

Review

# COVID-19: A Systematic Review of the Transmissibility, Pathogenesis, Entry Factors, and Signature Immune Response

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**Abstract:** Objectives: The emergence of coronavirus disease 2019 (COVID-19), caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a global health calamity unprecedented in the modern world. The disease spread worldwide, and to date, there have been over 230 million confirmed cases of COVID-19, including approximately 4.7 million deaths. Mutant variants of the virus have raised concerns about additional pandemic waves and threaten to reverse our progress thus far to limit the spread of the virus. These variants include Alpha, Beta, and Delta (first reported in December 2020 in the United Kingdom, South Africa, and India, respectively) and Gamma (reported in January 2021 in Brazil). In some cases, countries have even reported a rise in daily cases higher than the first wave in March 2020. Given the rapidly evolving nature of COVID-19 and subsequent new findings and updates each day, this review article aims to comprehensively summarize the etiology, pathophysiology, and clinical features of SARS-CoV-2 infection. Methods: A systematic review of the literature was performed in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to gain insight into the transmissibility, pathogenesis, entry factors, and immune response of COVID-19. Specifically, Pubmed and Google Scholar databases were searched to identify any relevant articles. References within the included articles were reviewed. Published articles related to search criteria from the onset of the COVID-19 pandemic to March 2022 were included. Results: Viral transmissibility is predominantly affected by the modes of transmission, various mutations on the nucleocapsid protein and endoRNase, gender, age, and other factors. The pathophysiological mechanism is generally unknown, although the clinical manifestations such as headache, loss of smell and taste, vomiting, diarrhea, multiorgan failure, and dermatological and cardiovascular complications are well documented. The progression of infection depends on the immunopathological response and the innate/adaptive immunity. Conclusion: Our review has summarized the latest knowledge about SARS-CoV2. However, as the pandemic continues to spread across the continents, there is an urgent need for more research on potentially emerging coronaviruses and the development of a universal coronaviruses vaccine to put the pandemic behind us.

**Keywords:** COVID-19; SARS-CoV-2; viral transmissibility; entry factors; viral pathogenesis; ACE2 receptor; delta variant



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## 1. Introduction

The emergence of the coronavirus disease 2019 (COVID-19), caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a global health calamity that is unprecedented in the modern world. Part of the Genus ‘Coronaviruses’ belonging to the family ‘Coronaviridae’, SARS-CoV-2 is the third virus of its kind responsible for an outbreak (albeit with greater global impact), succeeding SARS-CoV in 2003 and MERS-CoV (the Middle East Respiratory Syndrome Coronavirus) in

2012–2015 and 2020 [1]. COVID-19 emerged as novel pneumonia in Wuhan, Hubei Province, China, in December 2019 and is believed to have originated via zoonotic transmission. The disease quickly spread worldwide, prompting the World Health Organization (WHO) to declare the outbreak a pandemic on 11 March 2020. To date, there have been over 230 million confirmed cases of COVID-19, including approximately 4.7 million deaths [2]. The pandemic has also disrupted the global economic landscape due to widespread lockdowns, leading to loss of income for individuals and precipitating staggering negative trends in global stock markets [3].

Although substantial progress has been made regarding our understanding and treatment of SARS-CoV-2, governments are still struggling to vaccinate their populations to herd immunity levels. Moreover, mutant variants of the virus have raised concerns about additional pandemic waves and threaten to reverse our progress thus far to limit the spread of the virus. These variants include Alpha, Beta, and Delta (first reported in December 2020 in the United Kingdom, South Africa, and India, respectively) and Gamma (reported in January 2021 in Brazil) [4]. In some cases, countries have even reported a rise in daily cases that is higher than the first wave in March 2020 [5].

Given the rapidly evolving nature of COVID-19 and subsequent new findings and updates each day, this review article aims to comprehensively summarize the etiology, pathophysiology, and clinical features of SARS-CoV-2 infection.

## 2. Materials and Methods

### 2.1. Eligibility Criteria

For the selection of the papers, the following inclusion criteria were defined: i. articles focused on COVID-19, transmissibility, pathogenesis, immune system, variants, and ii. articles containing original data. The review studies that were doubles from various databases, studies from other SARS-CoV family members, studies with errata correspondence, and abstract presentations were excluded.

### 2.2. Information Sources

We have utilized various databases such as PubMed, MedRxiv, Scholar, Scopus, and Web of Science databases to conduct our literature research up to March 2022. The research strategy adopted included different combinations of the following terms: “SARS-CoV-2”, “COVID-19”, “transmissibility”, “pathogenesis”, “pathophysiology”, “entry factors”, “ACE2”, “COVID variant”, and “immune system”. Further filtering of reference lists was conducted manually by the authors.

### 2.3. Study Selection

All studies identified with the electronic and manual searches were listed by citation. We have followed the PRISMA and reporting guidelines. The PRISMA flow diagram of the selection process is provided in Figure 1.

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

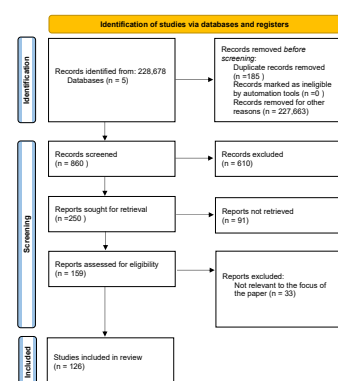


Figure 1. PRISMA chart of the literature flow of the study.

All the authors independently thoroughly researched the titles and abstracts of non-duplicated papers and excluded those that were not relevant to the topic and that were of the initial stages of discovery. Following this, the authors also independently reviewed the final list of the papers by reading the papers.

#### 2.4. Data Extraction

All the authors of this manuscript conducted the data extraction using a predefined form, including the following information: author, month and year, study location, period, design, and setting. During the data extraction, the outcomes of interest were classified into five major themes: COVID-19 transmissibility, pathogenesis and pathophysiology, entry factors, ACE2 receptor, and various SARS-CoV-2 variants.

#### 2.5. Assessment of Risk of Bias

The overlapping risk was assessed through a manual search which led us to the exclusion of 185 articles.

### 3. Results

#### 3.1. Viral Transmissibility

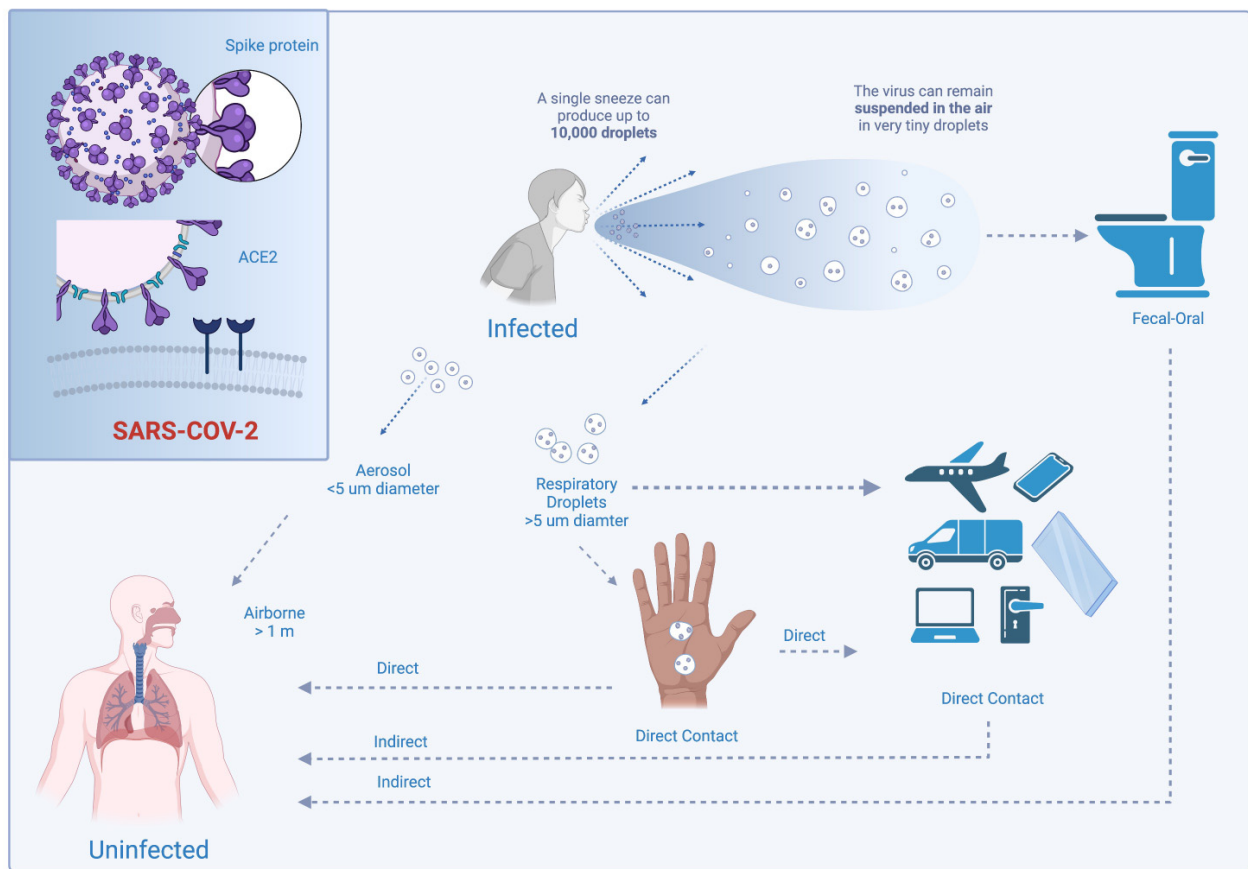
The primary mode of transmission of SARS-CoV-2 is human-to-human via respiratory droplets produced during exhalation, as shown in Figure 2 [6–8]. However, there is also evidence that suggests the virus is transmissible via viral aerosol particles produced by individuals with COVID-19 disease [9–11]. In order to assist in the development of prevention guidelines, a group from the Nebraska Medical Center further investigated the transmission routes of the virus. Their studies reveal that viral RNA is present in nearly two-thirds of the air samples from rooms housing COVID-19 patients [11]. However, there is insufficient evidence at this time that would suggest that these particles indicate a viable virus that could be transmitted [11]. While the transmissibility of the virus via inanimate surfaces is generally low [12,13], aerosol to surface studies reveal that SARS-CoV-2 is more stable on plastic and stainless steel surfaces, showing a presence for up to several days, Figure 2 [14].

