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Spotting Therapeutic Targets against SARS-CoV-2 and COVID-19

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Abstract

To control the SARS-CoV-2 pandemic infection, worldwide vaccination has been implemented. However, no specific therapeutic drugs are available for the treatment of COVID-19 yet. To find the effective therapeutic targets for COVID-19, efforts should be focused on the pathogenesis of COVID-19. SARS-CoV-2 induced COVID-19 involves the processes of viral entry into the host cells, viral replication in host cells, and induction of cytokine storm. Therefore, the potential targets may include the structural proteins of the virus and host cell proteins that play roles in the viral entry, associated enzymes in viral replication, and associated signaling proteins for the release of inflammatory cytokines that mediate the induction of cytokine storm. The development of candidates based on these targets may formulate the future treatment of COVID-19 or other associated diseases.

Keywords: Spike Protein, Cytokine Storm, Toll-Like Receptors, Janus Kinase, NLRP3 Inflammasome

1. Introduction

Since December 2019, SARS-CoV-2 infection had once become the major worldwide health crisis. SARS-CoV-2 infection not only causes severe respiratory impairment but also leads to complications in other systems of the body, including the cardiac and nervous systems, which contribute to the increased mortality in patients with COVID-19 [1-4]. Although the pandemic is being controlled with the implementation of vaccination, there is still a lack of specific drugs for the treatment of COVID-19 and its chronic complications. Therefore, developing therapeutic drugs for COVID-19 is critically important.

Although multiple therapeutic targets have been proposed and various associated agents have been tested in the clinical setting, an effective treatment for COVID-19 remains lacking. Finding specific therapeutic targets and developing effective drugs is an essential task for the control of COVID-19 and the reduction of mortality.

The induction of COVID-19 by SARS-CoV-2 infection involves several steps that include viral entry into the host cells, replication of the virus, and induction of pathogenic processes such as cytokine storm. To block either one of the steps should reduce the severity of infection and lower the mortality of COVID-19 patients. As a result, targeting these processes should be an effective strategy to fight against COVID-19. The review will focus on the following pathogenic processes and specific proteins that are involved in these processes as potential targets for developing therapeutic drugs for COVID-19.

2. Viral Invasion

Viral entry into the host cells, such as epithelial cells in the respiratory tract, is the critical step for infection. Blockade of the machinery that mediates cell entry of SARS-CoV-2 can prevent the infection or reduce the load of the virus and the severity of COVID-19. The following proteins that play important roles in the viral entry into the host cells may function as therapeutic targets for COVID-19 (Table 1).

2.1. Spike Protein

The coronavirus structural proteins include the spike glycoprotein (S-protein), envelope protein (E-protein), membrane protein (M-protein), and the nucleocapsid protein (N-protein). The S-protein of SARS-CoV-2 is similar to the coronavirus strain SARS-CoV, with over 72% amino acid sequence similarity [5]. SARS-CoV-2 can bind to angiotensin-converting enzyme 2 (ACE2) through the receptor-binding domain (RBD) of the S-protein with a higher affinity relative to SARS-CoV [6]. In addition, SARS-CoV-2 does not use receptors, such as aminopeptidase and dipeptidyl peptidase-4, which other coronaviruses employ [7].

The S-protein of SARS-CoV-2 consists of two subunits: a globular S1 domain at the N-terminal region and the membrane-proximal S2 domain. The RBD within the S1 subunit is essential for the virus attachment to the host cell receptor ACE2, while S2 is critical for virus entry by regulating viral membrane fusion to the host cell membrane [8,9]. The subunit S1 is composed of four domains named the N-terminal domain (NTD), the receptor-binding domain (RBD), and two subdomains, SD1 and SD2. The S2 subunit is divided into an N-terminal hydrophobic

fusion peptide (FP), two heptad repeats (HR1 and HR2), a transmembrane domain (TM), and a cytoplasmic tail (CT) (Figure 1A).

Both S1-RBD and S2 domains represent important potential targets for the development of SARS-CoV-2 vaccines and therapeutic drugs [10]. In fact, many of the currently developed vaccines target S-protein of SARS-CoV-2 [11,12]. RBD seems to be a good target for SARS-CoV-2 vaccine, since a recombinant RBD protein of SARS-CoV-2 prepared from insect cells was reported to induce serum antibodies that could bind to the RBD and neutralize viral infection in nonhuman primates [13]. However, the emerging SARS-CoV-2 variants have resulted in an alteration of RBD binding properties of neutralizing antibodies [14].

The current important variants include B.1.1.7 (UK variant with mutations of N501Y, S494P*, E484K*), P.1 (Brazil variant with mutations of K417N/T, E484K, N501Y), B.1.351 (South Africa with mutations of K417N, E484K, N501Y), B.1.427/B.1.429 (California variants with mutations of L452R), B. 1.617 (Indian variant with critical mutations of L452R and E484Q). The different mutations have changed the binding affinity of RBD and viral infectivity. To develop a vaccine that produces neutralizing antibodies to be effective against all variants is an important strategy to control the pandemic. Anyway, the development of monoclonal antibodies against or inhibitors of the S-protein of the primary strain and variants might hold great promise to find therapeutic candidates for COVID-19.

2.2. ACE2

The S-protein of both SARS-CoV and SARS-CoV-2 has been known to utilize ACE2 as a receptor for host cell entry [15]. ACE2 is a metallopeptidase that is expressed on major viral target cells, such as type II pneumocytes and enterocytes [16]. ACE2 is expressed not only in type II alveolar cells, but also in myocardial cells, proximal tubule cells of the kidney, ileum and esophagus epithelial cells, and bladder urothelial cells, as well as in brain cells, establishing the basis for possible extrapulmonary invasion of SARS-CoV-2

The catalytic domain of ACE2 binds to S-protein with high affinity [17]. Binding of S-protein to ACE2 triggers conformational rearrangements in S-protein, which are believed to increase the sensitivity of the S-protein to proteolytic enzymes, such as late mentioned furin, at the border between the S1 and S2 subunits for priming to facilitate the viral entry into the cells.

ACE2 has been demonstrated to play a role in viral invasion. A clinical-grade soluble recombinant human ACE2 protein (srhACE2) was shown to inhibit attachment of SARS-CoV-2 to simian Vero-E6 cells and prevent SARS-CoV-2 infection of engineered human capillary organoids and kidney organoids (Vero-E 6 cells) [18]. However, whether srhACE2 administration in vivo can prevent SARS-CoV-2 infection remains unknown. The roles of ACE2 inhibitors, such as nicotinamide and other small molecular inhibitors, in SARS-CoV-2 infection should be further investigated [19,20].

An ACE2 decoy has been designed to have combined T27Y and H34A mutations that increase the affinity of ACE2 to RBD of S-protein and have an H374N mutation that abrogates ACE2 enzymatic activity. The ACE2 triple decoy has been demonstrated to inhibit infection by competing with endogenous ACE2 for binding to the S-protein RBD domain of SARS-CoV-2 variants. The ACE2 triple decoy also showed an increased affinity for the RBD domain expressing N501Y (B.1.1.7 variant) or L452R (B.1.427/B.1.429 variants) mutations [21].

Coagulation factor Xa, a protease, cleaves the S-protein of SARS-CoV-2 and prevents its binding to ACE2, resulting in decreased viral entry. In contrast, rivaroxaban, a direct inhibitor of coagulation factor Xa, facilitates the viral entry [22].

Recently, tripartite motif containing 28 (TRIM28) has been shown to regulate ACE2 expression. Co-expression of TRIM28 with ACE2 and TMPRSS2 was found in type II pneumocytes [23]. TRIM28 seems to negatively mediate the expression of ACE2, since knockdown of TRIM28 induces ACE2 expression and increases pseudotyped SARS-CoV-2 cell entry in A549 cells and primary pulmonary alveolar epithelial cells [24], suggesting that upregulation of TRIM28 might be a possible approach to limit the viral entry, not the host cells.

2.3. Furin and Transmembrane Serine Protease 2

Binding of the S-protein of SARS-CoV-2 with ACE2 is followed by host furin and transmembrane serine protease 2 (TMPRSS2)-mediated priming of the viral S-protein. The priming process involves the cleavage of the S-protein at the S1/S2 border and S2' sites, facilitating the viral fusion with the host cell membrane [25].

Furin is a ubiquitously expressed calcium-dependent host cell protease that proteolytically activates many proproteins, including pathogenic agents and physiological proteins. Furin cleaves S-protein at the S1/S2 site into two segments, the S1 (1-685) and the S2 (686-1273) domains. Although furin cleavage of S-protein is not essential for SARS-CoV-2 infection and cell-cell fusion, it has been shown to promote the infection and demonstrated to be involved in transmission in ferrets, since SARS-CoV-2 virus lacking the S1/S2 furin cleavage site had lower titers in infected ferrets [26,27].

TMPRSS2, a transmembrane serine protease in airway epithelial cells and alveolar cells, plays a critical role in viral entry into host cells. Like ACE2, TMPRSS2 is also expressed in the heart, digestive tract, liver, kidney, brain, and other organs [28]. TMPRSS2 contains a type II transmembrane domain (TM), an LDL receptor class A domain (LDLRA) for calcium binding, a scavenger receptor cysteine-rich domain (SRCR) for binding to other cell surface or extracellular molecules, and a serine protease domain (SPD) that cleaves at arginine or lysine residues [29]. The cytoplasmic domain at N terminal interacts with cytoskeletal components and cell signaling molecules. SPD domain is the location for the proteolytic activity of TMPRSS2. LDLRA and SRCR domains may function to regulate protein binding and protein–protein interaction [25].

Viral S-protein is one of the targets of TMPRSS2. Both SARS-CoV and SARS-CoV-2 use the serine protease TMPRSS2 for S protein priming [25]. The binding of RBD within the S1 domain to ACE2 could trigger the effects of TMPRSS2 on the cleavage of S-protein at the S2′ site and facilitate cell membrane fusion for viral entry [30].

