# Secondary Publication: The Pathophysiology of COVID-19 (Coronavirus Disease 2019) Caused by the Infection of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2)

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**Review**

# Secondary Publication: The Pathophysiology of COVID-19 (Coronavirus Disease 2019) Caused by the Infection of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2)

**Ayako Nakamura-Ishizu**[1] and **Fumio Nakamura**[2]

[1]Department of Microscopic Anatomy, Tokyo Women's Medical University School of Medicine, Tokyo, Japan
[2]Department of Biochemistry, Tokyo Women's Medical University School of Medicine, Tokyo, Japan

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has a wide range of clinical manifestations, including acute respiratory distress syndrome, severe inflammation, abnormal blood coagulation, and cytokine storm syndrome. SARS-CoV-2 uniquely facilitates its entry and expansion in host cells through the spike protein consisting of S1 (receptor binding domain) and S2 (fusion peptide domain). The S1 binds to angiotensin-converting enzyme 2 (ACE2), the host cell receptor. The cleavage at the boundary of S1 and S2 by Furin protease and subsequent digestion within the S2 by TMPRSS2 activate the S2 fusion peptides, which are necessary for the entry of SARS-CoV-2 into host cells. After infection, SARS-CoV-2 RNA genome encodes viral proteins including structural proteins, RNA polymerases/helicases, and modulators of host- defense system, which inhibit type I interferon-related immune signaling and signal transducer and activator of transcription 1 (STAT1) signaling. In contrast, SARS-CoV-2 infection activates the proinflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor α (TNFα). In severe cases of COVID-19, these alterations in immune signaling may induce a state of systemic immune dysfunction. Recent studies also revealed the involvement of hematopoietic cells and alteration of cellular metabolic state in COVID-19. We here review the pathogenesis of COVID-19, primarily focusing on the molecular mechanism underlying SARS-CoV-2 infection and the resulting immunological and hematological alterations.

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**Keywords**: SARS-CoV-2, COVID-19, ACE2, TMPRSS2, cytokine storm

Corresponding Author:
Ayako Nakamaura-Ishizu, Department of Microscopic Anatomy, Tokyo Women's Medical University School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. ishizu.ayako@twmu.ac.jp
Fumio Nakamura, Department of Biochemistry, Tokyo Women's Medical University School of Medicine, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan. nakamura.fumio@twmu.ac.jp
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## Introduction

We here review the pathophysiological aspects of COVID-19 (coronavirus disease 2019) caused by the infection of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). We have focused on the imbalance of cytokine production and type I interferon (IFN) suppression, cytokine storm, and abnormal blood coagulation system in patients with COVID-19.

## Molecular Mechanism of SARS-CoV-2 Infection

SARS-CoV-2, classified into the Coronavirus group, possesses approximately 30,000 bp single-stranded RNA genome.[1] Coronaviruses are named so because of the characteristic appearance of spike (S) proteins on the viral surface envelope derived from host cell membranes. The viral genome of SARS-CoV-2 consists of genes encoding the spike, integral membrane (M), envelope (E), nucleocapsid (N), ORF1ab, and ORF3-10 proteins. ORF 1ab generates 16 non-structural proteins (NSPs) via post-translational processing by two proteases, main protease NSP5 and papain-like protease NSP3.

The S protein of SARS-CoV-2 is involved in the binding and fusing to host cells. Angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) on host cells serve as the main receptor and proteolytic processor of the S protein, respectively.[2] The S protein consists of the S1 domain (receptor binding domain: RBD) and S2 domain, which contains fusion peptide (FP) and transmembrane (TM) (**Figure 1**). Proteolytic sites for Furin and TMPRSS2 proteases on S protein form the boundaries of S1 and S2 and the S2 site within the S2 domain, respectively. SARS-CoV-2 attaches to host cells when the S1 domain binds to ACE2. Furin digests the S1/S2 junction of the S protein, and TMPRSS2 subsequently digests the S2' site to expose the FP. After invagination and fusion of the activated FP to host cell membrane, viral RNA and proteins are incorporated into the host cells. Hence, processing with Furin and TMPRSS2 is essential for FP activation and canonical viral infection. The virus also infects via endocytosis wherein the internalized viruses are digested by cysteine proteases Cathepsin-B/L (CTSB/L) within the endosomes and the viral genome is released into the cytoplasm after fusing with the endosome membranes.[2]