An additional potential transmission route is fecal-oral transmission, Figure 2 [15–18]. Clinical characterization data from pediatric patients in China has suggested that rectal swab testing is more efficacious than nasopharyngeal testing. Several patients had tested negative for the nasopharyngeal swabs while positive for the rectal swabs [16,17,19]. However, the number of SARS-CoV-2 particles and the length of exposure necessary to cause infection are unknown [9].

Yet, the epidemiological and clinical findings indicate that the transmissibility of COVID-19 depends on the viral load at the time of the onset of the disease. Researchers have observed a short serial interval (time between symptom onset of the index case and secondary case) of 4–5 days for COVID-19 and a decreasing risk for secondary infection over time. This suggests that there is a high risk of secondary transmission right before or at the onset of symptoms [6,20,21]. The recent data from South Korea reveals that the mean serial interval in the Delta variant is one day longer than when compared to the wild-type [22,23]. This trend is consistent with the viral shedding duration, as viral load peaks around symptom onset and decreases thereafter [6,20,24–26]. However, in more severe diseases, viral load was found to have peaked later in the second week of illness, suggesting a potentially longer period of infectiousness in cases of severe disease [27].

Recent discovery-based control design studies reveal that mutations in the viral nucleocapsid protein bring to light an association with the hospitalization rate [28]. Some single nucleotide variants increased the hospitalization rate, whereas a handful decreased it. Specifically, nonsynonymous variants R203K, R203S, and G204R occur in the linker region between the N-terminal RNA binding domain and the C-terminal dimerization domain, however this region has yet to be resolved [28,29]. It is hypothesized that the mutations in the linker region may potentially affect RNA binding interactions [28]. In

addition, nucleocapsid mutations in the endoRNase are also found to play an important role in the hospitalization status of the patients.



**Figure 2.** Modes of transmission of Sars-Cov-2. Modified from Harrison et al., 2020. Created with BioRender.com.

Several studies have examined the relationship between viral load and secondary transmission among symptomatic versus asymptomatic COVID-19 patients, although these findings have been inconsistent and contradictory. For instance, one study found comparable viral load in nasal and throat swabs at symptom onset among symptomatic and asymptomatic patients [26], while another found higher viral load in nasopharyngeal swabs in symptomatic patients [20]. Notably, a study by Hasanoglu et al. identified significantly higher viral load in asymptomatic patients in six types of specimens (nasopharyngeal/oropharyngeal, oral cavity, saliva, rectal, urine, and blood), suggesting higher infectiousness for asymptomatic patients than previously thought [30]. These findings highlight an important gap in the literature regarding the viral load and transmission risk of symptomatic versus asymptomatic COVID-19 patients that warrant further investigation.

Similarly, there are a limited number of studies in the literature that assess the relationship between SARS-CoV-2 viral load and disease severity. As was previously stated, disease severity may be associated with a higher risk of secondary transmission; however, this hypothesis must be examined further. Nonetheless, several studies have identified an association between disease severity and SARS-CoV-2 RNA shedding [31–33], while others report no such correlation [10,18]. For instance, the longer the duration of SARS-CoV-2 RNA shedding, the longer it takes to recover body temperature (when the fever was present at illness onset) compared to patients with early SARS-CoV-2 RNA clearance [33]. Additionally, patients admitted to the Intense Care Unit experienced longer viral shedding time than non-ICU patients [34]. A similar pattern is identified for the relative amounts of viral load over time, where higher viral load is associated with increased disease severity

and mortality [35,36]. However, other studies have found that viral load in severe patients is lower [30] than in mild cases, encouraging further research in this area.

One possible reason for these discrepancies in the literature relating to viral load and transmissibility may be due to the wide range of reported viral shedding times for SARS-CoV-2. For instance, the average reported duration of viral shedding from the onset of the illness ranges from 11 days [31] to 17 days [33] to 31 days [37]. The median duration of viral shedding also differs in the type of specimen collected. Major shedding routes for SARS-CoV-2, such as the nasopharyngeal, sputum, and stools [32], are expected to show a longer median duration of viral shedding than other collected specimens. Interestingly, sputum specimens exhibit longer viral shedding than nasopharyngeal specimens [32,38], almost twice as long according to some accounts [38]. Hindson et al., 2020 determined that the digestive system may exhibit longer viral shedding than the respiratory tract [16]. These findings are supported by the fact that patients with COVID-19 can simultaneously test positive for SARS-CoV-2 RNA on some samples but not on others. For instance, patients simultaneously tested positive for COVID-19 on rectal swabs but tested negative on nasopharyngeal swabs within the same testing period [15,16]. COVID-19 patients may also test negative for SARS-CoV-2 on samples that later test positive [16,39], further limiting the generalizability of viral shedding times in the literature.

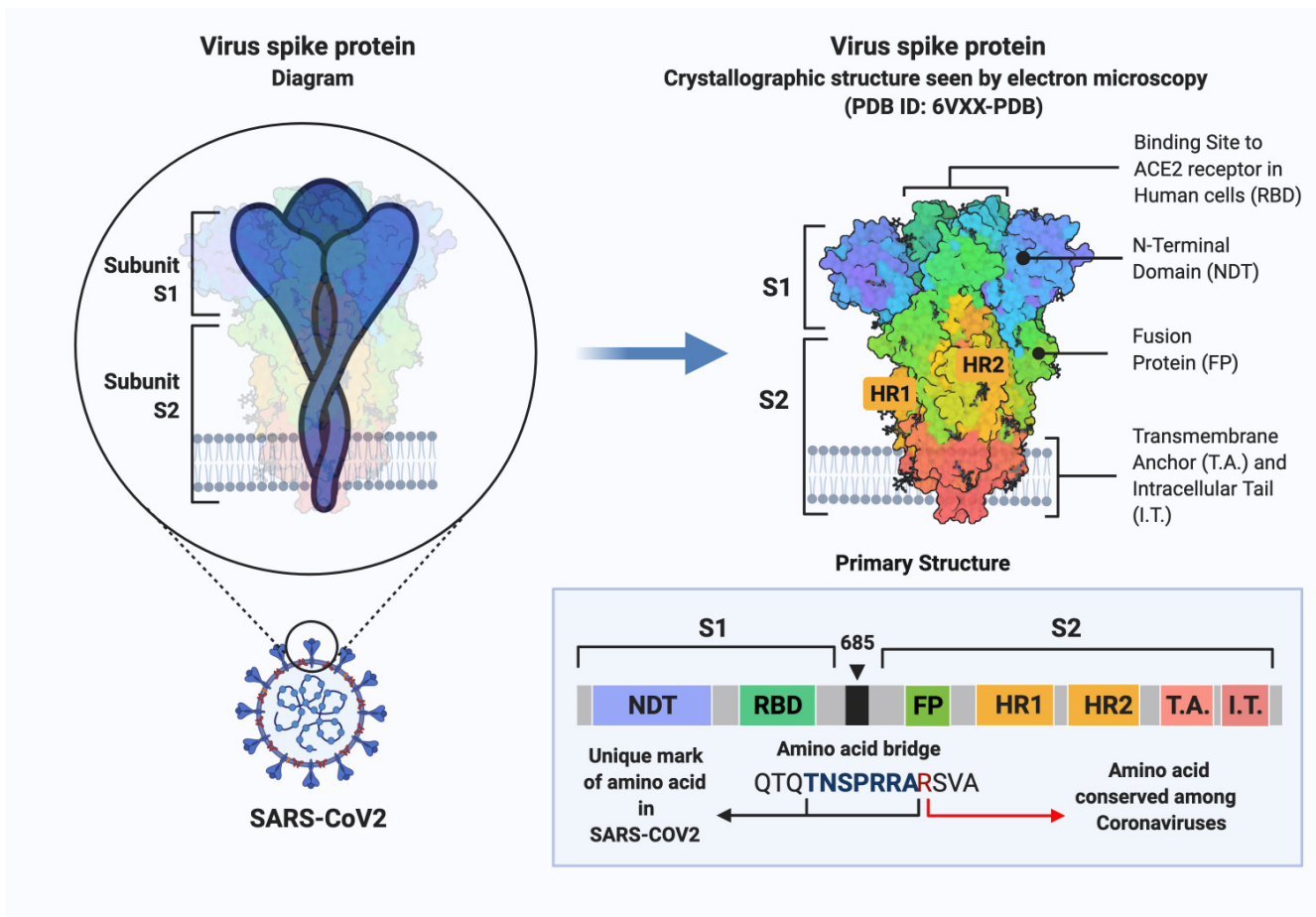
There are other factors that have been associated with the transmission. One of them is individuals' behaviors toward public health measures, such as physically distancing, masking, or staying home while they are sick. For example, those who had lower concerns about spreading the virus reflected the least uptake of public health measures [40]. Transmission dynamics were also affected among close contacts. Specifically, the transmission potential was at its peak in the first two days and after three days of the onset of the symptoms. When individuals were exposed to mild and moderate COVID-19 patients, they were exposed to a higher risk of COVID-19 when compared to asymptomatic carriers, and it worsens when exposed to patients with moderate cases of COVID-19 [41]. Moreover, environmental conditions play an important two-fold role; the initial viral load, and the immune response. Generally, higher temperatures and humidity have been associated with a lower fatality rate [42]. Another factor that has played an important role in the Sars-CoV-2 transmission are also the long diagnostic delays (LDDs). A study in Japan concluded that the portion of the long diagnostic delays with unknown exposure was correlated with a significant increase in the virus spread [43].

Moreover, the range of reported viral shedding times of SARS-CoV-2 may also be explained by independent risk factors such as age and gender. In some reports, old age—a major risk factor for COVID-19 severity—is associated with prolonged viral shedding duration [33,38]. Contrary to Xu K et al., 2020 and Wang K et al., 2020, another study finds no significant difference in viral shedding time between patients less than 65 years of age and those aged 65 years and older [37]. Similarly, some studies find viral shedding duration in males to be higher [33] or equal to females [37]. Concomitant hypertension has also been identified as a potential risk factor for prolonged viral shedding [33].