TMPRSS2 is essential for activation of SARS-CoV-2 S-protein in Calu-3 human airway epithelial cells, since antisense-mediated knockdown of TMPRSS2 expression abolished the infection [31]. TMPRSS2 inhibitor camostat mesylate significantly reduced infection of SARS-CoV-2 in human lung Calu-3 cells [25]. Nafamostat mesylate, which has been demonstrated to inhibit TMPRSS2-dependent host cell entry of MERS-CoV, can also prevent SARS-CoV-2 entry into host cells with roughly 15-fold-higher efficiency than camostat mesylate [25,32]. Another TMPRSS2 inhibitor, α-1antitrypsin, also blocks SARS-CoV-2 from entering host cells.

2.4. CD147

CD147, which is known as basigin or extracellular matrix metalloproteinase inducer, is another host cell receptor that binds to S-protein to mediate viral invasion. The S-protein of SARS-CoV-2 has recently been demonstrated to bind to CD147, the direct interaction of which regulates virus infection in host cells [33]. In in vitro studies, the loss of CD147 by stable knockdown or inhibition of CD147 by meplazumab lowered SARS-CoV-2 load in Vero E6 and BEAS-2B cell lines; in contrast, overexpression of CD147 promotes virus infection in BHK-21 cells. Human CD147 extracellular fragment that competes with CD147 for the binding to S-protein inhibited pseudovirus entry into BHK-21-CD147 cells. In addition, viral loads were detected in the lungs infected with SARS-CoV-2 in transgenic mice expressing human CD147, but not in wild type mice [33]. The results of these studies suggest that NRP1 play a role in viral entry and that NRP1 may be considered as a potential target for the development of specific anti-SARS-CoV-2 drugs.

2.5. Tyrosine-Protein Kinase Receptor UFO

Tyrosine-protein kinase receptor UFO (AXL) is a cell surface receptor tyrosine kinase, part of the TAM family of kinases, expressed in normal and cancer cells. AXL is a key factor that assists cancer cells in immune escape and drug resistance, leading to aggressive and metastatic cancers. Recently, AXL has been identified as a receptor for SARS-CoV-2 that promotes infection in pulmonary and bronchial epithelial cells. AXL specifically interacts with the N-terminal domain of the S-protein of SARS-CoV-2 in HEK293T cells, and AXL overexpression promoted viral entry as efficiently as ACE2 overexpression. Downregulating AXL, but not ACE2, significantly reduced infection of pulmonary cells by SARS-CoV-2. Soluble human recombinant AXL, but not ACE2, blocked SARS-CoV-2 infection in cells expressing high levels of AXL [34].

2.6. Neuropilin 1 (NRP1)

Neuropilin 1 (NRP1) is a host cell protein that is widely distributed throughout the body tissues. The higher expression of NRP1 was found in the respiratory and olfactory epithelium with highest expression in endothelial and epithelial cells [35].

NRP1 acts as a multifunctional co-receptor for a variety of ligand proteins and has been demonstrated to play significant roles in both physiological roles and pathological conditions. Interestingly, the ability NRP1 to facilitate the entry of SARS-CoV-2 has been recently identified [36].

NRP1 is a single-pass transmembrane glycoprotein that comprises a large N-terminus extracellular domain (NCD), a membrane spanning transmembrane domain (TM), and a short cytoplasmic tail (SCT) in the inner side of the cell membrane. The NCD domain containing two CUB (complement binding factors C1r/C1s, Uegf, bone morphogenetic protein 1) (a1/a2), two factor V/VIII coagulation factor homology (b1/b2) domains, and one MAM (homologous to meprin protease, A5 antigen, receptor tyrosine phosphatase μ and K) (c domain) functions to bind different ligands. The transmembrane domain is a single-pass protein consisting of a conserved GXXXG repeat crossing the cell membrane. Intracellular domain at the C-terminal contains a PDZ binding motif (SEA) that can act as a docking site for interacting partners [37].

NRP1 promotes SARS-CoV-2 infection through interacting with a furin-cleaved fragment of the S-protein of the virus. NRP1 can potentiate SARS-CoV-2 infection in the presence of other host factors, such as ACE2 and TMPRSS2 [35]. The host protease furin cleaves the full-length precursor S-protein into S1 and S2 peptides [38]. During the separation, the cell surface receptor NRP1 predominantly binds to the C-terminal RRAR motif (682-685) of the S1 domain and functions to stabilize the S1 C-terminal region (640-685) to assist the detachment of S2 N-terminal region (686-700). The liberated S2 domain mediates the fusion of virus and host membranes, suggesting that NRP1 increase virus infectivity by facilitating the S1/S2 separation [39]. Blocking this interaction by monoclonal antibody against NRP1 or selective inhibitors reduced SARS-CoV-2 entry and infectivity in epithelial cell culture [40].

2.7. Glucose Regulated Protein 78

Glucose-regulated protein 78 (GRP78), one of the members of the heat shock protein 70 (HSP70) family, is a cell stress response protein that is normally located in the lumen of the endoplasmic reticulum and plays roles in protein synthesis, protein folding, and cell death. In the endoplasmic reticulum, GRP78 binds to three inactivating enzymes, including activating transcription factor 6, inositol-requiring enzyme 1, and protein kinase RNA-like endoplasmic reticulum kinase [41].

Upon the stimulation of accumulated unfolded or misfolded proteins, GRP78 is released from the binding and translocated to the plasma membrane. GRP78 on the membrane functions to recognize and mediate entry of viruses via the substrate-binding domain [42]. The results of combined molecular modeling docking and structural bioinformatics indicated that four regions, including region I (C336-C361), region II (C379-C432), region III (C391-C525), and region IV (C480-C488) of the S-protein can fit tightly in the substrate binding domain β (SBDβ) of GRP78; In particular. the region IV with nine residues is the main driving force for GRP78 binding, which might be used to develop therapeutics specific inhibitors against COVID-19 [43].

2.8. High-Density Lipoprotein (HDL) Scavenger Receptor B Type 1 (SR-B1)

High-density lipoprotein (HDL) scavenger receptor B type 1 (SR-B1) is a cell-surface HDL receptor that mediates the selective uptake of cholesteryl esters and other lipid components of receptor-bound HDL particles [44]. Co-expression of SR-B1 and ACE2 has been found in human pulmonary and extrapulmonary tissues [45].

SR-B1 may promote the attachment of SARS-CoV-2 on ACE2-expressing cells. HDL can interact with the S1 domain of S-protein. Blockade of the cholesterol-binding site on the S1 domain with either a monoclonal antibody or SR-B1 inhibitors prevents HDL-enhanced SARS-CoV-2 infection [45]. Although the interaction between HDL and S-protein may not be the major mechanism of viral entry, blockade of this interaction should be beneficial to reduce the viral load.

3. Viral Replication

3.1. RNA Dependent RNA Polymerase

SARS-CoV-2 is an RNA virus, and its replication is dependent on RNA-dependent RNA polymerase (RdRp). Besides its 4 structural proteins, SARS-CoV-2 has 16 non-structural proteins (NSPs). NSP12 is one of the conserved NSPs among coronaviruses and is a vital enzyme that functions as an RdRp [46]. In addition, NSP8 functions as a primase that is associated with RNA synthesis. NSP8 interacts with NSP7 for their primer-dependent RdRp activity. The presence of NSP7 and NSP8 is necessary for the binding activity of NSP12 to the template-primer RNA [47]. In addition to its activities for viral replication, SARS-CoV-2 RdRp has also been shown to attenuates type I IFNs production [48].

RdRp is an attractive target to control SARS-CoV-2 infection. Inhibition of RdRp prevents the replication of the RNA of the virus without affecting the host cells. since host cells have no equivalent enzyme to RdRp. Effective inhibition of RdRp should lower the viral load without initiating target-related side effects. The RdRp is mainly composed of palm, thumb, and finger domains. Seven catalytic motifs (A, B, C, D, E, F, G) are located within the finger and palm domains [47]. In addition, NSP12 of SARS-CoV-2 has a newly identified β-hairpin domain at its N terminus. The N-terminal portion of NSP12 also contains a nidovirus-specific extension domain (NiRAN).

Targeting RdRp for inhibition, either using nucleoside analogue or nonnucleoside analogue is the choice for the development of RdRp inhibitors. Nucleoside analogues can compete with nucleosides that are required for RNA synthesis to inhibit RNA synthesis, resulting in termination of chain elongation and RNA replication. While non-nucleoside analogues can bind to allosteric sites on the surface of RdRp to inhibit the activity of RdRp and prevent the replication of RNA.

The catalytic site of RdRp is a conserved sequence among different organisms. In addition, there exist 96.35%, 98.8% and 97.5% similarities in NSP12, NSP7 and NSP8 between SARS-CoV and SARS-CoV-2, respectively [49]. As a result, inhibitors of the NSP12–NSP7–NSP8 complex for SARS-CoV may also inhibit the complex of SARS-CoV-2 [47]. For example, the NSP12–NSP7–NSP8 complex and RdRp activity inhibitor remdesivir has also been investigated in SARS-CoV-2 (47).

Remdesivir was originally developed for the treatment for of Ebola virus infection. Remdesivir is covalently incorporated into the primer strand of the virus at the first replicated base pair of RdRp to prevent the chain elongation [47]. It functions through its active form, metabolite remdesivir triphosphate to compete with the incorporation of nucleotide counterparts and inhibits transcription of viral RNA. Although remdesivir has not been shown to reduce the mortality in COVID-19 patients, it appears to accelerate the recovery when its administration is initiated early [50]. Remdesivir has been authorized in the USA for patients with severe COVID-19 since an NIH clinical trial demonstrated that it can accelerate the recovery and shorten the hospital stay of severe COVID-19 patients [50]. A recent report indicates that remdesivir given within 9 days from symptom-onset was associated with a decrease in mortality in moderate-to-severe COVID-19 patients [51].

However, an earlier clinical trial in China did not show significant benefit of remdesivir in severe COVID-19 patients [52]. The discrepancy of results between China and the USA has been ascribed to the difference in genetic backgrounds of patients with COVID-19 [53]. More effective inhibitors of SARS-CoV-2 RdRp are required for better management of COVID-19.

Through the above discussion, RdRp should be regarded as a promising target for the development of the treatment of COVID-19. Since the conserved nature of RdRp, RdRp inhibitors not only have great prospects in the fight against COVID-19 but also benefit other coronaviruses that have an equivalent enzyme as RdRp. In addition, the enzymatic function of RdRP also plays an important role in virus-associated tumor development, suggesting that RdRp inhibitors may hold therapeutic potential for cancers [54].