Angiotensin-II (AngII, amino-acid sequence DRVHIYPF) is the activated form of angiotensin (Ang). In the host cells, ACE2 is an endopeptidase that converts AngII to Ang(1-7) by removing the C-terminal Phe (F) from AngII.[3] Ang(1-7) is considered to exert vasodilatory and organ-protecting effects through its interaction with the Mas receptor. ACE2 is also involved in the inactivation of bradykinin (BK) derivative, Des-Arg9-BK.[3]

The binding of SARS-CoV-2 to ACE2 inhibits latter's enzymatic activity. Viral internalization also reduces the surface expression of ACE2. These changes lead to an increase in levels of AngII and Des-Arg9-BK in the blood and tissues, which may induce damage to various organs in patients with COVID-19.[4] The protective effect of ACE2 is revealed in *Ace2* knockout mice, which exhibit acute respiratory distress syndrome.[5] TMPRSS 2, a membrane-bound serine protease, is involved in limited proteolysis, maturation, and infection of capsid proteins of various viruses including influenza and coronaviruses.[6] TMPRSS2 is considered an aggravating factor in male patients with COVID-19 because the gene encoding this

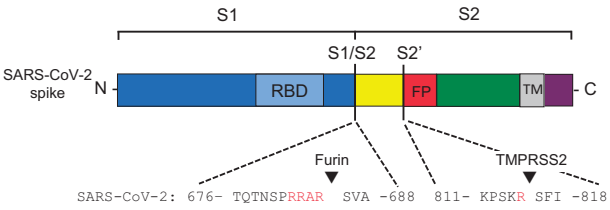


**Figure 1.** Spike protein of SARS-CoV-2 and its proteolytic sites.
Furin and TMPRESS2 proteases cleave S1/S2 and S2' sites, respectively.
RBD, receptor binding domain; FP, fusion peptides; TM, transmembrane domain.
Adapted from Shen LW et al, Biochimie (2017)[6] and Hoffman M et al, Cell (2020).[2]

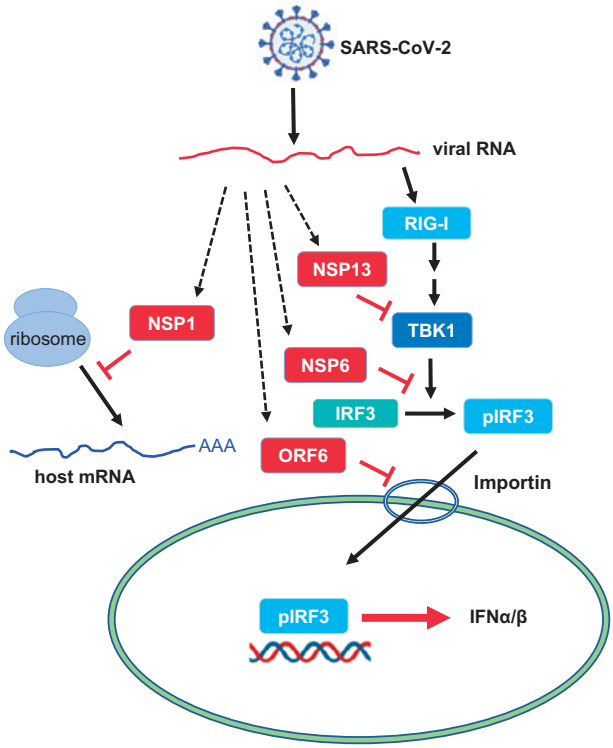


**Figure 2.** Suppression of IFN expression by of SARS-CoV-2 products.
Viral RNA activates RIG-1-TBK1-IRF3 cascade and facilitates the transcription of IFN-stimulated genes. The viral proteins (NSP1, NSP6, NSP13, ORF6) from SARS-CoV-2 genome interfere with signaling.

enzyme possesses an androgen-responsive element.[7]