Conversely, the differences in the duration of viral shedding may be due to features that vary on a case-by-case basis, such as a longer time from the onset of symptoms to hospitalization or treatment, which increases the risk for prolonged viral shedding [31,33,44]. Other factors that are associated with an increased duration of viral shedding include cough [32], fever, and hydrocortisone use [31], specifically high-dose corticosteroids [45]. The presence of diseases other than COVID-19, such as diabetes mellitus and chronic lung disease, is associated with viral RNA detection [20]. Overall, these studies highlight the need for additional research on the factors affecting viral shedding duration and the detection of SARS-CoV-2.

As is the case with other coronaviruses, SARS-CoV-2 relies on the spike (S) glycoprotein to successfully bind to and enter host cells. Components of the SARS-CoV-2 S protein include subunit S1, subunit S2, the transmembrane anchor, and the intracellular tail [46–48]. However, while SARS-CoV-2 related coronaviruses today contain a monobasic cleavage site between S1 and S2, SARS-CoV-2 garners a multibasic cleavage site, Figure 3 [49–51], believed to be

the result of a recombination event [18,37,49]. This specialized motif enables SARS-CoV-2 to exploit a greater variety of widespread cellular proteases in the body, thereby allowing the virus to have a more rapid spread [46,48]. Additionally, newly synthesized virions can bypass the requirement of binding to host cell receptors and still continue to infect cells as they can be secreted in a preactivated state [48].



**Figure 3.** Schematic representation of SARS-CoV-2 spike protein primary structure. Domains are depicted with different colors. NTE, N-terminal domain; RBD, receptor-binding domain; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane domain; IT, intracellular tail. Created with [BioRender.com](https://www.biorender.com/).

Moreover, the SARS-CoV-2 S protein can trigger cellular entry independent of proteases but dependent on receptor binding, leading to augmented viral spread [48]. However, when the S1/S2 site is damaged, it can severely inhibit the S protein cleavage and proteolytic processing [49]. Moreover, structural studies suggest that the addition of basic residues to the S1/S2 site of SARS-CoV-2 exhibits an increased viral spread via cell–cell fusion but no change to virus–cell fusion [49]. In sum, the structural studies stress the importance of recognizing which viral mutants lead to inhibition or augmentation of viral spread to develop effective therapeutic strategies against SARS-CoV-2 and its variants.

For viral spread to occur, the S protein of SARS-CoV-2 must first be activated. This process starts when the receptor-binding domain (RBD) of the S1 subunit binds to the host cell surface receptor angiotensin-converting enzyme-2 (ACE2) [46,48,49,52] via its peptidase domain [53,54]. Compared to SARS-CoV, the receptor binding capacity of SARS-CoV-2 to ACE2 is at least 10-fold higher [46,51]. This high affinity is partially due to the specialized RBD of SARS-CoV-2, which contains a residue motif at 482–485 (Gly–Val–Glu–Gly) that allows for better contact with ACE2 and two key residues (Gln493 and Leu455)

that stabilize ACE2 binding [48,55]. Interestingly, the data reveals that this high-affinity results in increased virulence of SARS-CoV-2 [6,50,51]. Once SARS-CoV-2 and ACE2 bind together, it alters the conformation of the S protein, exposing a cleavage site on the S2 subunit, which the host cell proteases will act upon in the next step [48,56]. Then, the host cell proteases, such as transmembrane protease serine S1 member 2 (TMPRSS2), carry out proteolysis of the S protein at the cleavage site between the S1/S2 boundary, resulting in a new S2 site (S2') [48,57,58]. Following the S2' cleavage and the release of the S2 subunit, the activation of the protein concludes, allowing for the subsequent initiation of the endocytosis of the virus through the fusion of the viral and cell membranes [48,56–58].

### 3.2. Pathophysiology

Based on the taxonomical and phylogenetical assessment from the International Committee on Taxonomy of Viruses, SARS-CoV-2 is classified as a virus in the species severe acute respiratory syndrome-related coronavirus (SARSr-CoV) in the genus Betacoronavirus of the family Coronaviridae [59]. Other viruses that belong to the  $\beta$ -coronavirus genus include SARS-CoV and MERS, of which SARS-CoV-2 shares 79% and 50% similarity, respectively [60]. While the precise origin of SARS-CoV-2 is currently unknown, several lines of evidence suggest that bats were the original host of this virus as they share an almost exact SARSr-CoV genome sequence identity with SARS-CoV-2 [60–62].

While SARS-CoV-2 is genetically like other human coronaviruses, its clinical manifestations are rather broad. They range from asymptomatic infection to mild symptoms, severe illness, and mortality [63,64]. Commonly reported symptoms include fever, cough, fatigue, dyspnea, myalgia, and headache [63–65], whereas gastrointestinal symptoms include nausea, diarrhea, and vomiting [63,64,66–68]. Another clinical manifestation is headache which is significantly associated with the loss of smell (anosmia) and taste (ageusia) [69,70]. Headache is often a prodromal symptom and can persist long after COVID-19 resolution, most commonly in females and those with a prior history of headaches [69]. However, little is known regarding the pathophysiological mechanism of headache in COVID-19 infection, which remains a debate in the literature. While clinicians often attribute fever as the cause of headache in viral infections [69], several studies have demonstrated that fever and headache present independently in COVID-19 [69,71]. It has been suggested, therefore, that the cause of headache in COVID-19 may be due to the binding of SARS-CoV-2 to ACE2 in trigeminal nerve endings in the nasal cavity, triggering the activation of the trigeminovascular system or neuroinflammation (as a result of systemic inflammation) that subsequently stimulates trigeminal terminals [69,72]. Similarly, this mechanism takes place at the olfactory epithelium to cause anosmia [69,73]. Nonetheless, further research is needed in order to fully understand the mechanism of headaches in COVID-19. In addition to the fever-like symptoms, gastrointestinal manifestations, and headaches, other commonly reported clinical manifestations are neurological symptoms of COVID-19, whereby patients are experiencing olfactory and gustatory dysregulation [63,74,75] in addition to dermatological dysregulation, with erythematous and a chickenpox rash [63,76,77]. Finally, severe COVID-19 infection is characterized by cardiovascular complications (i.e., arrhythmia, cardiomyopathy), respiratory complications (i.e., pneumonia, respiratory failure), and multi-organ failure (i.e., septic shock, acute respiratory distress syndrome) [78,79].

While the symptomatic manifestations of COVID-19 are well documented, its pathophysiological mechanism is still poorly understood. The current state of knowledge reveals that its infection progression relies on several pathophysiological mechanisms that are intertwined, including immunopathological response to the SARS-CoV-2 infection as well as the involvement of innate and adaptive immunity. To name them more specifically, some of the mechanisms involve cytopathic effects such as cellular rounding, detachment, degeneration, and multinucleate cells [80]. Other mechanisms are the ACE2 downregulation and renin-angiotensin-aldosterone system (RAAS) disturbances [81]; hyperactivation of the inflammatory system leading to the cytokine storm [80–84]; increased coagulopathy associated with sepsis-induced coagulopathy and disseminated intravascular coagulation [85–87];

endothelial dysfunction associated with endothelial injury, endotheliitis, impaired micro-circulatory function and endothelial cell dysfunction [88,89]; and immunothrombosis mediated by pyroptosis—an inflammatory programmed cell death mechanism [89,90].

The first step of the pathophysiological mechanisms starts with SARS-CoV-2 entering the body by binding to the host cell receptor angiotensin-converting enzyme 2 (ACE2) and its priming by proteases furin and transmembrane serine protease-2 (TMPRSS2) [49,89,91–93]. Once the SARS-CoV-2 spike (S) protein binds to ACE2 in lung cells, its activity is significantly reduced [94,95]. Since ACE2 is an important regulator of the renin-angiotensin system (RAS), it forms an antagonistic relationship with the angiotensin-converting enzyme (ACE) to maintain physiological homeostasis and regulate the function of several organs [95–97]. In the ACE2 pathway, angiotensin I (1–20) and angiotensin II (1–8) convert into angiotensin (1–9) and angiotensin (1–7), respectively, which then leads to the binding to angiotensin II type 2 receptor (AT2R) to promote aldosterone inhibition, hyperkalemia, and pulmonary epithelium protection [97]. As a result of ACE2 downregulation, ACE2-mediated RAS activity is reduced, resulting in higher levels of angiotensin I (1–20) and angiotensin II (1–8), which will subsequently bind to angiotensin II type 1 receptor (AT1R) causing induction of antagonistic effects compared to AT2R such as vasoconstriction, apoptosis, lung inflammation, and degradation [97–99]. These cytopathic effects cause an imbalance in physiological homeostasis resulting in an increase in the inhibition of gas exchange.

As shown in Figure 4 the cytokine storm is also believed to arise from SARS-CoV-2 infection of monocytes, macrophages, and dendritic cells, resulting in the release of pro-inflammatory cytokines and chemokines [100–102]. Their release is not only associated with the disease severity [64] but it also contributes to the progression of acute respiratory distress syndrome (ARDS), which is the leading cause of death in fatal COVID-19 cases [103,104]. Notably, severe COVID-19 patients were found to have high levels of IL-2 (interleukin-2), IL-7 (interleukin-7), IL-10 (interleukin-10), G-CSF (Granulocyte colony-stimulating factor), TNF (Tumor necrosis factor), CXCL10 (C–X–C motif chemokine 10), MCP1 (monocyte chemoattractant protein-1), and MIP1 $\alpha$  (macrophage inflammatory protein 1-alpha) in their serum [105]. Once the cytokine and chemokine levels increase, more white blood cells are recruited to the area of infection [89], which in turn recruits even more cytokines and chemokines, creating a pro-inflammatory feedback loop [89,106].