3.2. Viral Proteases

The 3-chymotrypsin-like protease (3CLPro) and a papain-like protease (PLPro) function to process NSPs, including RdRp, and therefore, inhibition of these proteases should interfere with the activity of RdRp. 3CLPro cleaves the polyprotein at 11 distinct sites to generate various NSPs that play an important role in viral replication [55]. The antiviral and cell protection efficacy of 3CLPro inhibition has been illustrated in simian Vero cells infected by SARS-CoV-2 [56].

Several candidates have been found to have inhibitory effects on 3CLPro. Anti-hepatitis C virus (HCV) drug ravidasvir has the ability to bind and inhibit the 3CLPro of SARS-CoV-2 [57]. Similarly, HCV protease inhibitors paritaprevir and simeprevir were also identified as potential inhibitors of SARS-CoV-2 3CLPro [58]. Using computational molecular modeling to screen FDA approved drugs and subsequent studying for their inhibitory effects on SARS-CoV-2 3CLpro enzyme in vitro, boceprevir, ombitasvir, paritaprevir, tipranavir, ivermectin, and micafungin were found to exhibit inhibitory effect towards 3CLpro enzymatic activity [59].

The efficacy of 3CLPro inhibitors in COVID-19 need to be proved. Lopinavir is a highly potent inhibitor of the human immunodeficiency virus (HIV) protease essential for intracellular HIV assembly. Concomitant oral administration of lopinavir and ritonavir, which blocks the metabolism of lopinavir, increases the anti-viral potency of lopinavir. However, COVID-19 patients receiving the combined treatment lopinavir and ritonavir yielded no significant benefit compared to patients treated with standard-care [60]. More specific inhibitors of proteases for SARS-CoV-2 should be evaluated in COVID-19, since a study indicated that lopinavir and ritonavir did not significantly inhibit 3ClPro in an in vitro enzymatic assay [61]. However, the aforementioned 3CLPro inhibitors may benefit COVID-19 patients, which needs to be proven in future clinical trials.

In regard to PLPro, activity profiling and crystal structures of inhibitor study indicated that there is a very high level of sequence and structural similarity between SARS-CoV and SARS-CoV-2 PLPro in the substrate binding pocket, suggesting that SARS-CoV PLPro inhibitors can possibly inhibit SARS-CoV-2 PLPro [62]. Non-covalent small molecules of SARS-CoV PLPro inhibitors has been shown to inhibit SARS-CoV-2 PLPro and display antiviral activity in a SARS-CoV-2 infection model [63]. Biochemical, structural, and functional characterization investigation revealed that SARS-CoV and SARS-CoV-2 PLPro share 83% sequence identity but exhibit different host substrate preferences. SARS-CoV-2 PLPro preferentially binds to the ubiquitin-like interferon-stimulated gene 15 protein (ISG15), cleaving ISG15 from interferon-responsive factor 3 (IRF3) and attenuating type I interferon responses. Whereas SARS-CoV PLPro predominantly targets ubiquitin chains. Inhibition of SARS-CoV-2 PLPro impairs the virus-induced cytopathogenic effect, maintains the antiviral interferon pathway, and reduces viral replication in infected cells [64].

3.3. N-protein

To prevent the replication of SARS-CoV-2 RNA, the N-protein of the virus may also be a potential target. N-proteins comprise three domains, including an N-terminal RNA-binding domain (NTD), a Ser/Arg-rich central linker region, and a C-terminal dimerization domain (CTD). The N-proteins play a major role in packing the viral RNA into viral ribonucleoproteins. It also helps with viral RNA transcription and replication [65].

The NTD functions for RNA-binding, and the CTD domain plays a role in oligomerization, while the central linker is necessary for primary phosphorylation [66]. However, targeting N-protein for vaccine should be careful, since the increased titer of IgG antibody against N-protein has been associated with poor prognosis, increasing ICU admission and longer hospital stay [67]. But the higher N-protein antibody titer may indicate the higher viral load and more severe disease, which causes the poor outcomes, not the N-protein antibody itself, leading to the poor prognosis. Further studies could be performed to clarify the underlying association.

Recently, the sequences that interact with B and T cells in the NTD domain of N-protein have been found by single immuno-informatics and structure-based drug discovery techniques, suggesting that N-protein might be associated with overactive immune responses, and the development of the NTD inhibitors may hold some promise [68]. The role of N-protein inhibition in the treatment of anti-SARS-CoV-2 should be further evaluated.

4. Cytokine Associated Signaling Pathways

After infection with SARS-CoV-2, the activation of cytokine storm as a result of a hyperactive immune response leads to severe inflammation that has been closely associated with the severity and mortality in COVID-19 patients. SARS-CoV-2 induced cytokine storm was characterized by overproduction of inflammatory cytokines and inhibition of type I interferons [69]. Inflammatory cytokines induce cell death in various cell types, followed by associated pathological damages in many systems, such as respiratory, cardiovascular, and neurological disorders in the case of COVID-19, severe pneumonia and complications in other systems.

To fight against cytokine storm, the cytokine antagonists, such as cytokine monoclonal antibodies or inhibitors, have been well documented [70,71]. This review will focus on the cell signaling pathways that are associated with cytokines. The signal proteins that are involved in the cascade of cytokine storm may potentially function as therapeutic targets for COVID-19. Since these signal proteins play different levels of roles, candidates that target different signals may result in different efficacy in the treatment of COVID-19.

4.1. Interleukin -6

Interleukin -6 (IL-6) plays multiple biological roles, which include enhancing the synthesis of inflammatory response proteins, such as C-reactive protein (CRP), regulating both T and B immune cells to increase immune responses, and promoting the production of vascular endothelium growth factor (VEGF), which aggravate the inflammatory damage by inducing angiogenesis and impairing the vascular integrity. In addition to its immune and inflammatory regulatory activities, IL-6 also upregulates the activation of the coagulation pathway, increasing the thrombotic events [72].

IL-6 has been closely associated with SARS-CoV-2 induced cytokine storm. IL-6 is one of the key cytokines that is frequently reported to be increased in COVID-19 patients with hypercytokinemia [73]. The level of IL-6 is valuable as a prognostic parameter for the disease severity in COVID-19 patients.

The proinflammatory function of IL-6 is mediated through a series of cell signaling pathways. IL-6 binds to its receptor (IL-6R) to initiate intracellular signaling.

Two forms of IL-6R have been found: membrane IL-6 receptor (mIL-6R) and soluble IL-6 receptor (sIL-6R). The binding of IL-6 to both receptors results in the dimerization and activation of the glycosylated type I membrane protein of 130–150 kDa (gp130). IL-6/mIL-6R medicated activation of gp130 induces IL-6 classic signaling pathway; while IL-6/sIL-6R induced activation of gp130 leads to activation of IL-6 trans-signaling pathway [74]. Classic IL-6 signaling is generally regarded as

an anti-inflammation and protective pathway, while IL-6 trans-signaling is mainly a pro-inflammatory pathway.

Blockade of IL-6 trans-signaling, not the full blockade of IL-6 signaling, prevents inflammation [75]. A selective sIL-6 pathway blocker is more effective for controlling inflammation in mouse models when compared to the nonspecific IL-6R blockers. Soluble gp130 (sgp130) selectively inhibited trans-signaling dose-dependently increased survival in a murine polymicrobial sepsis model [75].

The dimerization of signaling receptor gp130 mediates the activation of Janus kinases (JAKs) and subsequent activation of phosphatase Src homology domains containing tyrosine phosphatase-2 (SHP-2), the ras/raf/MAPK pathway, signal transducer and activator of transcription factor-3 STAT-3, and PI3K/Akt, which subsequently activate target genes (Figure 1A).

The binding of IL-6 to either sIL-6R or mIL-6R has been considered to be dependent on the concentration of the receptors. During the inflammatory process, mIL-6R may be greatly increased up to 100,000-fold, while sIL-6R only increases by 2-5-fold. However, the change in IL-6Rs during COVID-19 has not yet been investigated.

The efficacy of tocilizumab, a humanized monoclonal antibody, is designed to bind both mIL-6R and sIL-6R, in COVID-19, has been closely associated with the severity of cases and its efficacy is controversial in milder cases of COVID-19, suggesting that the regulation of IL-6R during COVID-19 deserved to be investigated and that the development of regulators of trans-IL-6 signaling pathway may hold better promise for the treatment of COVID-19 [76,77].

4.2. Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is another important cytokine that plays a critical role in SARS-CoV-2-induced cytokine storm. The function of TNF-α is mediated through binding to its two distinct membrane receptors on target cells: TNFR1 and TNFR2 (78). TNFR1 is ubiquitously expressed nearly all cells of the body, while TNFR2 expression is limited to certain lymphocyte populations including T-regulatory cells.

In the TNF-α induced cell signaling pathway, TNF-α binds to and activates TNFR1, promoting the interaction between the intracellular domains of TNFR1 with TNFR1-associated death domain protein (TRADD), which recruits receptor-interacting protein-1 (RIP-1) and TNF receptor-associated factor-2 (TRAF-2) to form a signal complex [79].

The signal complex can trigger the activation of the IκB kinase (IKK), which results in activation of NF-κB, and mitogen-activated protein kinase (MAPKs: JNK and p38), leading to the transcription of many different genes, including genes of inflammatory cytokines [80]. Binding of TNF-α to TNFR2 induces inflammation through the direct recruitment of TRAF2, which in turn recruits TRAF1, leading to activation of IKK and MAPKs (Figure 1B) [81].

Abnormal serum levels of soluble TNFR1 and TNFR2 (sTNFR1 and sTNFR2) have been observed in COVID-19 patients. An increased level of these two receptors in COVID-19 patients were identified. However, the levels were significantly lowered among patients who recovered from critical diseases, suggesting that sTNFR1 and sTNFR2 levels may be related to the severity of COVID-19 [82].

Another study also indicated that both serum TNF-α and sTNFRI levels were greatly increased in COVID-19 patients when compared with that in the healthy subjects [83]. Targeting TNF-α and its associated cell signaling pathways could be an important strategy for developing a therapy for COVID-19. In addition, since the expression of TNFR2 is limited and that of TNFR1 is ubiquitous, TNFR2 and its associated cell signaling are more attractive as targets for drug development.