## The Distribution of SARS-CoV-2 Receptors

Tissue expression patterns of ACE2 and TMPRSS2 have been extensively studied using various techniques, including single-cell RNA sequencing analyses during the COVID-19 pandemic.[8-11] In the respiratory system, the nasal epithelium strongly expresses both ACE and TMPRSS2.[8] The expression of ACE2 gradually decreases from the trachea and bronchi to the alveoli. In the alveoli, type-2 alveolar epithelial cells and secretory transitional cells express ACE2 and TMPRSS2; however, a minor population (1%) of type-2 alveolar epithelial cells express ACE2.[9-11] The abundant expression of ACE2 and TMPRSS2 in the nasal epithelium may contribute to the initial infection and propagation of SARS-CoV-2. ACE2 is also expressed in the intestinal epithelium, vascular endothelium, renal tubules, heart vessels, testes, and ovaries.[10, 12] In the immune system, macrophages express ACE2.[13] In the nervous system, ACE2 and TMPRSS2 are expressed in sustentacular cells of the olfactory nerve. This may be correlated with anosmia of COVID-19 patients.

In addition to ACE2 and TMPRSS2, Neuropilin-1 (NRP1) and Basigin (CD147) have been reported as accessory SARS-CoV-2 receptors.[14-16] NRP1 serves as a receptor for the axon guidance molecule Sema3A and vascular endothelial growth factor (VEGF).[17] NRP1 binds to the C-terminal residue (RRAR) of the S1 domain exposed by Furin-digestion.[14, 15] Despite the low expression of ACE2 and TMPRSS2 in the nervous system, neural infection with SARS-CoV-2 has been reported. This might be due to NRP1 and CD147 found in excitatory neurons and neuronal endothelial cells.[16] The expression of cathepsin B/L (CTSB/L) in neural endothelial cells and neurons may also contribute to the SARS-CoV-2 infection via the endosome route.[16]

## Suppression of Type I Interferon and Augmentation of Inflammatory Cytokines in COVID-19

In ordinal RNA-virus infection, the binding of viral RNA

to RIG-1 activates antiviral signaling (**Figure 2**), where TANK binding kinase (TBK1) phosphorylates interferon regulatory factor 3 (IRF3). Phosphorylated IRF3 translocates to cellular nuclei and acts as a transcriptional regulator to induce type I interferon (IFN) α/β, which subsequently activates IFN-stimulated genes (ISGs). However, SARS-CoV-2 infection suppresses anti-viral signaling but produces a favorable environment for virus propagation. In contrast, Toll receptors of innate immune cells recognize SARS-CoV-2 infection and activate nuclear factor-κB (NF-κB), which produces inflammatory cytokines, including interleukin (IL) -6 and TNFα. In the early stages of SARS-CoV-2 infection, the imbalance of IFN suppression and cytokine activation contributes to the aggravation of COVID-19.[18]

**1. Suppression of type I interferon by SARS-CoV-2 viral proteins**

SARS-CoV-2 viral proteins, including NSP1, NSP3, NSP6, NSP13, ORF3b, and ORF6, are involved in IFN-suppression (**Figure 2**). NSP1 binds to the 40S ribosomal subunit of host cells and interferes with the mRNA translation of RIG-1 and ISGs. This results in dysfunction of the innate immune system, especially retinoic acid-induced gene expression, which is activated by viral infection.[19]

NSP6, NSP13, and ORF6 inhibit signaling from RIG-1 to IRF3.[20] NSP13 suppresses the activation of TBK1, NSP6 inhibits the IRF3-phosphorylation by TBK1, and ORF6 binds importin to block the nuclear localization of IRF3. ORF6 also blocks nuclear translocation of STAT1/2 and the expression of ISGs. SARS-CoV-2 suppresses IRF3-nuclear localization and mRNA transcription of IFNα/β through these modulations.