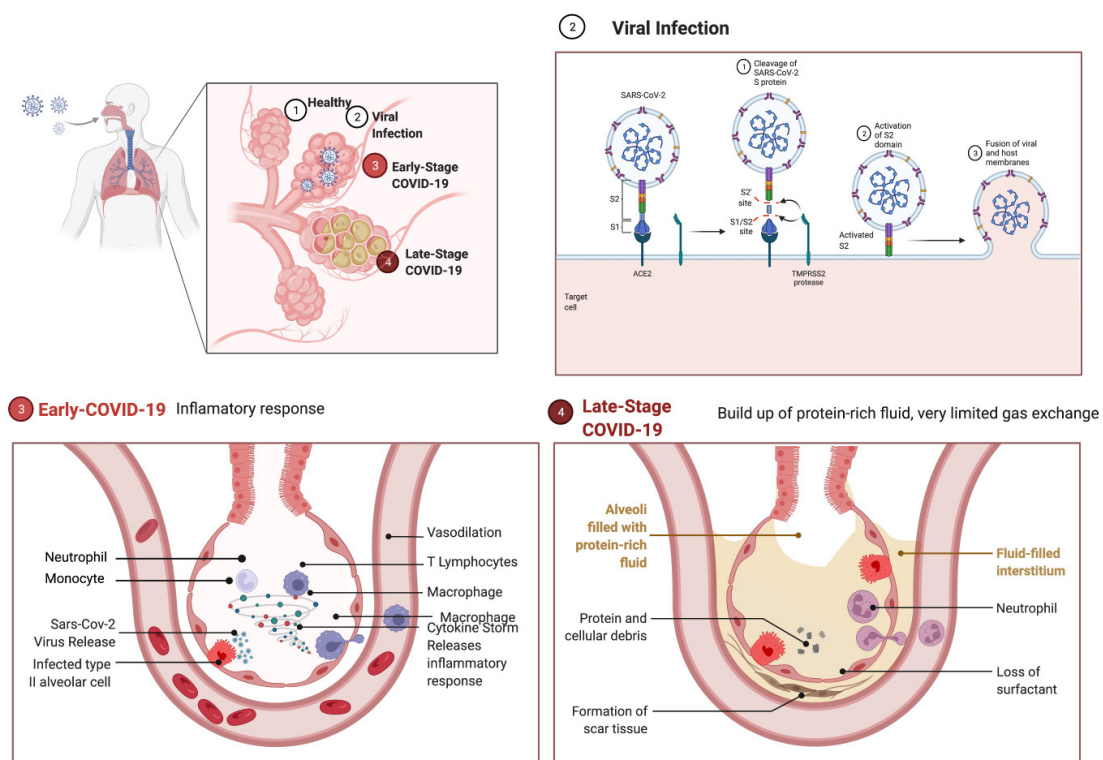
Type I interferon response (IFN-I) is critical for the activation of antiviral activities to host cells [106–109] and appears to be significantly reduced in early COVID-19 infection [106,110,111]. Studies have suggested that interferon protein deficiencies may result from inherited mutations in IFN-I related genes [112] or the development of neutralizing antibodies against IFN-I proteins [113]. As a result of this dampened innate immunity, it is believed that SARS-CoV-2 can replicate in high titers early in infection [106,114–116]. However, there is increasing evidence to suggest that severe COVID-19 patients have considerable IFN-I response [115,117,118], which can further contribute to hyperinflammation. This highlights the need for further research into the role of IFN-I in SARS-CoV-2 pathogenesis.

Regardless, in both cases, there is an accumulation of white blood cells, cytokines, and chemokines, which promotes apoptosis of lung and alveolar cell tissue [89,119], leading to pulmonary alveolar edema, vascular leakage [119,120], and in some severe cases hypoxia and ARDS [103]. To add to this, the increase in IL-6 levels and TNF- $\alpha$ -mediated cell apoptosis attenuates virus-specific T-cell response [121], contributing to the uncontrolled inflammatory response in the body [119] that can spread to other organs and ultimately result in multi-organ failure [89,120]. Collectively, these studies outline the critical role of the cytokine storm in the pathogenesis of SARS-CoV-2 and the need for further investigation.

Recent evidence has detected a hypercoagulable state in some COVID-19 patients [122–125], termed COVID-19-associated coagulopathy (CAC) [126]. Increased levels of D-dimer and fibrin/fibrinogen-degradation products appear to be the most frequently cited factors associated with CAC [123,125,127–129]. Additional factors include increased prothrombin time, elevated fibrinogen level, and increased or near-normal partial thromboplastin time [123,125,129]. Notably, increased levels of D-dimer are associated with illness severity



and 28-day mortality [122,130]. As of 10 March 2022, studies have investigated the pathophysiology of hypercoagulability in COVID-19 infection; however, the precise mechanism remains to be determined. One hypothesis proposes that the cytokine storm in the lungs leads to local inflammation-induced thrombosis, which can then progress into a generalized hypercoagulable state in the body [131,132]. Other studies interpret hypercoagulability through Virchow’s triad, a model that proposes three critical factors in the development of venous thrombosis: vascular damage, blood hypercoagulability, and blood [133]. Vascular damage arises from SARS-CoV-2 entry into endothelial cells via ACE2; a receptor expressed ubiquitously throughout the body [134]. As discussed above, this leads to inflammation (by the cytokine storm) in multiple organ systems, vascular permeability, and lung degradation. In addition to increased levels of cytokines and chemokines, the protective immune system releases the C reactive protein fibrinogen, D-dimer, and ferritin that exert prothrombotic effects, contributing to a hypercoagulable state [135,136]. A recent theory has also suggested the role of neutrophil extracellular traps (NETs) in triggering thrombosis [135,137]. Furthermore, attenuation of the ACE2-induced pathway in the RAS limits the protective capability of this pathway to exert antithrombotic effects [138]. Finally, blood stasis results from blood hyperviscosity [138], likely due to increased fibrinogen levels and prolonged bed rest due to strict isolation or hospitalization [135,136]. Considering all the evidence thus far, it appears CAC shows much distinctiveness in its interaction with the immune system and requires further research if antithrombotic therapies are to be successfully developed for COVID-19.



**Figure 4.** Schematic representation of SARS-CoV-2 viral infection. Upper left panel a representation of the bronchi in healthy, during viral infection, early-stage infection and late-stage infection. The right upper panel displays a schematic representation of SARS-CoV-2 viral spike protein and the cleavage sites for furin and TMPRSS2. The lower left panel represents the early stages of infection, where the macrophages are recruited and cause the cytokine storm as a result. The lower right panel represents the late-stage of COVID-19 infection where there is a protein-rich fluid inside the alveolus, triggering the alveolar collapse which in turn decreases the gas exchange. Neutrophils are also recruited to destroy the cells that have been infected, which could also lead the ARDS. Created with [BioRender.com](https://www.biorender.com).

However, it should be noted that not all COVID-19 patients will experience the extent of complications previously described. The largest risk factor to predict COVID-19 severity and mortality is aging [44,139]. While the basis for this disparity is currently not understood, one interesting hypothesis attributes the defense of young age to the high activity of endothelial nitric oxide synthase (eNOS), and thus, increased nitrous oxide (NO) levels, as eNOS activity and NO availability declines with age [139,140]. Importantly, low levels of eNOS-derived NO have been associated with endothelial dysfunction, leading to proinflammation, vasoconstriction, and hypercoagulation [139,141]—all characteristic of severe COVID-19 cases. This hypothesis was supported by recent evidence showing that inhaled NO improves oxygenation in COVID-19-induced ARDS patients [142].

Furthermore, NOS3 polymorphism, the gene that codes for eNOS [139,143], may help explain the discrepancy in COVID-19 death rates amongst different populations. One study found that populations with an increasing prevalence of the wild-type haplotype of the NOS3 gene (T-4b-Glu) are associated with a decreasing COVID-19 death rate compared to other populations [139]. In addition to age, other established risk factors for COVID-19 severity include male sex and the presence of comorbidities [144,145], such as obesity, hypertension, chronic lung disease, and immunodeficiencies [144]. Some reports have also identified risk factors for disease severity in the early stage of infection, including increased length of fever and partial pressure of oxygen less than 80 mmHg [18]. Finally, increasing evidence has suggested that abnormal liver function is associated with greater disease severity [146–149] and increased levels of inflammatory markers [147]. These reports conclude that severely ill COVID-19 patients exhibited increased levels of ALT (alanine transaminase), TBIL (total bilirubin), AST (aspartate aminotransferase) [146–149], and GGT (gamma-glutamyl transferase) [146,148,149]. As a result of this disturbed liver function, other cellular functions could malfunction, including liver inflammation via the cytokine storm, ARDS, medication-induced hepatotoxicity, and direct viral entry of SARS-CoV-2 into ACE2-expressing liver cells [146,150–152].

### 3.3. SARS-CoV-2 Entry Factors

Infection of target cells by coronaviruses requires binding the spike (S) glycoprotein to cell surface receptors and S protein priming by host cell proteases [46,48,49,52,91,92]. The entry receptor for SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2) [49,62,91,92,153], and entry activators for S protein priming include proteases furin and transmembrane serine protease-2 (TMPRSS2) [49,91,92]. Interestingly, following the cleavage of the full length S protein into two peptides, S1 and S2, by the host originated furin, the C terminus S1 peptide generated binds to the receptor neuropilin-1 (NRP1), which is involved in the vascular permeability, angiogenesis, and axon guidance [154–159]. As a result, cells highly co-expressing these proteins are a likely target for SARS-CoV-2 infection [92,160].

Following SARS-CoV-2 binding to the entry receptor ACE2, receptor neuropilin-1 (NRP1) and the host cell proteases furin and TMPRSS2 play an important role as entry activators. Both furin and TMPRSS2 are two protein-cleaving enzymes involved in priming the SARS-CoV-2 S protein, a process required for cellular entry [49,91]. Protein-proofed single-cell RNA profiling (psc-RNA) studies reveal that ACE2, furin, and TMPRSS2 highly co-express in lung macrophages and AT2 followed by cardiomyocytes, stromal cells of the adrenal gland, testes, ovary, and thyroid, identifying tissue-dependent SARS-CoV-2 infection vulnerability [161]. Following furin-mediated S protein cleavage at the S1/S2 boundary results in two noncovalently associated proteins (S1 and S2) [48,49,91,162,163]. This process exposes a cleavage site on S2, which TMPRSS2 acts upon to activate the S protein, which initiates the fusion of the viral and cell membranes [56,162]. Moreover, S protein cleavage by furin exposes an [R/K]XX[R/K] motif within the C-terminus of the S1 protein contains, designated as the “C-end rule” (CendR) [156,164]. The exposure of the CendR allows for direct binding to the transmembrane receptor NRP1 and its homolog neuropilin-2 (NRP2) on target cells [156,157,164]. Interestingly, NRP1 and NRP2 show the highest expression in endothelial and epithelial cells, whereas the lower expression is

observed in the respiratory and olfactory cells [157]. The binding of CendR to NRP1 was determined to significantly enhance SARS-CoV-2 infectivity, while NRP2 binding has yet to be investigated [157,164]. Nonetheless, the RNA expression of both NRP1 and NRP2 were elevated in bronchoalveolar lavage fluid (BALF) cells infected with SARS-CoV-2 compared to adjacent non-infected cells, suggesting a similar role for NRP2 [157]. Additionally, furin and TMPRSS11A expression were also increased in SARS-CoV-2 infected BALF cells [157].