4.3. Interleukin-1

Interleukin-1 (IL-1) is a family of cytokines that plays an important role in T-helper-1 (Th1) cell response and innate immunity. IL-1 has been associated with acute and chronic inflammation. In the inflammatory cascade, IL-1 is an upstream cytokine that promotes the activation of other cytokines, including IL-6 and TNF-α.

IL-1α and IL-1β are the most investigated IL-1 cytokines. IL-1α is released from dying epithelial and endothelial cells, while IL-1β comes from activated macrophages, monocytes, and neutrophils. IL-1 induces cell signaling pathways through binding its receptors (IL-1Rs). There are ten receptors for IL-1. IL-1α or IL-1β mainly binds to IL-1R1, resulting in the recruitment of a co-receptor named as IL-1R3 to IL-1R1. IL-1Rs and Toll-like receptors (TLRs) share the cytoplasmic Toll-IL-1-Receptor (TIR) domain.

The formation of a trimeric complex of IL-1/ILR1/IL-R3 promotes the approximation of the TIR domains, leading to the binding of myeloid differentiation primary-response 88 (MyD88) to the TIR domains and activation of NF-κB, followed by transcription of pro-inflammatory genes. Infection of SARS-CoV-2 activates IL-1, increasing the secretion of TNF-α, IL-6, and other cytokines, contributing to the induction of cytokine storm and inflammatory pulmonary and systemic injury [84]. In addition, IL-1 has also been shown to induce thromboxane-A2 in COVID-19 to enhance inflammation and facilitate the micro-thrombosis [84].

Inhibition of IL-1 can be achieved by direct inhibitors of IL-1 and antagonists of IL-1Rs, which have been investigated in cases of COVID-19 patients with efficacy [85-88].

4.4. Toll-Like Receptors

Toll-like receptors (TLRs) are the major pattern recognition receptors (PRRs) that identify pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs). The function of TLRs is closely related to the process of inflammatory cytokine release. Expressed on monocytes, macrophages, dendritic cells, neutrophils, B cells, T cells, fibroblasts, endothelial cells, epithelial cells, and even

on neurons, TLRs are type I transmembrane proteins that contain three structural domains: a leucine-rich repeats (LRRs) motif as an extracellular domain, a transmembrane domain, and a cytoplasmic Toll/IL-1R (TIR) domain. The LRRs motif is responsible for identifying PAMPs, while the TIR domain interacts with signal transduction adaptors to initiate downstream signaling pathways [89].

TLRs mediated cell signaling pathway plays a significant role in cytokine storm-associated diseases including severe COVID-19. Upon the stimulation by microbial-associated molecular patterns (MAMPs) of the pathogens or DAMPs of the host cells, TLRs are activated. Bacteria, fungi, protozoa, and viruses all express MAMPs. After activation, TLRs recruit cytoplasmic TIR domain-containing adaptor proteins, such as MyD88 and TIR-containing adapter-inducing interferon-β (TRIF), leading to the activation of NF-κB, MAPKs, or interferon-regulatory factor (IRF). Subsequently, the transcription of genes that are responsible for synthesis and the release of proinflammatory cytokines is activated, promoting the release of cytokines, such as TNF-α, IL-1β, IL-6, IL-18, chemokines, and interferons (Figure 1C) [90].

In SARS-CoV-2-induced cytokine storm, TLR4 seems to play a role [91]. Recombinant proteins of SARS-CoV-2 have been demonstrated to promote the expression of pro-inflammatory cytokines/chemokines and NF-κB signaling activation in human primary peripheral blood mononuclear cells and monocyte-derived macrophages [92]. Activation of TLR4 in lung macrophages resulted in a concentration- and time-dependent increase in the production of chemokines and cytokines [93]. In patients with COVID-19, the expression of TLR 4 and NF-κB target genes was upregulated [92]. In addition, TLR4 may bind to and interact with S-protein to promote the expression of ACE2, facilitating the viral entry into the host cells [94]. Antagonists of TLR4 may attenuate the cytokine storm and reduce the viral entry in severe COVID-19 patients [94].

4.5. Interleukin-1 Receptor–Associated Kinase 4

Interleukin-1 receptor–associated kinase 4 (IRAK4) plays a critical role in TLRs and IL-1R-mediated signaling pathways that regulate the induction of hyperinflammation. IRAK4 is one of the family members of mammalian IRAKs that include IRAK1-4. IRAKs share a similar structure containing a conserved death domain (DD) and a central kinase domain (KD). The N-terminal lobe of IRAK4 consists of a five-stranded antiparallel beta-sheet and one alpha helix, while the C-terminal lobe is composed mainly of a number of alpha helices.

IRAK4 has been demonstrated to promote the induction of inflammatory cytokines in human monocytes through activating transforming growth factor-β-activated kinase 1 (TAK1) and IKK, which leads to the nuclear translocation of interferon regulatory factor 5 (IRF5) [95]. Upon activation of TLR7/8, MyD88 gets recruited in, along with IRAK1/4 and TNF receptor-associated factor 6 (TRAF6), which leads to the autophosphorylation of IRAK4 and ubiquitination of IRF5 by TRAF6. IRAK4 then phosphorylates and activates TAK1, which then phosphorylates IKK. The ubiquitinated IRF5 is then phosphorylated by IKK (or other kinases) [96,97]. The activated IKK phosphorylates IRF5 at Ser462, resulting in its dimerization and nuclear translocation in myeloid cells to induce cytokine gene transcription [98]. In addition, IKK and also phosphorylate IκB to release NF-κB (Figure 1B); however, the kinase activity of IRAK4 is not sole factor for NF-κB activation [95]. As a consequence, blocking IRAK4 with a specific kinase inhibitor abolishes IRF5 activation but still permits NF-κB activation by other pathways [97]. Inhibition of IRAK4 or IRF5 both prevents proinflammatory responses [95]. IRF5 inhibition has been demonstrated to prevent viruses, such as influenza A, induced cytokine storm [99].

The IRAK4 could also have a major role in the induction of a hyperinflammatory state associated with severe COVID-19, and IRAK4 inhibitors might be considered as potential therapeutic candidates for the cytokine storm induced by SARS-CoV-2 [100]. Due to the role of IRAK4 in COVID-19, the efficacy of IRAK4 inhibitors in COVID-19 with ARDS is under investigation, which has been registered in ClinicalTrials.gov (Identifier: NCT04575610).

4.6. Angiotensin 2

The renin-angiotensin system (RAS) plays an important role in regulating vascular and kidney functions. In this system, angiotensin I (Ang I) is generated by cleavage of angiotensinogen by renin, and then ACE converts Ang I to angiotensin 2 (Ang II). ACE2 functions to convert Ang II to Ang 1-7. Ang II activates Ang II receptor type 1 (AT1R) and Ang II receptor type 2 (AT2R). Activation of AT1R induces detrimental effects, such as inflammation with release of cytokines, fibrosis, impaired redox balance, and vasoconstriction, while activation of AT2R leads to anti-inflammatory, anti-fibrotic, and vasodilation [101]. In addition, Ang II may regulate the TLR4-mediated pathway by upregulating the expression of TLR4 and the downstream pathways (Figure 2) [102].

Ang II-induced cell signaling pathway is a potential mechanism underlying the cytokine storm caused by SARS-CoV-2. The binding of the S-protein of SARS-CoV-2 to ACE2 on the cell surface caused downregulation of ACE2 expression. In the plasma of prolonged viral shedders of SARS-CoV-2, higher concentrations of Ang II and Ang I have been found when compared with normal subjects [103]. The reduced expression and activity of ACE2 resulted in an increase in Ang II, leading to elevated inflammatory responses (Figure 4). Consequently, the application of Ang II inhibitors might benefit COVID-19. It should be noted that Ang II receptor blocker (ARBs) may not be applicable for this purpose, since ABRs have been shown to upregulate the expression of ACE2 and increase the SARS-CoV-2 infection and replication in infected Vero E6 cells [104]. Although clinical trials demonstrated that discontinuation of ARBs in COVID-19 patients had no significant effect on the severity of COVID-19, stopping use of ARBs indeed accelerated the recovery [105]. The use of ARBs in hypertensive patients with SARS-CoV-2 infection should be further evaluated for the risk and benefit in COVID-19.

4.7. Janus-Associated Kinases

The Janus-associated kinase (JAK) family is one of ten recognized families of non-receptor tyrosine kinases. Mammalian members

of JAK family are Jak1, Jak2, Jak3 and Tyrosine kinase 2 (Tyk2). JAK-STAT-pathway mediates the downstream signals of multiple cytokines and inhibitors of JAK/STAT have been used in the treatment of inflammatory diseases [106]. JAK associates with cytokine receptors to mediate cytokine-induced signaling pathways. JAK1 and JAK2 regulate cell signaling pathways of cytokines, such as IL-2, IL-4, IL-7, IL-9, and IL-15, that share a common γ chain (γc) as receptor subunit [107]. JAK1 also regulates cell signaling downstream of IL-6, which activates its receptor to initiate the dimerization of receptor subunit gp130. JAK2 activation is essential for the signaling transduction of erythropoietin and other growth factors [108].

Since multiple cytokines are involved in SARS-CoV-2-induced cytokine storm, JAK inhibitors have been therapeutically used in COVID-19 patients [109]. Ruxolitinib is a potent and selective inhibitor of JAK1/2 with some levels of inhibitory effects on TYK2 and JAK3. COVID-19 patients who received ruxolitinib plus standard-of-care showed a faster clinical improvement on CT images with decreased levels of cytokines, including IL-6, nerve growth factor β, IL-12, migration inhibitory factor, MIP-1α, MIP-1β, and VEGF, although it was not associated with significantly accelerated clinical improvement in severe cases [110].

Treatment with ruxolitinib in COVID-19 patients with severe systemic hyperinflammation, who suffered from progression to ARDS and multiorgan failure, significantly reduced the inflammatory score with sustained clinical improvement [111]. For example, ruxolitinib, given to a 65-year-old Asian woman with COVID-19-induced ARDS, not only potently reduced ARDS-associated inflammatory blood cytokine levels such as IL-6 and the acute phase protein ferritin, but also associated with a rapid respiratory and cardiac improvement and clinical stabilization baricitinib is an oral JAK inhibitor [112]. Clinical studies have demonstrated the ability of baricitinib to reduce the viral titers and decrease IL-6 levels with the resolution of fever and cough symptoms in COVID-19 patients [113,114].