NSP3, a papain-like protease, is involved in the maturation and digestion of viral proteins. NSP3 also suppresses IRF3 via enzymatic activity. ISG15, a ubiquitin-like protein, attaches to viral proteins to inhibit viral production.[21] ISG15 also attaches to IRF3 to enhance its nuclear localization. However, NSP3 removes the ISG15-conjugation, in turn, to suppress IFN production by IRF3.[22] In addition, ORF3b, a splice variant of ORF3, also suppresses IFN production, although its molecular mechanism remains unknown. A variant form of ORF3b found in severely infected and dead patients was revealed to potently inhibit IFN production.[23] It is worth noting that the expression level of type I IFN is not downregulated but increased in moderate and severe cases of COVID-19.[24] This suggests that IFN-suppression in early stages augments viral production, and IFN-overproduction in advanced stages aggravates COVID-19.[25]

**2. Augmentation of inflammatory cytokine production by SARS-CoV-2 infection**

It has been reported that moderate and severe cases of COVID-19 show an increase in inflammatory cytokines, including IL-6, IL-8, and TNFα, as well as the type II interferon, IFNγ.[24] This may reflect the secretion of inflammatory cytokines from immune and host cells infected with SARS-CoV-2.

In usual RNA-viral infections, immune cells, including dendritic cells and macrophages, recognize the viral RNA using pattern-recognizing receptors TLR3, 7, and 8. These receptors activate NF-κB through intracellular signaling via MYD88 and TRF4. Nuclear-translocated NF-κB induces expression of inflammatory cytokines, such as IL-6 and TNFα, in both dendritic cells and macrophages.[25] In the case of SARS-CoV-2 infection, macrophages express cytokines, including TNFα, IL-1b, and IL-8. However, in dendritic cells, the immune response and expression of these cytokines are suppressed by a mechanism similar to that of type I IFN suppression.[26] This results in the overproduction of inflammatory cytokines without suppressing the viral replication. The increase in INFγ after a SARS-CoV-2 infection is considered antiviral Th1 response generated by a subset of $CD4^+$ T cells.[27]

SARS-CoV-2 infection also induces the secretion of inflammatory cytokines from non-immune cells, such as type-2 alveolar epithelial cells. In particular, viral S proteins are involved in TNFα secretion.[28] TNFα, which is cleaved from the membrane-bound TNFα precursor by a metalloprotease ADAM17 (TACE), forms a soluble trimer. ACE2 complex and the S fragment of SARS were found to activate ADAM17.[29] Therefore, a similar mechanism could be utilized for SARS-CoV-2 infection. Released TNFα augments the inflammatory response through the activation of TNF receptor signaling. ADAM17 also digests membrane-bound ACE2 and IL-6 receptors and generates their soluble forms.[28] The soluble

IL-6 receptor is capable of IL-6 binding, associated with gp130, and activates IL-6 intracellular signaling. Therefore, SARS-CoV-2 infection may result in the downregulation of ACE2 and augmentation of the IL-6 response through an increase in soluble IL-6 receptors.

In addition, a recent study suggested that synergism between TNFα and IFNγ triggers cell death and tissue damage by the hyperactivation of JAK/STAT1 signaling.[30] This may explain the organ failure observed following the cytokine storm in severe cases of COVID-19.

## SARS-CoV-2-Induces Macrophage Activation and Cytokine Storm

Patients with severe COVID-19 suffer respiratory failure accompanied by a fulminant inflammatory response involving the activation of various hematopoietic cells. By binding to ACE2, SARS-CoV-2 infects pulmonary alveolar epithelial cells and alveolar macrophages.[2] In severe cases of SARS-CoV-2 infection, virus-infected pulmonary alveolar epithelial cells and alveolar macrophages produce an array of inflammatory cytokines. SARS-CoV-2 damages alveolar epithelial cells and stimulates the secretion of factors, such as VEGF, which increases vascular permeability. Enhanced tissue damage and vessel leakage facilitate the release of the virus and cytokines into the blood circulation.[31] Therefore, an increase in serum levels of IL-6, IL-1, IFNγ, and pathogen-associated molecular pattern (PAMP) are observed in severe cases of COVID-19.[32] Upregulation of ACE2 expression by IFN signaling further aggravates COVID-19 pathogenesis.[33]