It was discovered that the interaction between NRP1 and the S1 protein could be blocked with a small-molecule inhibitor or monoclonal antibodies, which reduced viral infectability, suggesting a potential target for future antiviral therapies [157,164]. Mutational analysis revealed NRP1 requires a furin cleaved site to function properly during infection [157]. Moreover, proteases involved in other coronaviruses have been suggested as potential SARS-CoV-2 entry activators, including ANPEP of HCoV-229E [92,165] and DPP4 of MERS-CoV [92,166]; however, these possibilities for SARS-CoV-2 have not yet been extensively explored in the literature [92].

However, a multitude of other SARS-CoV-2 entry factors is currently under investigation. Notably, utilizing single-cell transcriptomics, Singh et al. compiled a comprehensive profile of 28 SARS-CoV-2 and coronavirus-associated receptors and factors (SCARFs) [167]. Members of the SCARFs, which include entry factors, including cell surface receptors, proteases, restriction factors, post-entry factors, replication, and trafficking factors, have been shown to either mediate or restrict SARS-CoV-2 infections, and this will aid in predicting the vulnerability of certain tissues and cell types in adults and embryos [167]. Cells such as intestinal goblet cells, enterocytes, and kidney proximal tubule cells are most permissive to SARS-CoV-2 infection [167]. Unlike in adults, in embryos, cytotrophoblasts (CTBs) exhibited the highest proportion of receptor-protease co-expression, suggesting CTB is most vulnerable to SARS-CoV-2 infection in the first-trimester placenta [167]. SARS-CoV-2 susceptibility and severity also depend on other clinical factors such as age, sex, and comorbidity because they influence the SARS-CoV-2 entry factors. Some studies have explored the influence of age on the expression of SARS-CoV-2 entry factors; however, their findings are often in disagreement or limited by the conditions of the study. For example, one study found TMPRSS2 expression in lung tissue increases with aging in humans [168], while deconvolution analysis determined that TMPRSS2 mildly decreases in the lungs with age [167,169]. Other studies looked at age and co-expression of SARS-CoV-2 entry factors, finding a higher proportion of ACE2-TMPRSS2/4 co-expression in human nasal epithelium cells of older patients but lower ANPEP-TMPRSS2/4 co-expression [167,169]. However, these results are limited by a sample size and potential confounding factors. Patient sex has also been reported as a potential regulator of SARS-CoV-2 entry factors. The male sex appears to have increased levels of ACE2 expression [167,170].

Furthermore, the gene location of the ACE2 gene may play a role in sex dependency. Since the location of the ACE2 gene is on the X chromosome, it is hypothesized that ACE2 expression is regulated differently in males and females due to a variable X-inactivation [167,170,171]. However, evidence for the relationship between ACE2 expression and factors such as age, sex, and comorbidity have been conflicting [170]. From these limited and contradictory findings, it is clear that shifts in SARS-CoV-2 entry factor expression must be explored further. Additionally, time-resolved transcriptomics studies have identified that temperature dependence may influence entry factor expression [172]. V'kovski et al. have found that SARS-CoV-2 replicated more efficiently at temperatures encountered in the upper respiratory tract (33 °C) than in the lower respiratory tract (37 °C) in human airway epithelial cell culture models, contrasting SARS-CoV [172]. The high transmissibility of SARS-CoV-2 is thought to be facilitated by the early replication of SARS-CoV-2 in the upper respiratory tract of infected individuals [172].

### 3.4. ACE2 Receptor

SARS-CoV-2 enters host cells by binding to the metalloproteinase angiotensin-converting enzyme 2 (ACE2) receptor via its spike (S) protein and proteases such as the

transmembrane serine protease 2 (TMPRSS2) that assists in this process by activating the S protein [46,48,49,52]. Unlike other coronaviruses, the SARS-CoV-2 receptor-binding domain (RBD) binds to the receptor with 10–20 fold more binding capacity, and once bound, it triggers an increase in the expression of ACE2 [46]. On the other hand, some reports suggest that SARS-CoV-2 binding reduces ACE2 activity and remains an unresolved issue in the literature [94,95].

Upon transmission, SARS-CoV-2 quickly binds to host cells since ACE2 is expressed on the surface of the lung alveolar epithelium and goblet cells of the nasal epithelium [114,173,174]. ACE2 expression encompasses a wide selection of cells such as respiratory epithelial cells, esophageal epithelial cells, cardiomyocyte cells, renal tubular epithelial cells, urothelial bladder cells, and ileum epithelial cells, as well as in the vascular endothelium of numerous human organs such as the mucosa of the mouth and nose, nasopharynx, lung, stomach, small intestines, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain [114,175,176]. Single-cell transcriptomics studies identified type II pneumocytes, ciliated cells, and transient secretory cells as the primary cell types expressing ACE2 [160,174]. Interestingly, SARS-CoV-2 can still invade tissues with a low ACE2 expression to a high degree compared to SARS-CoV, which may be attributed to the additional cleavage site on SARS-CoV-2 that results in an increase in viral infectability [116].

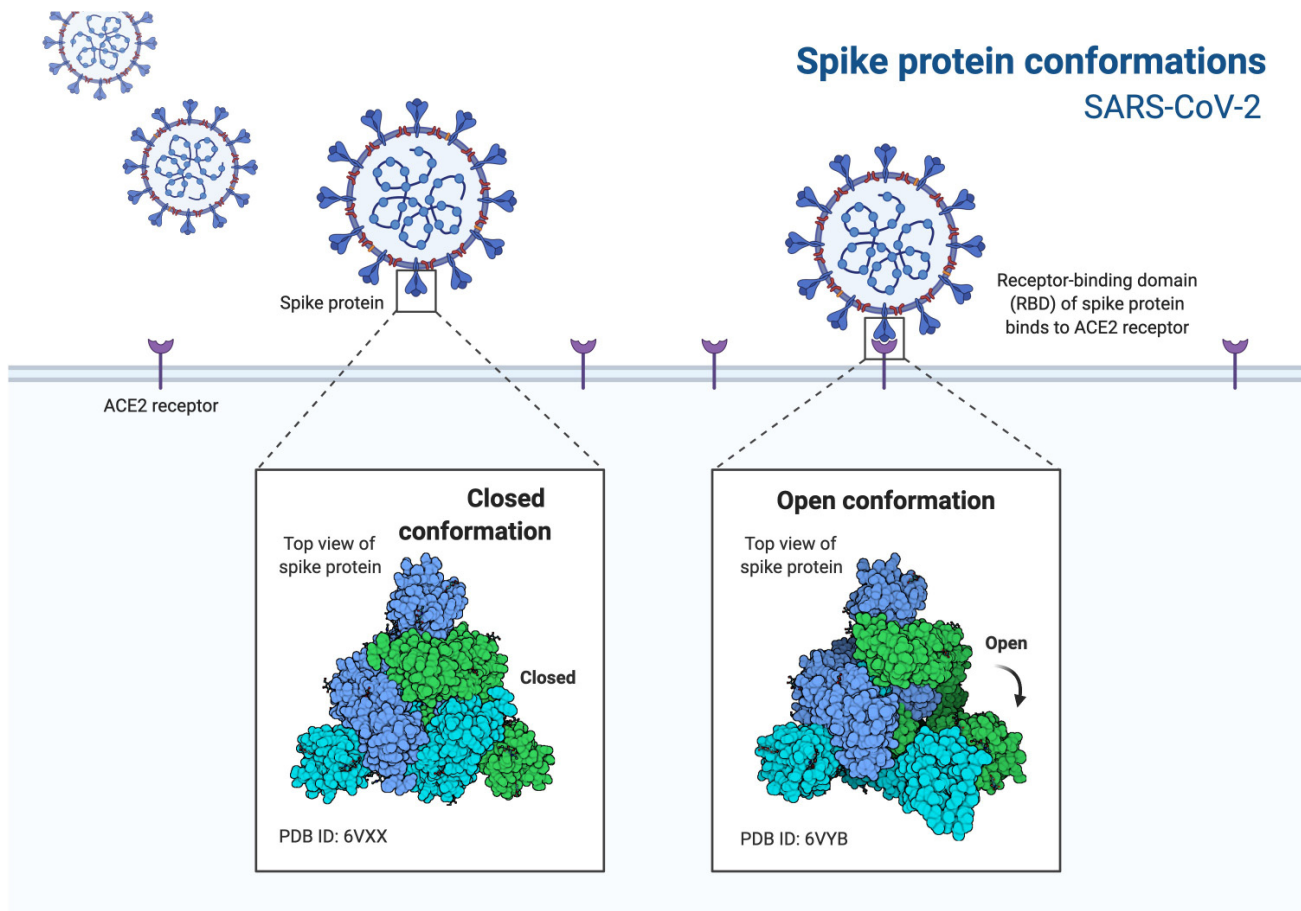
It is elucidated that ACE2 is an interferon-driven gene, and its expression during lung injury increases, resulting in additional SARS-CoV-2 internalization via receptor-mediated endocytosis [174,177,178]. High expression of ACE2 in such regions may explain the increased immune response to SARS-CoV-2 in those areas [179,180]. This hypothesis is supported by the research on ACE2 expression across different cohorts, which suggests ACE2 expression is associated with vulnerability to SARS-CoV-2 infection [175,181]. Moreover, ACE2 expression on the uterus and placental villi suggests a potential maternal–fetal transmission of SARS-CoV-2 [175,182].