In COVID-19 pneumonia, baricitinib treatment reduced the serum levels of IL-6, IL-1β, and TNF-α, promoted the recovery of circulating T and B cell frequencies, increased antibody production against the SARS-CoV-2 S-protein, and reduced patients' need for oxygen therapy [115]. In moderate to severe SARS-CoV-2 pneumonia, baricitinib has an add-on effect when combined with corticosteroids in improving pulmonary function [116]. Baricitinib exerts both anti-inflammatory and anti-viral effects, and one additional advantage is its ability to cross the BBB into the CNS [113].

4.8. NOD-like Receptor Pyrin Domain Containing 3

As a well characterized member of the NOD-like receptor family of the innate immune system, NOD-like receptor pyrin domain containing 3 (NLRP3) has been implicated in chronic inflammation associated with obesity, diabetes, cancer, etc. NLRP3 contains a pyrin domain, a nucleotide-binding site (NBS) domain, and a leucine-rich repeat (LRR) motif. The NLRP3 inflammasome is a multi-protein complex that recruits pro-caspase-1 via the adaptor, apoptosis-associated speck-like protein containing caspase activation and recruitment domains (ASC) and then cleaves the cytokine precursor pro-IL-1β into mature IL-1β and then pro-IL-18 into IL-18 [117]. The following release of other inflammatory cytokines, such as IL-6, TNF-α, prostaglandins, and leukotrienes contributes to the induction of cytokine storm [118]. Inhibition of NLRP3 has been shown to attenuate the severity of inflammatory diseases [119].

SARS-CoV-2, similar to SARS-CoV, encodes viroporins that have been shown to activate NLRP3. Ang II, which can be reduced by ACE2, has been shown to induce the activation of NLRP3 in renal tubular epithelial cells [120]. The binding of S-protein to ACE2 to reduce the availability of ACE2 may increase Ang II, which is a possible mechanism that SARS-CoV-2 induces the activation of NLRP3. The different scenarios of NLRP3 activation in COVID-19 may reflect different levels of immune responses [121]. However, the role of NLRP3 in inflammatory response and the effectiveness of NLRP3 inhibitors in COVID-19 patients with hyperinflammation remains to be elucidated.

Colchicine, a currently used anti-gout drug, has an anti-inflammatory effect by inhibiting neutrophil chemotaxis and the inflammasome. The anti-inflammasome function of colchicine is mediated through its inhibiting activity of NLRP3 inflammasome [122].

Treatment with colchicine reduced cytokines and lowered the rate of clinical deterioration in COVID-19 patients [123]. In hospitalized COVID-19 patients with pneumonia, treatment with colchicine was associated with reduced mortality and accelerated recovery [124]. A recent randomized trial indicated that colchicine is effective as a proactive anti-inflammatory therapy in hospitalized COVID-19 patients and viral pneumonia [125]. The systematic review of a total of eight studies with 5778 COVID-19 patients demonstrated that the administration of colchicine was associated with improved outcomes of COVID-19 [126].

4.9. The Nuclear Factor Erythroid 2-Related Factor 2

The nuclear factor erythroid 2-related factor 2 (NRF2), a transcription factor, upregulates the genes that are associated with antioxidative stress and mitochondrial biogenesis. Cellular stress activates NRF2 leading to its translocation to the nucleus, where it binds to the antioxidant response element (ARE) to initiate the transcription of antioxidant genes to protect cells against inflammatory responses [127]. NFR2 also has a gene-repressing activity that inhibits the transcription of cytokine genes, resulting in a decrease in the expression of the inflammatory cytokines IL-1β, IL-6, and TNF-α in human macrophages [128]. In contrast, NRF2 knockout mice showed increased levels of proinflammatory cytokines in response to lipopolysaccharide stimulation [129]. In addition, NRF2 induces the expression of heme oxygenase-1 (HO-1) and increases the activity of HO-1 [130]. HO-1 functions to catalyze the degradation of heme into carbon monoxide (CO), free iron, and biliverdin, which is then converted to bilirubin by biliverdin reductase. Free heme is pro-inflammatory, while CO, bilirubin, and HO-1 itself have significant anti-inflammatory [131].

CO can inhibit the production of proinflammatory cytokines, such as TNF-α and IL-1β, through mediating the p38MPAK pathway [132]. Increased NRF2-dependent HO-1 expression has been associated with anti-inflammatory activity [133]. Moreover, NRF2 has been shown to induce the quinone oxidoreductase (NQO1) expression and thereby inhibit NLRP3 inflammasome activation [134]. NRF2 also inhibits NF-κB transcriptional activity, since NRF2 knockdown significantly increases NF-κB-dependent gene transcription [135]. The downstream target of NRF2, HO-1 can also inhibit NF-κB activity [136].

In response to oxidative stress, activated IκB kinase (IKK) promotes the phosphorylation and degradation of IκB. In normal conditions, NF-κB is trapped in the cytoplasm by IκB binding. The loss of IκB frees NF-κB, which is translocated to the nucleus to promote the gene transcription of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1 (Figure 5) [137].

As a result, the proposed use of NRF2 inducers to prevent the development of an excessive inflammatory response in COVID-19 patients is rational [138]. Recently, NRF2 agonist 4-octyl-itaconate and dimethyl fumarate have been demonstrated to attenuate inflammatory responses to SARS-CoV2 infection [139]. NRF2 agonists may hold potential value as candidates in the treatment of SARS-CoV-2 infection.

4.10. Sterol Regulatory Element Binding Protein 2

Sterol regulatory element binding protein 2 (SREBP2), one of the basic-helix-loop-helix-leucine zipper transcription factors, functions to regulate the gene expression involved in lipid cholesterol biosynthesis [140]. SPEBP2 is composed of three segments. The N-terminal segment with approximately 480 amino acids projecting into the cytosol consists of an acidic transcription-activating domain and a basic-helix-loop-helix–leucine zipper for binding DNA. The middle membrane binding segment of approximately 80 amino acids consists of two hydrophobic transmembrane helices separated by a short loop that projects into the lumen of the endoplasmic reticulum and nuclear envelope. The C-terminal segment of about 590 amino acids in length projects into the cytosol, which functions to regulate SREBP2 translocation and regulatory activity [141].

In normal conditions, the C-terminal domain of SREBP2 binds to the WD-repeat domain of SREBP cleavage-activation protein (SCAP). SCAP binds to insulin-induced gene proteins (INSIGs), which are the residents of the endoplasmic reticulum (ER), by its NH2-terminal domain to keep SREBP2 in the ER.

In response to low sterol levels, SCAP dissociates with INSIGs and relocates SREBP2 from the ER to the Golgi apparatus, where SREBP2 is cleaved by two membrane-bound proteases, site-1 protease (S1P) and site-2 protease (S2P), to release its N-terminal domain. The N-terminal domain of SREBP2 is then translocated to the nucleus to regulate the transcription of its target genes [142].

SREBP2 has been associated with inflammatory responses. Tumor necrosis factor can stimulate macrophages, followed by activation of SREBP2. SREBP2 binds to inflammatory and interferon response target genes and promotes inflammation [143]. Upon the stimulation with endogenous damage- and pathogen-associated molecules such as cholesterol crystals and LPS, SREBP2 activated NLRP3 inflammasome through NADPH oxidase, leading to functionally disturbed endothelium with increased inflammation [142].

Recently, SREBP2 have been associated with viral infection. In COVID-19 patients, the C-terminal fragment of SREBP2 was progressively upregulated with increasing severity of the disease. SREBP-2-induced inflammatory responses was also upregulated in severe COVID-19 cases [144]. Further study indicated that direct pharmacological inhibition of SREBP2 with fatostatin A decreased the inflammatory cytokines, such as IL-1β and TNF-α, production and attenuated pulmonary damages in mice infected with the virus [144].

4.11. The Viral Proteins

S-protein has been shown to activate the mitogen-activated protein kinase (MEK)/ extracellular signal-regulated kinase (ERK) pathway and increase the downstream chemokine expression through ACE2 [145].

E-protein PDZ-binding motif activates p38 MAPK which is involved in over expression of inflammatory cytokines [146]. 2-E proteins were found to form a type of pH-sensitive cation channels in bilayer lipid membranes. As observed in SARS-CoV-2-infected cells, heterologous expression of 2-E channels induced rapid cell death in various susceptible cell types and robust secretion of cytokines and chemokines in macrophages. Intravenous administration of purified 2-E protein into mice caused ARDS-like pathological damage in the lung and spleen. A dominant negative mutation lowering 2-E channel activity attenuated cell death and SARS-CoV-2 production. Newly identified channel inhibitors exhibited potent anti-SARS-CoV-2 activity and excellent cell protective activity in vitro, and these activities were positively correlated with inhibition of the 2-E channel. Importantly, prophylactic and therapeutic administration of the channel inhibitor effectively reduced both the viral load and secretion of inflammatory cytokines in the lungs of SARS-CoV-2-infected transgenic mice [147].

SARS-CoV-2 M-protein has been associated with inhibition of the production of type I and III interferons (IFNs), which are the first lines of innate immune response to infection [148,149].M-protein interacted with the central adaptor protein mitochondrial antiviral signaling (MAVS), leading to the impairment of MAVS aggregation and its recruitment of downstream TRAF3, TANK-binding kinase 1 (TBK1), and retinoic acid-inducible gene-I (RIG-I) [150].

Further study indicated that SARS-CoV-2 M-protein could interact with melanoma differentiation-associated gene 5 (MDA5), TRAF3, IKKε, and TANK-binding kinase 1 (TBK1), and induce TBK1 degradation via K48-linked ubiquitination. The reduced TBK1 further impaired the formation of TRAF3–TANK–TBK1-IKKε complex that activates the transcription factors IFN-regulatory factor 3 (IRF3) and NF-κB leading to inhibition of IFN-I production [151].