The excessive systemic production of cytokines, termed cytokine storm syndrome,[34] is a frequent manifestation in severe COVID-19 cases and a potential therapeutic target. Cytokine storm syndrome involves the activation of phagocytic macrophages, resulting in macrophage activation syndrome (MAS) or hemophagocytic lymphohistiocytosis (HLH).[32] High serum levels of IL-6, IL-10, and TNFα, along with low expression levels of IFNγ in $CD4^+$ T cells, are reported as poor prognostic factors for COVID-19.[35,36] Therefore, immunosuppressive treatments ranging from systemic steroids to specific inhibition of IL-6, IL-1, and IFNγ signaling have been implicated.[34] However, investigation of the efficacy of these treatments for COVID-19 has been hampered by lack of appropriate SARS-CoV-2 animal models, as SARS-CoV-2 does not infect wild-type mice. Recently, the development of mice transduced with the human ACE2 adenovirus vector (Ad5 hACE2) allowed *in vivo* investigation of the pathophysiology of COVID-19 and the efficacy of anti-cytokine treatments.[37] The Ad5 hACE2 mouse model allowed entry of SARS-CoV-2 into murine lung cells, leading to severe pneumonia. IFNα receptor knockout mice transduced with Ad5 hACE2 exhibited significantly lower inflammatory response levels and decreased weight when infected with SARS-CoV-2. However, mice deficient in the downstream IFNα signal molecule STAT1, transduced with Ad5 hACE2, exhibited significantly higher inflammatory response levels and reduced viral clearance. Furthermore, treatment with poly I: C, an IFN signal stimulator drug, was effective in SARS-CoV-2 mice models. These data indicate that IFN-STAT1 signaling is critical for the pathophysiology of COVID-19 but is complexly regulated in severe COVID-19 cases. Furthermore, proteomic analysis of SARS-CoV-2-infected Vero E6 cells showed enrichment in p38/mitogen-activated protein kinase (MAPK) pathway-associated protein expression.[38] p38/MAPK activation is associated with inflammatory stress and may stimulate the production of inflammatory cytokines **(Figure 3)**. Consequently, SARS-CoV-2-infected cell lines exhibited decreased gene expression of IL-6 and TNFα upon treatment with p38/MAPK inhibitor (SB203580). Investigations should be conducted to discover novel targets that may control excessive cytokine production in severe cases of COVID-19.

We reported that the tumor suppressor gene, *folliculin* (*FLCN*, *Flcn*), regulates the activation of phagocytic macrophages by modulating TFE3 transcription and lysosomal biogenesis.[39] Mutations in *FLCN* cause Birt-Hogg-Dubé (BHD) syndrome, a syndrome presenting with tumor formation in the kidney, lungs, and skin.[40] *Flcn*-deficient mice exhibit increased nuclear translocation and activation of TFE3 and TFEB transcription factors which influence mTOR/AMPK signaling pathways and perturb cellular metabolic processes, such as autophagy and lysosomal biogenesis.[41] Furthermore, hematopoietic stem cells (HSCs) from hematopoietic cell-specific *Flcn*-deficient mice ($Flcn^{fl/fl}$; $MxCre1^{+/-}$) exhibit

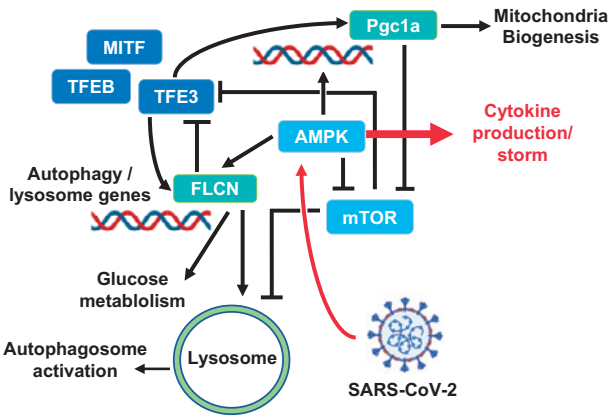


**Figure 3.** Schematic representation of SARS-CoV-2-induced cytokine production and related metabolic pathways.
SARS-CoV-2 infection activates p38/MAPK-AMPK pathway, which subsequently stimulates cytokine production in host cells.The AMPK/mTOR pathway is also related to TFE3/FLCN pathway which involves metabolic changes within a cell.

impaired stem cell potential and bone marrow failure.[42] *Flcn*$^{fl/fl}$; *MxCre1*$^{+/-}$ mice also exhibited a significant increase in the number and activity of phagocytic macrophages in the bone marrow.[39] The abnormal hematopoietic phenotype in *Flcn*$^{fl/fl}$; *MxCre1*$^{+/-}$ mice were rescued when the mice were crossbred with *Tfe3*$^{-/-}$ mice. One interesting feature of *Flcn*-deficient macrophages is the dysregulation of glyconeogenesis, suggesting a crucial role of glucose/glycogen metabolism in inflammatory phagocytosis. As COVID-19 is associated with cytokine storm syndrome and MAS/HLH, investigating the changes in metabolic pathways, such as FLCN/TFE3 signaling, may reveal novel treatment targets **(Figure 3)**.