However, many individual factors can influence ACE2 expression. For example, Radzikowska U et al. found that male patients that suffered from asthma, COPD, hypertension, smoking, or obesity generally expressed higher levels of ACE2-related genes in the lungs, bronchoalveolar lavage, or blood [173]. Additionally, patients with atopic dermatitis exhibited higher expression levels of ACE2-related genes in the skin. In contrast, the co-expression of ACE2 and TMPRSS2 appears primarily localized in the skin and lungs, with ACE2 more predominantly widespread in the lungs [173]. More specifically, type II pneumocytes widely express ACE2, with one report identifying type II pneumocytes composed 83% of human lung cells that express ACE2 [174,175,180]; therefore, the lungs are a primary target of SARS-CoV-2 infection.

Such widespread ACE2 expression allows for it to carry out various functions in the body. Firstly, ACE2 acts as a negative regulator of the renin-angiotensin system (RAS), responsible for maintaining homeostasis in the body. Following degradation of Angiotensin (Ang) I (1–20) and Ang II (1–8) by ACE2 and converting it into Ang (1–9) and Ang (1–7), respectively, it promotes vasodilation and anti-proliferation [175,183,184]. In addition to regulating the amino acid absorption in the gut and the kidney [175,183], ACE2 is also an interferon-stimulated gene (ISG) in human airway epithelial cells, and IFN- $\alpha$  drives ACE2 expression [174]. Since SARS-CoV-2 can elevate the expression of ISGs [180], it is, therefore, possible that SARS-CoV-2 could take advantage of the interferon-driven upregulation of ACE2 by the host IFN response to strengthen ACE2 as a viral target and, in turn, intensify the spread of infection [174].

When SARS-CoV-2 binds to ACE2, its spike (S) protein will be activated via cleavage by host cell proteases, and as a result, it yields two subunits, S1 and S2. S1 serves as an attachment site to ACE2, whereas S2 is important for viral-host cell membrane fusion [185,186]. Structural studies reveal that S1 contains a receptor-binding domain (RBD) and N-terminal domain (NTD) of unknown function [51,186,187]. The S2 subunit contains the fusion peptide (FP), heptad repeat 1 (HR1), a central helix (CH) Connector domain (CD), another heptad repeat (HR2), a transmembrane domain <sup>TM</sup>, and an intracellular tail (IT) [188]. The RBD domain

interacts with the ACE2 peptidase domain by adjusting its configuration from a closed to an open state to regulate this interaction by utilizing atomic force microscopy, as shown in Figure 5 [51,186,187,189]. Once in the open conformation, SARS-CoV-2 fuses with the host cell membranes leading to infection [190].



**Figure 5.** Spike-protein trimer in closed (left) and open (right) states of the SARS-CoV-2 S glycoprotein. Created with [BioRender.com](https://www.biorender.com).

Furthermore, SARS-CoV-2 has a high affinity for ACE2, suggesting high specificity in this binding interaction [189]. Interestingly, bilayer interferometry experiments have revealed the importance of two N-glycan sites, N165 and N234, in the SARS-CoV-2 spike protein, stabilizing this interaction and assisting the virus in evading the immune system [186,191].

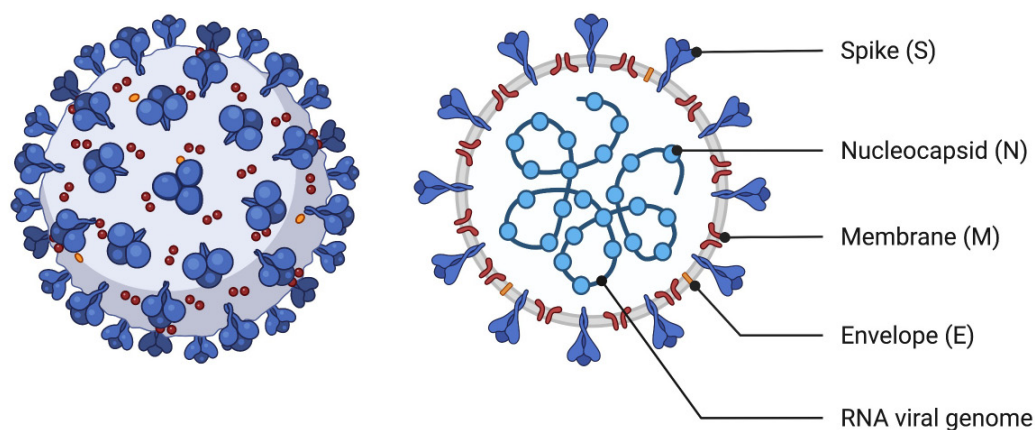
Together these studies provide important insights into potential therapies against SARS-CoV-2 that target the RBD-ACE2 receptor interaction. One potential target is the linoleic acid (LA) binding pocket on the SARS-CoV-2 S protein. LA binding restricts the S protein to the closed configuration, preventing it from binding to the ACE2 receptor, Figure 4 [186,192]. Another option currently being investigated is the addition of peptide inhibitors to target viral attachment in host cells. Structural studies reveal that adding a peptide fragment derived from the N-terminal ACE2 helix was the best at attenuating SARS-CoV-2 binding [189]. As a result, ACE2-focused drug therapies have become a central focus in COVID-19 research.

### 3.5. Immune Response

Both innate and adaptive immune mechanisms are investigated in this review to provide a bird's eye view of the overall immune mechanism response to SARS-CoV-2. To date, SARS-CoV-2 has shown lasting immunity 6 to 8 months post-infection [193–195].

SARS-CoV-2 genome encodes for 23 putative nonstructural proteins, besides the spike protein S, the envelope E, the membrane M, and nucleoprotein N structural proteins, as shown in Figure 6 [196]. The body defense mechanism produces antibodies against different parts of the virus, including the spike (S) protein and its receptor-binding domain (RBD), the nucleocapsid, but also non-structural proteins such as ORF8 and ORF3b [193,197]. Interestingly, the antibodies produced against the non-structural proteins are especially important for SARS-CoV-2 antibody testing since their regions are the least identical to SARS-CoV proteins, allowing for increased specificity in the detection of SARS-CoV-2 [197]. Overall, reports agree that antibody levels follow a classical signature decline characteristic of primary immune responses [193–195]. Nevertheless, the detectable levels of specific antibodies vary widely in the literature. At six months post-infection, some COVID-19 patients show a significant decrease in anti-SARS-CoV-2 RBD IgM (Immunoglobulin M) and IgG (Immunoglobulin G) levels, but a small change in IgA (Immunoglobulin A) levels [195], while others exhibit a significant decrease in IgM and IgA levels [194]. Interestingly, anti-RBD IgG levels are associated with neutralizing activity, a finding that is inconclusive for both IgM and IgA levels [194,198]. Furthermore, adaptive immune response memory CD4<sup>+</sup> T and CD8<sup>+</sup> T cells and memory B cells persist long after SARS-CoV-2 infection [193,195,199]. Six months post-infection, memory B cells have been found to express antibodies that have more potent neutralizing activity and cover a wider variety of SARS-CoV-2 variants [195]. While the precise mechanism of this finding remains unclear, it suggests a long-lasting response of SARS-CoV-2 immunity.

## Human Coronavirus Structure



**Figure 6.** A schematic representation of SARS-CoV-2 structure. It is enveloped and contains a positive-sense RNA virus with four main structural proteins such as the spike (S), membrane (M) glycoproteins, the envelope (E) and the nucleocapsid (N) proteins. Created with [BioRender.com](https://www.biorender.com/).

COVID-19 patients can have immunological memory cells from previously encountered pathogens before infection that can recognize the epitope on SARS-CoV, SARS-CoV2 and other human coronaviruses [200–204]. Specifically, recent analyses have identified cross-reactive memory T cells of the adaptive immune system that recognize SARS-CoV-2 in addition to other viruses [201,203,204]. In two separate studies of blood samples, researchers found that people unexposed to SARS-CoV-2 had memory CD4<sup>+</sup> T cells endemic to human coronaviruses, including (HCoV)-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 that also recognized SARS-CoV-2 [201,204]. These HCoV-specific T cells recognize common epitopes between SARS-CoV-2 and common cold coronaviruses, with S proteins the most frequently recognized antigen [201,204].

Other research has focused on prior exposure to SARS-CoV, which shares 79% of its identity with SARS-CoV-2 [60]. Notably, anti-SARS-CoV nucleocapsid (N) protein T cells were found to be reactive to the N protein of SARS-CoV-2 as well [203]. Conversely, it was found that patients that had neither been exposed to SARS-CoV nor SARS-CoV-2 also possessed reactive T cells to the (N) protein and non-structural regions of SARS-CoV-2, suggesting that such T cells could have been induced by other beta coronaviruses or were simply naive T cells [203].

Another investigation that has been a central focus of SARS-CoV-2 immunity has been “trained immunity”, a concept proposed by Netea and colleagues [205]. Trained immunity refers to enhancing innate immune cells, such as macrophages, monocytes, and natural killer cells, to re-infection, a process driven by epigenetic modifications and metabolic reprogramming of innate immune cells [205]. As a result, a faster activation of genes for the innate immune system occurs when re-encountering the pathogen [205]. Several lines of evidence suggest that trained immunity provided by the bacilli Calmette–Guérin (BCG) tuberculosis vaccine enhances overall innate immunity, attenuation of SARS-CoV-2 infectability and severity, possibly mediated through the nitric oxide [200,206,207]. Among 55 countries, populations with higher BCG vaccination rates were associated with a slower spread of SARS-CoV-2 and lower death rates [202]. Moreover, a population of hospital workers who received a booster BCG vaccine in March 2020 had no cases of SARS-CoV-2 after four months, while a comparable unvaccinated group had an infection rate of 8.6% [200]. While the BCG tuberculosis vaccine may attenuate the spread and the severity of SARS-CoV-2, it is still not recommended to be used as a therapy outside of clinical trials [207].