The expression of the membrane-anchored PLpro domain (PLpro-TM) from SARS-CoV inhibits STING/TBK1/IKKε-mediated activation of type I IFNs and disrupts the phosphorylation and dimerization of IRF3, which are activated by STING and TBK1. Meanwhile, we showed that PLpro-TM physically interacts with TRAF3, TBK1, IKKε, STING, and IRF3, the key components that assemble the STING-TRAF3-TBK1 complex for activation of IFN expression. Suggesting that PLpro negatively regulates IRF3 activation by interaction with the STING-TRAF3-TBK1 complex [152].

5. Conclusive Remarks

Multiple pathogenic processes are involved in COVID-19 after SARS-CoV-2 infection. The associated proteins or signals ruling in the viral entry into the host cells, viral replication in host cells, and induction of cytokine storm might function as therapeutic targets for COVID-19. As shown in Table 1, both viral and host cell proteins have been associated with viral invasion. These proteins may also contribute to the viral RNA replication via promoting cell entry and assisting the RdRp and proteases. Multiple cell-signaling proteins have been involved in the induction of proinflammatory cytokines and in the regulation of cytokines-induced cell injury. Further elucidation of mechanisms underlying the viral invasion, replication and cytokine storm may prompt to find novel therapeutic targets, which is a critical strategy for developing treatments of COVID-19. Establishing an effective platform to develop therapeutic drugs for COVID-19 is beneficial for coping with not only COVID-19, but also for future possible virus-associated pandemics.

References

1. Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., ... & Hu, B. (2020). Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA neurology, 77*(6), 683-690.
2. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., ... & Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet, 395*(10223), 497-506.
3. Helms, J., Kremer, S., Merdji, H., Clere-Jehl, R., Schenck, M., Kummerlen, C., ... & Meziani, F. (2020). Neurologic features in severe SARS-CoV-2 infection. *New England Journal of Medicine, 382*(23), 2268-2270.
4. Thepmankorn, P., Bach, J., Lasfar, A., Zhao, X., Souayah, S., Chong, Z. Z., & Souayah, N. (2021). Cytokine storm induced by SARS-CoV-2 infection: The spectrum of its neurological manifestations. *Cytokine, 138*, 155404.
5. Chen, Y., Guo, Y., Pan, Y., & Zhao, Z. J. (2020). Structure analysis of the receptor binding of 2019-nCoV. *Biochemical and biophysical research communications, 525*(1), 135-140.
6. Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., ... & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *nature, 581*(7807), 215-220.
7. Zhou, J. H., Wu, B., Wang, W. X., Lei, F., Cheng, X., Qin, J. J., ... & Li, H. L. (2020). No significant association between dipeptidyl peptidase-4 inhibitors and adverse outcomes of COVID-19. *World journal of clinical cases, 8*(22), 5576.
8. Wrapp, D., De Vlieger, D., Corbett, K. S., Torres, G. M., Wang, N., Van Breedam, W., ... & McLellan, J. S. (2020). Structural basis for potent neutralization of betacoronaviruses by single-domain camelid antibodies. *Cell, 181*(5), 1004-1015.
9. Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science, 367*(6485), 1444-1448.
10. Huang, A. T., Garcia-Carreras, B., Hitchings, M. D., Yang, B., Katzelnick, L. C., Rattigan, S. M., ... & Cummings, D. A. (2020). A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nature communications, 11*(1), 4704.
11. Logunov, D. Y., Dolzhikova, I. V., Shcheblyakov, D. V., Tukhvatulin, A. I., Zubkova, O. V., Dzharullaeva, A. S., ... & Gintsburg, A. L. (2021). Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *The Lancet, 397*(10275), 671-681.
12. Folegatti, P. M., Ewer, K. J., Aley, P. K., Angus, B., Becker, S., Belij-Rammerstorfer, S., ... & Hamlyn, J. (2020). Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *The Lancet, 396*(10249), 467-478.
13. Yang, J., Wang, W., Chen, Z., Lu, S., Yang, F., Bi, Z., ... & Wei, X. (2020). A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. *Nature, 586*(7830), 572-577.
14. Deshpande, A., Harris, B. D., Martinez-Sobrido, L., Kobie, J. J., & Walter, M. R. (2021). Epitope classification and RBD binding properties of neutralizing antibodies against SARS-CoV-2 variants of concern. *Frontiers in immunology, 12*, 691715.
15. Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., ... & Qi, J. (2020). Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell, 181*(4), 894-904.
16. Mossel, E. C., Wang, J., Jeffers, S., Edeen, K. E., Wang, S., Cosgrove, G. P., ... & Mason, R. J. (2008). SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. *Virology, 372*(1), 127-135.
17. Wong, S. K., Li, W., Moore, M. J., Choe, H., & Farzan, M. (2004). A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. *Journal of Biological Chemistry, 279*(5), 3197-3201.
18. Monteil, V., Dyczynski, M., Lauschke, V. M., Kwon, H., Wirnsberger, G., Youhanna, S., ... & Mirazimi, A. (2021). Human soluble ACE2 improves the effect of remdesivir in SARS-CoV-2 infection. *EMBO molecular medicine, 13*(1), e13426.
19. Takahashi, S., Yoshiya, T., Yoshizawa-Kumagaye, K., & Sugiyama, T. (2015). Nicotianamine is a novel angiotensin-converting enzyme 2 inhibitor in soybean. *Biomedical Research, 36*(3), 219-224.
20. Ye, M., Wysocki, J., Gonzalez-Pacheco, F. R., Salem, M., Evora, K., Garcia-Halpin, L., ... & Batlle, D. (2012). Murine recombinant angiotensin-converting enzyme 2: effect on angiotensin ii–dependent hypertension and distinctive

angiotensin-converting enzyme 2 inhibitor characteristics on rodent and human angiotensin-converting enzyme 2. *Hypertension, 60*(3), 730-740.
21. Tanaka, S., Nelson, G., Olson, C. A., Buzko, O., Higashide, W., Shin, A., ... & Soon-Shiong, P. (2021). An ACE2 Triple Decoy that neutralizes SARS-CoV-2 shows enhanced affinity for virus variants. *Scientific Reports, 11*(1), 12740.
22. Dong, W., Wang, J., Tian, L., Zhang, J., Settles, E. W., Qin, C., ... & Yu, J. (2023). Factor Xa cleaves SARS-CoV-2 spike protein to block viral entry and infection. *Nature communications, 14*(1), 1936.
23. Ziegler, C. G., Allon, S. J., Nyquist, S. K., Mbano, I. M., Miao, V. N., Tzouanas, C. N., ... & Zhang, K. (2020). SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. *Cell, 181*(5), 1016-1035.
24. Wang, Y., Fan, Y., Huang, Y., Du, T., Liu, Z., Huang, D., ... & Zhang, P. (2021). TRIM28 regulates SARS-CoV-2 cell entry by targeting ACE2. *Cellular Signalling, 85*, 110064.
25. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., ... & Pöhlmann, S. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *cell, 181*(2), 271-280.
26. Papa, G., Mallery, D. L., Albecka, A., Welch, L. G., Cattin-Ortolá, J., Luptak, J., ... & James, L. C. (2021). Furin cleavage of SARS-CoV-2 Spike promotes but is not essential for infection and cell-cell fusion. *PLoS pathogens, 17*(1), e1009246.
27. Peacock, T. P., Goldhill, D. H., Zhou, J., Baillon, L., Frise, R., Swann, O. C., ... & Barclay, W. S. (2021). The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nature microbiology, 6*(7), 899-909.
28. Dong, M., Zhang, J., Ma, X., Tan, J., Chen, L., Liu, S., ... & Zhuang, L. (2020). ACE2, TMPRSS2 distribution and extrapulmonary organ injury in patients with COVID-19. *Biomedicine & Pharmacotherapy, 131*, 110678.
29. Thunders, M., & Delahunt, B. (2020). Gene of the month: TMPRSS2 (transmembrane serine protease 2). *Journal of clinical pathology, 73*(12), 773-776.
30. Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., & Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell, 181*(2), 281-292.
31. Bestle, D., Heindl, M. R., Limburg, H., Pilgram, O., Moulton, H., Stein, D. A., ... & Böttcher-Friebertshäuser, E. (2020). TMPRSS2 and furin are both essential for proteolytic activation of SARS-CoV-2 in human airway cells. *Life science alliance, 3*(9).
32. Yamamoto, M., Matsuyama, S., Li, X., Takeda, M., Kawaguchi, Y., Inoue, J. I., & Matsuda, Z. (2016). Identification of nafamostat as a potent inhibitor of Middle East respiratory syndrome coronavirus S protein-mediated membrane fusion using the split-protein-based cell-cell fusion assay. *Antimicrobial agents and chemotherapy, 60*(11), 6532-6539.
33. Wang, K. E., Chen, W., Zhang, Z., Deng, Y., Lian, J. Q., Du, P., ... & Chen, Z. N. (2020). CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal transduction and targeted therapy, 5*(1), 283.
34. Wang, S., Qiu, Z., Hou, Y., Deng, X., Xu, W., Zheng, T., ... & Li, X. (2021). AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. *Cell research, 31*(2), 126-140.
35. Cantuti-Castelvetri, L., Ojha, R., Pedro, L. D., Djannatian, M., Franz, J., Kuivanen, S., ... & Simons, M. (2020). Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science, 370*(6518), 856-860.
36. Chekol Abebe, E., Mengie Ayele, T., Tilahun Muche, Z., & Asmamaw Dejenie, T. (2021). Neuropilin 1: a novel entry factor for SARS-CoV-2 infection and a potential therapeutic target. *Biologics: Targets and Therapy*, 143-152.
37. Roy, S., Bag, A. K., Singh, R. K., Talmadge, J. E., Batra, S. K., & Datta, K. (2017). Multifaceted role of neuropilins in the immune system: potential targets for immunotherapy. *Frontiers in immunology, 8*, 1228.
38. Xia, S., Lan, Q., Su, S., Wang, X., Xu, W., Liu, Z., ... & Jiang, S. (2020). The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin. *Signal transduction and targeted therapy, 5*(1), 92.
39. Li, Z. L., & Buck, M. (2021). Neuropilin-1 assists SARS-CoV-2 infection by stimulating the separation of Spike protein S1 and S2. *Biophysical Journal, 120*(14), 2828-2837.
40. Daly, J. L., Simonetti, B., Klein, K., Chen, K. E., Williamson, M. K., Antón-Plágaro, C., ... & Yamauchi, Y. (2020). Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science, 370*(6518), 861-865.
41. Ibrahim, I. M., Abdelmalek, D. H., & Elfiky, A. A. (2019). GRP78: A cell's response to stress. *Life sciences, 226*, 156-163.
42. Mediate, C. C. I. M. W. (2002). GRP78, a Coreceptor for Coxsackievirus A9. J. *Virol, 76*(2), 633.
43. Ibrahim, I. M., Abdelmalek, D. H., Elshahat, M. E., & Elfiky, A. A. (2020). COVID-19 spike-host cell receptor GRP78 binding site prediction. *Journal of infection, 80*(5), 554-562.
44. Shen, W. J., Asthana, S., Kraemer, F. B., & Azhar, S. (2018). Scavenger receptor B type 1: expression, molecular regulation, and cholesterol transport function. *Journal of lipid research, 59*(7), 1114.
45. Wei, C., Wan, L., Yan, Q., Wang, X., Zhang, J., Yang, X., ... & Zhong, H. (2020). HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. *Nature metabolism, 2*(12), 1391-1400.
46. Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., ... & Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B, 10*(5), 766-788.
47. Yin, W., Mao, C., Luan, X., Shen, D. D., Shen, Q., Su, H., ... & Xu, H. E. (2020). Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science, 368*(6498), 1499-1504.
48. Wang, W., Zhou, Z., Xiao, X., Tian, Z., Dong, X., Wang, C., ... & Wang, J. (2021). SARS-CoV-2 nsp12 attenuates type I interferon production by inhibiting IRF3 nuclear translocation. *Cellular & molecular immunology, 18*(4), 945-953.
49. Ruan, Q., Yang, K., Wang, W., Jiang, L., & Song, J. (2020).

Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive care medicine, 46*(5), 846-848.
50. Beigel, J. H., Tomashek, K. M., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., ... & Lane, H. C. (2020). Remdesivir for the treatment of Covid-19—preliminary report. *New England Journal of Medicine, 383*(19), 1813-1836.
51. Mehta, R. M., Bansal, S., Bysani, S., & Kalpakam, H. (2021). A shorter symptom onset to remdesivir treatment (SORT) interval is associated with a lower mortality in moderate-to-severe COVID-19: a real-world analysis. *International Journal of Infectious Diseases, 106,* 71-77.
52. Wang, Y., Zhang, D., Du, G., Du, R., Zhao, J., Jin, Y., ... & Wang, C. (2020). Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The lancet, 395*(10236), 1569-1578.
53. Wang, L. Y., Cui, J. J., Ouyang, Q. Y., Zhan, Y., Guo, C. X., & Yin, J. Y. (2020). Remdesivir and COVID-19. *The Lancet, 396*(10256), 953-954.
54. Machitani, M., Yasukawa, M., Nakashima, J., Furuichi, Y., & Masutomi, K. (2020). RNA-dependent RNA polymerase, RdRP, a promising therapeutic target for cancer and potentially COVID-19. *Cancer science, 111*(11), 3976-3984.
55. Rathnayake, A. D., Zheng, J., Kim, Y., Perera, K. D., Mackin, S., Meyerholz, D. K., ... & Chang, K. O. (2020). 3C-like protease inhibitors block coronavirus replication in vitro and improve survival in MERS-CoV–infected mice. *Science translational medicine, 12*(557), eabc5332.
56. Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., ... & Yang, H. (2020). Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature, 582*(7811), 289-293.
57. Bera, K. (2022). Binding and inhibitory effect of ravidasvir on 3CLpro of SARS-CoV-2: A molecular docking, molecular dynamics and MM/PBSA approach. *Journal of Biomolecular Structure and Dynamics, 40*(16), 7303-7310.
58. Alamri, M. A., Tahir ul Qamar, M., Mirza, M. U., Bhadane, R., Alqahtani, S. M., Muneer, I., ... & Salo-Ahen, O. M. (2021). Pharmacoinformatics and molecular dynamics simulation studies reveal potential covalent and FDA-approved inhibitors of SARS-CoV-2 main protease 3CLpro. *Journal of Biomolecular Structure and Dynamics, 39*(13), 4936-4948.
59. Mody, V., Ho, J., Wills, S., Mawri, A., Lawson, L., Ebert, M. C., ... & Taval, S. (2021). Identification of 3-chymotrypsin like protease (3CLPro) inhibitors as potential anti-SARS-CoV-2 agents. *Communications biology, 4*(1), 93.
60. Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G., ... & Wang, C. (2020). A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. *New England journal of medicine, 382*(19), 1787-1799.
61. Mahdi, M., Mótyán, J. A., Szojka, Z. I., Golda, M., Miczi, M., & Tőzsér, J. (2020). Analysis of the efficacy of HIV protease inhibitors against SARS-CoV-2′ s main protease. *Virology journal, 17*, 1-8.
62. Rut, W., Lv, Z., Zmudzinski, M., Patchett, S., Nayak, D., Snipas, S. J., ... & Olsen, S. K. (2020). Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti–COVID-19 drug design. *Science advances, 6*(42), eabd4596.
63. Klemm, T., Ebert, G., Calleja, D. J., Allison, C. C., Richardson, L. W., Bernardini, J. P., ... & Komander, D. (2020). Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. *The EMBO journal, 39*(18), e106275.
64. Shin, D., Mukherjee, R., Grewe, D., Bojkova, D., Baek, K., Bhattacharya, A., ... & Dikic, I. (2020). Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. *Nature, 587*(7835), 657-662.
65. Cong, Y., Ulasli, M., Schepers, H., Mauthe, M., V'kovski, P., Kriegenburg, F., ... & Reggiori, F. (2020). Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle. *Journal of virology, 94*(4), 10-1128.
66. Kang, S., Yang, M., Hong, Z., Zhang, L., Huang, Z., Chen, X., ... & Chen, S. (2020). Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. *Acta Pharmaceutica Sinica B, 10*(7), 1228-1238.
67. Batra, M., Tian, R., Zhang, C., Clarence, E., Sacher, C. S., Miranda, J. N., ... & Mirsaeidi, M. (2021). Role of IgG against N-protein of SARS-CoV2 in COVID19 clinical outcomes. *Scientific reports, 11*(1), 3455.
68. Kwarteng, A., Asiedu, E., Sylverken, A. A., Larbi, A., Sakyi, S. A., & Asiedu, S. O. (2021). Molecular characterization of interactions between the D614G variant of SARS-CoV-2 S-protein and neutralizing antibodies: a computational approach. *Infection, Genetics and Evolution, 91,* 104815.
69. Chong, Z. Z., & Souayah, N. (2022). SARS-CoV-2 induced neurological manifestations entangles cytokine storm that implicates for therapeutic strategies. *Current Medicinal Chemistry, 29*(12), 2051-2074.
70. Jamilloux, Y., Henry, T., Belot, A., Viel, S., Fauter, M., El Jammal, T., ... & Sève, P. (2020). Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions. *Autoimmunity reviews, 19*(7), 102567.
71. Roshanravan, N., Seif, F., Ostadrahimi, A., Pouraghaei, M., & Ghaffari, S. (2020). Targeting cytokine storm to manage patients with COVID-19: a mini-review. *Archives of medical research, 51*(7), 608-612.
72. D'Alessandro, A., Thomas, T., Dzieciatkowska, M., Hill, R. C., Francis, R. O., Hudson, K. E., ... & Hansen, K. C. (2020). Serum proteomics in COVID-19 patients: altered coagulation and complement status as a function of IL-6 level. *Journal of proteome research, 19*(11), 4417-4427.
73. Kaman, K., Azmy, V., Chichra, A., Britto-Leon, C., & Price, C. (2021). Cytokine profiles in severe SARS-CoV-2 infection requiring extracorporeal membrane oxygenation support. *Respiratory Medicine Case Reports, 33*, 101376.
74. Ebihara, N., Matsuda, A., Nakamura, S., Matsuda, H., & Murakami, A. (2011). Role of the IL-6 classic-and trans-signaling pathways in corneal sterile inflammation and wound healing. *Investigative ophthalmology & visual science, 52*(12), 8549-8557.
75. Barkhausen, T., Tschernig, T., Rosenstiel, P., van Griensven, M., Vonberg, R. P., Dorsch, M., ... & Waetzig, G. H. (2011). Selective blockade of interleukin-6 trans-signaling improves survival in a murine polymicrobial sepsis model. *Critical*

