury.[44] There are numerous of drugs under study, from those that block cell entry and viral replication to the drugs oriented against non-adapted cytokine storm and so on. Along with increasing our knowledge about the molecular basis of the virus attack, in near future effective treatment is not far to reach.

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