Although SARS-CoV-2 immunity shows promising potential long-term protection against the disease, the threat of re-infection is still possible. To and colleagues provided the first documented case of COVID-19 re-infection supported by genetic analysis of a 33-year-old man in Hong Kong, who experienced an asymptomatic episode 142 days after his initial symptomatic case [208]. The patient’s re-infection was caused by a coronavirus strain different from the one responsible for the first case and thus triggered the creation of new antibodies [208]. However, for most COVID-19 patients, re-infection is uncommon, and antibodies produced during the primary immune response can protect against future re-infection [209,210]. More specifically, the National Cancer Institute found that among more than 3 million people, only 0.3% of those who initially had SARS-CoV-2 serum antibodies later tested positive for re-infection, compared to 3% who lacked these antibodies [209]. Moreover, in a longitudinal cohort study of over one thousand healthcare workers, the presence of anti-spike or anti-nucleocapsid IgG antibodies was associated with a reduced risk of SARS-CoV-2 re-infection in the next six months [210].

Based on SARS-CoV-2 immunity research, one potential therapy that has been suggested against COVID-19 is antibody transfer. Transfer of IgG antibodies from SARS-CoV-2 infected rhesus macaques (*Macaca mulatta*) to uninfected macaques boosted immune response against the infection in recipient animals [211]. Additionally, reduced CD8<sup>+</sup> T cell levels in macaques resulted in attenuated protection against re-infection, indicating that T cells play a key role in SARS-CoV-2 immunity [211]. These findings suggest the potential for plasma transfers as a treatment against COVID-19.

### 3.6. SARS-CoV-2 Variants

Four variants of SARS-CoV were listed as variants of concern (VOC) as of mid-September 2021. They were named alpha (B.1.1.7 lineage), beta (B.1.351 lineage), gamma (P.1 lineage), and delta (B.1.167.2) by the World Health Organization (WHO). The alpha variant emerged in the United Kingdom (UK) in September 2020 [212]. It was found to be 43%–90% more transmissible than its predecessor, and by March 2021, the UK was experiencing a SARS-CoV-2 surge, with 98% of cases being the alpha variant [213]. The beta variant appeared in South Africa in October 2020 [214] and the gamma variant first appeared in Brazil in November 2020 [215]. Both beta and gamma variants appear to have

higher transmissibility than the wild-type SARS-CoV-2. The delta variant emerged in India in October 2020 and became the predominant strain globally, perhaps due to its enhanced replication ability and rapid infectivity [216,217]. The delta variant has become so dominant that on 21 September 2021, the US CDC downgraded the alpha, beta, and gamma strains to variants being monitored, leaving the delta variant as the only VOC [218].

All four variants have the D614G mutation in the spike protein. The D614G mutation was reported in samples from as early as March 2020 and, by the end of May 2020, was the predominant global circulating form of the spike protein [219]. While D614G did not increase disease severity, it did increase viral replication, viral load, transmission, and infectivity [220,221]. It has been suggested that this mutation increased the chance that those infected would lose their sense of taste and smell compared to WT S-protein [222]. This mutation abolishes the interaction between D614 on the S1 subunit and T859 on the S2 subunit [223]. It was shown that the D614G mutation caused higher affinity binding to human ACE2 receptor [224] and that it increases the likelihood that the S-protein will be in its open conformation [223]. It was also shown to increase proteolysis between S1 and S2 [225]. Yet another study showed that D614G S-protein facilitated increased infection of various human cell lines from the lung, colon, and liver [226]. Taken together, these findings suggest a mechanism by which this mutation enhances the fitness of the virus.

The alpha, beta, and gamma strains all have the N501Y spike protein mutation. The spike protein structure bound to human ACE2 shows that N501 forms a hydrogen bond to ACE2 Y41 and interacts with ACE2 K353, G354, and D355 [227]. Computational data predicts that while the N501Y mutation abolishes the hydrogen bond to ACE2 Y41, it creates a ring stacking interaction between the two tyrosine residues, and new hydrogen bonds form to ACE2 D38 and K353 [228]. A separate computational study notes that the N501Y mutation seems to increase interactions at some locations like ACE2 Y41 and K353 while decreasing interaction with others, like ACE2 Q498 [229]. The authors theorized that the interface between the spike protein and ACE2 reshapes itself in response to N501Y mutation, a hypothesis verified with a solved cryo-electron microscopy structure of the alpha variant spike protein in complex with ACE2 [230]. Two different studies used surface plasmon resonance (SPR) in order to determine that the N501Y mutation resulted in a 10-fold decrease in  $K_d$  for ACE2 binding, which is a possible mechanism of increased infectivity and transmissibility for the variants with this mutation [228,231]. Both studies agree that the increased affinity is primarily due to a decreased off rate.

In addition to increased binding affinity to ACE2, the alpha variant has mutations that weaken the interaction between the three subunits of the S-protein, A570D, S982A, and the aforementioned D614G [232]. Destabilizing the interaction between the three subunits is thought to enhance viral fusion. In addition, the P681H mutation is near the protease cleavage site between S1 and S2. As furin cleavage sites increase viral transmissibility [233], it was thought that the P681H mutation could increase cleavage and, therefore, transmissibility, but literature reports are mixed. While molecular docking simulations show increased binding of furin to the alpha variant S-protein [234], a 2022 study found that the P681H mutation didn't alter furin cleavage or viral entry, implying the other mutations are more responsible for increased viral transmissibility [235].

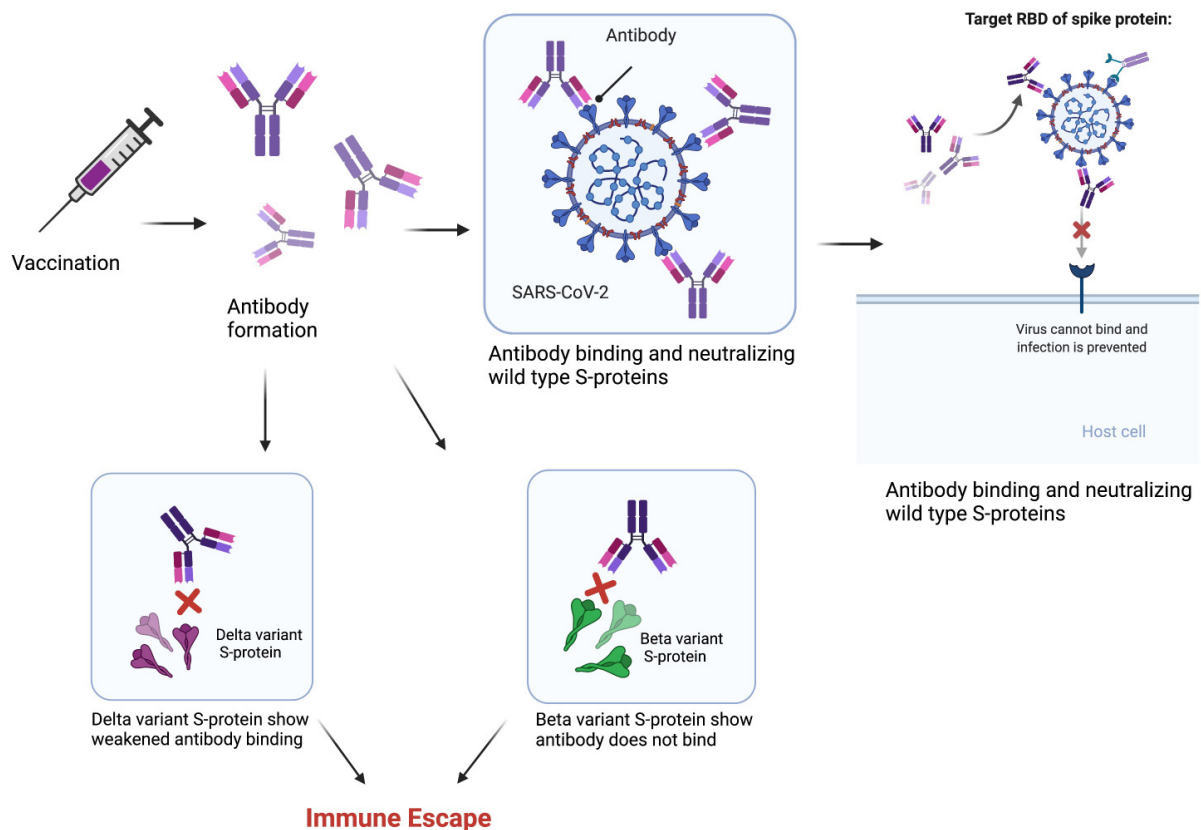
The beta and gamma strains both have spike K417N or K417T and E484K mutations. When compared to Spike RBD with only the N501Y mutation, one study found that the addition of the mutations at residues E484 and K417 lowered ACE2 binding affinity to only 2-fold greater than wild-type RBD rather than 10-fold greater with just N501Y [236]. In contrast, another SPR study showed that RBD with N501Y mutation and RBD with all three residues mutated both had a 10-fold greater affinity for ACE2 [231]. Both SPR studies used purified protein, but the Tian study used aa 319–591, whereas the Liu study used aa 319–541. A recent computational study that mapped contacts between the RBD of various spike proteins and ACE2 showed that while the N501Y mutation increased contacts, the N417N and E484K mutations in the beta variant have fewer contacts with



ACE2 than the wild type, which would support the hypothesis of a lower binding affinity than Y501 alone [237].

Unlike the alpha, beta, and gamma variants, the delta variant does not have the N501Y mutation but has other mutations in the RBD and another D950 N-terminal domain of S protein mutation that sits at the trimer interface of the spike protein and potentially affects the stability of the trimer [238]. One study found that the delta variant had increased replication compared to the alpha variant and increased cell fusion and entry compared to wild-type [217]. This may be due to increased cleavage observed in delta variant viral particles [217]. It was also found that the delta variant can infect target cells more rapidly and at lower levels of ACE2 than other variants, despite the finding that the delta spike protein did not bind any tighter to ACE2 than other variants [216]. In another study, it was found that the delta variant was able to enter lung and colon cell lines more easily than WT and also caused increased cell-to-cell fusion [239]. Taken together, these conclusions provide a plausible rationale for the enhanced viral transmissibility of the delta variant over previous variants.