## Changes in Platelet Function and Coagulation Process in COVID-19

SARS-CoV-2 infection not only affects immune cells but also dysregulates thrombotic and coagulation processes in the hematopoietic system. Patients with COVID-19 frequently present with significantly high circulatory levels of D-dimer, fibrinogen, and FDP, indicating an abnormal coagulation response.[43] Furthermore, enhanced cytokine production observed in severe cases of COVID-19 further disconcerts thrombosis and coagulation processes.[44] Furthermore, inflammation-stimulated complement-mediated thrombotic microangiopathy (TMA) is frequently a complication of severe COVID19[45]. Moreover, patients with COVID-19 and thrombocytopenia have a poor prognosis.[46, 47] Proteomic analysis of SARS-CoV-2-infected Vero E6 cells showed enrichment of platelet-related proteins, such as APOH, CD9, TSPAN4, and AHSG.[38] Furthermore, proteomic analysis of serum samples from patients with COVID-19 revealed the enrichment of 105 proteins compared to healthy controls; many of these proteins were associated with complements, macrophages, and platelets.[48] Interestingly, a significant decrease in platelet factor 4 (PF4) was observed in serum samples from patients with severe COVID-19. PF4 is known to inhibit host cell entry of the human immunodeficiency virus (HIV) -1 virus, and a decline in PF 4 levels is a poor prognostic factor in SARS.[49, 50]

Several mechanisms have been proposed for SARS-CoV-2 infection-induced changes in hemostasis. High levels of circulatory IL-6 stimulate acute phase protein (CRP, fibrinogen) production in the liver, which subsequently stimulates platelet production in the bone marrow. Live imaging of transgenic reporter mice has identified platelet-producing megakaryocytes within the lung tissue.[51] This report identifies that approximately 50% of circulating platelets are produced at a rate of $1 \times 10^7$ cells/h in murine lungs. As the lung is a major site of platelet production, pulmonary injury due to SARS-CoV-2 infection may directly influence hemostasis in COVID-19.

IFN signaling upregulates platelet production by promoting the commitment of bone marrow HSCs to platelet-lineage differentiation.[52] We reported that the

platelet-lineage commitment of HSCs is regulated through the activation of mitochondrial respiration.[53] To date, a detailed analysis of metabolic changes in hematopoietic cells during SARS-CoV-2 infection has not been conducted. Metabolomic screening of serum from patients with COVID-19 has revealed alterations in apolipoprotein (APOA1, APOA2, APOH, and APOL1) and sphingolipids, which may implicate systemic metabolic changes as a result of SARS-CoV-2 infection.[48] Future studies should address how metabolic changes are associated with COVID-19 pathophysiology.

## Lymphocytes in COVID-19

Lymphopenia is another manifestation frequently observed in cases of severe COVID-19.[54,55] Compared to other viral infections, SARS-CoV-2 infection frequently manifests a significantly low number of circulating T lymphocytes. Serum levels of IL-6, IL-10, and TNFα negatively correlate with the degree of lymphopenia, suggesting that cytokine signaling may influence lymphocyte numbers in patients with COVID-19.[56] Lymphocytes may be sequestered within damaged pulmonary tissues, lowering the number of circulating lymphocytes.[57] Furthermore, the proliferative capacity of lymphocytes is known to be associated with longer telomere length, suggesting a relationship between lymphocytopenia and the poor prognosis of aged individuals with COVID-19.[58]

## Conclusion

We reviewed the current understanding of the pathophysiology of SARS-CoV-2 infection with a focus on alterations in the immune response and hemostasis. As the world combats COVID-19 through the development and distribution of vaccines, further investigation of COVID-19 pathophysiology is necessary, particularly to develop novel treatments for severe cases of COVID-19.

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