There is evidence that COVID-19 variants, especially beta and, to a lesser extent, delta, may be able to escape immune system neutralization, as shown in Figure 7. It has been observed that while plasma from both those previously infected with COVID-19 and those vaccinated with the Pfizer-BioNTech vaccine could neutralize the alpha strain of the virus, it was much less effective against the beta strain [238,240]. Lowered neutralization of both the beta and gamma strains was attributed to the mutations at amino acids 417, 484, and 501, all of which appear important for antibody recognition of the spike protein receptor-binding domain [241]. Antibody binding studies show that the mutation at E484 is the crucial substitution responsible for abolishing bamlanivimab antibody binding to the RBD [236]. Mapping RBD mutations that affect the binding of neutralizing antibodies also reveals E484 to be the most crucial residue [242].



**Figure 7.** Mechanisms of antibody-mediated neutralization of viruses by blocking the binding to cell surface receptors and as a result inhibiting or dampening the infectivity. Created with [BioRender.com](https://www.biorender.com).

The delta variant also evades the immune system. Several studies have found that antibodies from either those with previous SARS-CoV-2 infection or those vaccinated show reduced activity against the delta variant [216,217,238,243]. It should be noted that the neutralization potential is reported as better for delta than for beta across these studies. Sera from unvaccinated individuals who recovered from infection 12 months prior could neutralize wild-type and alpha variants at high doses, but the sera had no effect on the beta and delta variants [238]. The T478K mutation in delta is near the E484K mutation found in the beta and gamma variants; this area of the RBD is an epitope for neutralizing antibodies and likely contributes to immune system escape [238]. Delta's L452R mutation was also characterized in spike protein variants escaping antibody neutralization [244].

COVID-19 variant escape of antibody neutralization appears to be partial, and the time between vaccine doses may play a role in the strength of immune response. An April 2021 study on health care workers found that those that were previously infected with COVID-19 had a surge of antibodies to the spike protein after one Pfizer-BioNTech vaccine dose. COVID-19 naïve patients experienced a similar spike, but only after two vaccine doses, three weeks apart [245]. None of the participant infections had the N501Y mutation, so were not alpha or beta variants. The authors found that the antibodies from participant sera were able to bind and neutralize the original Wuhan strain, the alpha variant, and the beta variant at various time points post-infection or vaccination, although binding and neutralization of the beta strain was consistently lower [245]. In addition, a February 2022 study in health care workers indicates that delaying the second vaccination for 8–16 weeks, rather than the indicated 3-week interval for the Pfizer-BioNTech vaccine, results in increased neutralization ability for WT, alpha, beta, and delta S-proteins compared to those on the standard dosing schedule [246]. Using PRNT50 and PRNT90 neutralization tests, they showed that those that had delayed their second vaccine dose 8–16 weeks had a consistently higher average neutralization ability than those on the standard dosing schedule. As in previous studies, the strongest response was to the WT and the alpha variant, with the beta variant having the lowest neutralization response of those tested [246]. This indicates that longer periods between vaccine doses may be helpful for lowering infection rates and could influence recommendations about the frequency of vaccine boosters.

While it appears antibody recognition of the beta variant is lowered or abolished, T-cell mediated response remains high with both the alpha and beta variants after infection and one dose of vaccine or two doses of vaccine [245]. Infection and/or vaccination increased the amount of S-protein specific antibody cellular cytotoxicity and also increased both CD4<sup>+</sup> and CD8<sup>+</sup> S-protein specific T-cells [245]. The authors report that CD4<sup>+</sup> activation in the presence of peptides from mutated regions of the alpha and beta S-protein is similar to activation with peptides from the WT S-protein, indicating that T-cell mediated immunological response is similar across these variants [245]. A separate study determined that mutations in the beta S-protein affected CD4<sup>+</sup> T-cell recognition [247]. Despite that decline in recognition, there was a comparable T-cell immune response as compared to WT COVID-19.

Recent studies show that individuals maintain robust T-cell responses for at least 6-months post-vaccination with either the Pfizer-BioNTech or Moderna vaccines and that these T-cells were able to recognize portions of the S-protein from the alpha, beta, and delta variants [248–250]. The T-cell response is thought to prevent SARS-CoV-2 infection from becoming severe, but without antibody neutralization, there is a partial immune escape, and breakthrough cases still occur [250]. Memory B cells in both vaccinated and recovered and vaccinated individuals were able to produce antibodies reactive to not only WT, but the alpha, beta, and delta variants as well [248]. After analyzing somatic hypermutations in B-cell clones and establishing lineage relationships, the authors determined that B-cells producing antibodies that bound the RBD of the beta S-protein were likely evolving from B-cells producing antibodies binding only the RBD of the wild-type S-protein [248]. This finding is encouraging as it suggests that vaccination against the original SARS-CoV-2 strain may prime the immune system's ability to quickly produce altered antibodies to

neutralize mutated S-proteins, limiting the severity of disease caused by infection with SARS-CoV-2 variants.

As the COVID-19 pandemic continues, more variants will continue to arise. Characterization of the clinical characteristics of these variants, and the biochemical mechanisms from which the clinical characteristics arise, will be critical to our understanding of how to prevent and treat further COVID-19 infection and disease.

#### 4. Discussion

The review focuses on viral transmissibility, and based on the literature, it reveals that the primary route of viral transmission is through aerosol particles and respiratory droplets. What plays an important role in viral transmissibility is also the viral load at the time of the onset of the disease and its particular importance in the secondary transmission. The viral load peaks around system onset, and it decreases later on; however, in cases of severe COVID-19 the viral load peaks much later, usually during the second week. As a result, the period of infectiousness is highly increased in these cases. It would be beneficial to investigate the viral load peaks amongst all the future variants to potentially predict the length of the infectiousness and relate that to the different variants but also the severity of the variants. Additionally, the range of the viral shedding time should also be further investigated to account for other independent risk factors such as age and gender.

While the COVID-19 symptomology is thoroughly investigated and similarly documented, the pathophysiological mechanism is poorly understood. To name a few, some of these mechanisms involve cytopathic events, ACE downregulation, hyperactivity of the inflammatory system causing the cytokine storm, increased coagulopathy, endothelial dysfunction, and immune-thrombosis. The precise pathophysiological mechanism is yet to be determined; however, it has been elucidated that a cytokine storm may play a role in concert with other such mechanisms such as the neutrophil extracellular traps (NET) to springboard other downstream pathways such as inflammation-induced thrombosis. While further investigating the COVID-19 pathophysiological mechanism of future variants, it is important to pay close attention to the other risk factors such as obesity, hypertension, chronic lung disease, various immunodeficiencies, and previous liver dysfunction as a way to establish a mechanistic baseline and view it through the lens of each comorbidity.

Moreover, other potential viral entry factors may need to be further investigated. Once the SARS-CoV-2 virus binds to the ACE receptor and is followed by the cleavage and final entry into the host cells, it may be worth investigating the role of the ACE expression at this particular point in the entry mechanism. While there is evidence that following the action of proteases, the S protein RBD domain binds to the ACE2 receptor with 10–20 fold more binding capacity, which in turn triggers an increase in the expression of ACE2. However, there are other reports that suggest that SARS-CoV-2 binding reduces ACE2 expression. As a result, we believe that further studies should shed light on the ACE expression and the various factors that may play a role.

The COVID-19 immune response is twofold; innate and adaptive. When it comes to the innate response, there is an increasing body of evidence that agrees on the fact that the antibody levels follow a classical signature decline, however there are also some reports that disagree on the detectable levels of these specific antibodies. When it comes to the adaptive immune response, the literature reveals that it persists much longer, however the mechanism by which it functions is yet to be determined.

Many important facets of viral transmissibility of SARS-CoV-2 remain unclear, including length of exposure, viral load, and viral shedding times. An overview of SARS-CoV-2 host cell entry is known, but we need a better understanding of the pathophysiology as this affects entry factors, immune responses, and potential therapeutic outcomes. Additionally, research into variants will clarify some gaps in the literature as they exemplify how specific mutations and amino acids affect the viral pathway. Further studies are needed to continue developing effective treatments and vaccines to combat this pandemic and finally put it behind us.

## 5. Conclusions

Transmission of SARS-CoV-2 primarily occurs human-to-human via respiratory droplets, however other potential transmission routes include the airborne route, fecal–oral route, and direct contact with contaminated surfaces. The risk of transmissibility depends on viral load at the time of disease onset, with the highest risk/viral load right before or at symptom onset. Other factors that may influence the transmissibility of COVID-19 include adherence to public health measures, close contact, and environmental conditions like temperature and humidity, however further investigation is required to understand the differences in viral shedding times presented in the literature.

While the symptomology of COVID-19 is well characterized, the pathophysiological mechanism of SARS-CoV-2 is less understood. Current scientific knowledge reveals that SARS-CoV-2 infection leads to ACE2 downregulation and renin-angiotensin-aldosterone system disturbances, which results in cytopathic effects including vasoconstriction, apoptosis, and lung inflammation. SARS-CoV-2 infection is also characterized by a cytokine storm which can contribute to acute respiratory distress syndrome, the leading cause of death in fatal COVID-19 cases. Further research is required to understand the role of the cytokine storm in the pathogenesis of SARS-CoV-2, as well as the age disparity in COVID-19 severity and mortality.

The alpha, beta, gamma, and delta variants of SARS-CoV-2 all show higher transmissibility than the wild-type SARS-CoV-2. All four strains have the D614G mutation which causes higher affinity binding to ACE2 and, thus, increases viral replication, viral load, transmission, and infectivity. Moreover, the alpha, beta, and gamma strains have the N501Y spike protein mutation which results in a 10-fold decrease in  $K_d$  for ACE binding, likely increasing infectivity and transmissibility. Unlike the other variants, delta can infect target cells more rapidly and at lower levels of ACE2. While COVID-19 variants may be able to escape immune system neutralization as their mutations are important for antibody recognition, vaccination against the original strain may prime the immune system's ability to produce altered antibodies to mutated S-proteins.

Our review has summarized the latest knowledge about SARS-CoV-2. However, as the pandemic continues to spread across the continents rapidly, there is an urgent need for more research focusing on the relationship between the length of exposure and the viral loads of symptomatic and asymptomatic patients. We need a better understanding of the different viral mutants that could illuminate the development of effective therapies against the virus.

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