care medicine, 39(6), 1407-1413.
76. Nasa, P., Singh, A., Upadhyay, S., Bagadia, S., Polumuru, S., Shrivastava, P. K., ... & Patidar, S. (2020). Tocilizumab use in COVID-19 Cytokine-release syndrome: retrospective study of two centers. *Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine, 24*(9), 771.
77. Chong, Z. Z., & Souayah, N. (2022). SARS-CoV-2 induced neurological manifestations entangles cytokine storm that implicates for therapeutic strategies. *Current Medicinal Chemistry, 29*(12), 2051-2074.
78. Horiuchi, T., Mitoma, H., Harashima, S. I., Tsukamoto, H., & Shimoda, T. (2010). Transmembrane TNF-α: structure, function and interaction with anti-TNF agents. *Rheumatology, 49*(7), 1215-1228.
79. Bradley, J. (2008). TNF-mediated inflammatory disease. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 214*(2), 149-160.
80. Yang, S., Wang, J., Brand, D. D., & Zheng, S. G. (2018). Role of TNF–TNF receptor 2 signal in regulatory T cells and its therapeutic implications. *Frontiers in immunology, 9,* 784.
81. López-Urrutia, E., Campos-Parra, A., Herrera, L. A., & Pérez-Plasencia, C. (2017). Alternative splicing regulation in tumor necrosis factor-mediated inflammation. *Oncology letters, 14*(5), 5114-5120.
82. Bowman, E. R., Cameron, C. M. A., Avery, A., Gabriel, J., Kettelhut, A., Hecker, M., ... & Cameron, M. J. (2021). Levels of soluble CD14 and tumor necrosis factor receptors 1 and 2 may be predictive of death in severe coronavirus disease 2019. *The Journal of Infectious Diseases, 223*(5), 805-810.
83. Mortaz, E., Tabarsi, P., Jamaati, H., Dalil Roofchayee, N., Dezfuli, N. K., Hashemian, S. M., ... & Adcock, I. M. (2021). Increased serum levels of soluble TNF-α receptor is associated with ICU mortality in COVID-19 patients. *Frontiers in Immunology, 12,* 592727.
84. Conti, P., Caraffa, A., Gallenga, C. E., Ross, R., Kritas, S. K., Frydas, I., ... & Ronconi, G. (2020). Coronavirus-19 (SARS-CoV-2) induces acute severe lung inflammation via IL-1 causing cytokine storm in COVID-19: a promising inhibitory strategy. *J Biol Regul Homeost Agents, 34*(6), 1971-5.
85. Huet, T., Beaussier, H., Voisin, O., Jouveshomme, S., Dauriat, G., Lazareth, I., ... & Hayem, G. (2020). Anakinra for severe forms of COVID-19: a cohort study. *The Lancet Rheumatology, 2*(7), e393-e400.
86. Cauchois, R., Koubi, M., Delarbre, D., Manet, C., Carvelli, J., Blasco, V. B., ... & Kaplanski, G. (2020). Early IL-1 receptor blockade in severe inflammatory respiratory failure complicating COVID-19. *Proceedings of the National Academy of Sciences, 117*(32), 18951-18953.
87. Franzetti, M., Forastieri, A., Borsa, N., Pandolfo, A., Molteni, C., Borghesi, L., ... & Piconi, S. (2021). IL-1 receptor antagonist anakinra in the treatment of COVID-19 acute respiratory distress syndrome: a retrospective, observational study. *The Journal of Immunology, 206*(7), 1569-1575.
88. Cavalli, G., & Dagna, L. (2021). The right place for IL-1 inhibition in COVID-19. *The Lancet Respiratory Medicine, 9*(3), 223-224.
89. Kawasaki, T., & Kawai, T. (2014). Toll-like receptor signaling pathways. *Frontiers in immunology, 5*, 461.
90. Costa, A. G., Ramasawmy, R., Val, F. F. A., Ibiapina, H. N. S., Oliveira, A. C., Tarragô, A. M., ... & Lacerda, M. V. G. (2018). Polymorphisms in TLRs influence circulating cytokines production in Plasmodium vivax malaria: TLR polymorphisms influence cytokine productions in malaria-vivax. *Cytokine, 110*, 374-380.
91. Aboudounya, M. M., & Heads, R. J. (2021). COVID-19 and toll-like receptor 4 (TLR4): SARS-CoV-2 may bind and activate TLR4 to increase ACE2 expression, facilitating entry and causing hyperinflammation. *Mediators of inflammation, 2021*(1), 8874339.
92. Sohn, K. M., Lee, S. G., Kim, H. J., Cheon, S., Jeong, H., Lee, J., ... & Jo, E. K. (2020). COVID-19 patients upregulate toll-like receptor 4-mediated inflammatory signaling that mimics bacterial sepsis. *Journal of Korean medical science, 35*(38).
93. Grassin-Delyle, S., Abrial, C., Salvator, H., Brollo, M., Naline, E., & Devillier, P. (2020). The role of toll-like receptors in the production of cytokines by human lung macrophages. *Journal of innate immunity, 12*(1), 63-73.
94. Choudhury, A., & Mukherjee, S. (2020). In silico studies on the comparative characterization of the interactions of SARS-CoV-2 spike glycoprotein with ACE-2 receptor homologs and human TLRs. *Journal of medical virology, 92*(10), 2105-2113.
95. Cushing, L., Winkler, A., Jelinsky, S. A., Lee, K., Korver, W., Hawtin, R., ... & Lin, L. L. (2017). IRAK4 kinase activity controls Toll-like receptor–induced inflammation through the transcription factor IRF5 in primary human monocytes. *Journal of Biological Chemistry, 292*(45), 18689-18698.
96. Ren, J., Chen, X., & Chen, Z. J. (2014). IKKβ is an IRF5 kinase that instigates inflammation. *Proceedings of the National Academy of Sciences, 111*(49), 17438-17443.
97. Scarneo, S. A., Hughes, P. F., Yang, K. W., Carlson, D. A., Gurbani, D., Westover, K. D., & Haystead, T. A. (2020). A highly selective inhibitor of interleukin-1 receptor–associated kinases 1/4 (IRAK-1/4) delineates the distinct signaling roles of IRAK-1/4 and the TAK1 kinase. *Journal of Biological Chemistry, 295*(6), 1565-1574.
98. Lopez-Pelaez, M., Lamont, D. J., Peggie, M., Shpiro, N., Gray, N. S., & Cohen, P. (2014). Protein kinase IKKβ-catalyzed phosphorylation of IRF5 at Ser462 induces its dimerization and nuclear translocation in myeloid cells. *Proceedings of the National Academy of Sciences, 111*(49), 17432-17437.
99. Wang, Q., Fang, P., He, R., Li, M., Yu, H., Zhou, L., ... & Liu, S. (2020). O-GlcNAc transferase promotes influenza A virus–induced cytokine storm by targeting interferon regulatory factor–5. *Science Advances, 6*(16), eaaz7086.
100. Stoy, N. (2021). Involvement of interleukin-1 receptor-associated Kinase 4 and interferon regulatory Factor 5 in the immunopathogenesis of SARS-CoV-2 infection: Implications for the treatment of COVID-19. *Frontiers in Immunology, 12,* 638446.
101. D'Ardes, D., Boccatonda, A., Rossi, I., Guagnano, M. T., Santilli, F., Cipollone, F., & Bucci, M. (2020). COVID-19 and RAS: unravelling an unclear relationship. *International*

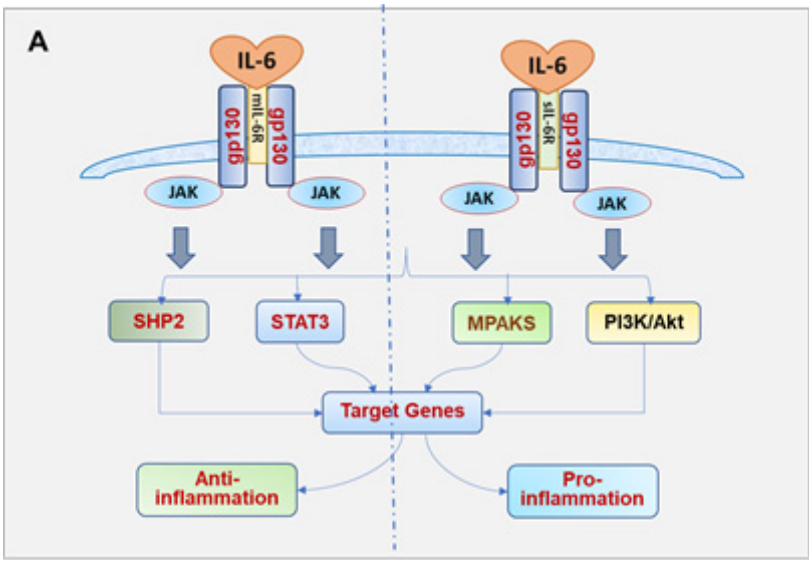
Journal of Molecular Sciences, 21(8), 3003.
102. Wu, J., Yang, X., Zhang, Y. F., Zhou, S. F., Zhang, R., Dong, X. Q., ... & Yu, X. Q. (2009). Angiotensin II upregulates Toll-like receptor 4 and enhances lipopolysaccharide-induced CD40 expression in rat peritoneal mesothelial cells. *Inflammation Research, 58,* 473-482.
103. Osman, I. O., Melenotte, C., Brouqui, P., Million, M., Lagier, J. C., Parola, P., ... & Devaux, C. A. (2021). Expression of ACE2, soluble ACE2, angiotensin I, angiotensin II and angiotensin-(1-7) is modulated in COVID-19 patients. *Frontiers in Immunology, 12*, 625732.
104. Pires de Souza, G. A., Osman, I. O., Le Bideau, M., Baudoin, J. P., Jaafar, R., Devaux, C., & La Scola, B. (2021). Angiotensin II receptor blockers (ARBs antihypertensive Agents) increase replication of SARS-CoV-2 in vero E6 cells. *Frontiers in cellular and infection microbiology, 11*, 639177.
105. Bauer, A., Schreinlechner, M., Sappler, N., Dolejsi, T., Tilg, H., Aulinger, B. A., ... & Zabernigg, A. (2021). Discontinuation versus continuation of renin-angiotensin-system inhibitors in COVID-19 (ACEI-COVID): a prospective, parallel group, randomised, controlled, open-label trial. *The Lancet Respiratory Medicine, 9*(8), 863-872.
106. Jamilloux, Y., El Jammal, T., Vuitton, L., Gerfaud-Valentin, M., Kerever, S., & Sève, P. (2019). JAK inhibitors for the treatment of autoimmune and inflammatory diseases. *Autoimmunity reviews, 18*(11), 102390.
107. Pesu, M., Laurence, A., Kishore, N., Zwillich, S. H., Chan, G., & O'Shea, J. J. (2008). Therapeutic targeting of Janus kinases. *Immunological reviews, 223*(1), 132-142.
108. Maiese, K., Chong, Z. Z., Shang, Y. C., & Wang, S. (2012). Erythropoietin: new directions for the nervous system. *International journal of molecular sciences, 13*(9), 11102-11129.
109. Seif, F., Aazami, H., Khoshmirsafa, M., Kamali, M., Mohsenzadegan, M., Pornour, M., & Mansouri, D. (2020). JAK inhibition as a new treatment strategy for patients with COVID-19. *International archives of allergy and immunology, 181*(6), 467-475.
110. Cao, Y., Wei, J., Zou, L., Jiang, T., Wang, G., Chen, L., ... & Zhou, J. (2020). Ruxolitinib in treatment of severe coronavirus disease 2019 (COVID-19): A multicenter, single-blind, randomized controlled trial. *Journal of Allergy and Clinical Immunology, 146*(1), 137-146.
111. La Rosée, F., & La Rosée, P. (2020). Ruxolitinib in COVID-19 hyperinflammation and haematologic malignancies. *Acta Haematologica, 1*.
112. Neubauer, A., Wiesmann, T., Vogelmeier, C. F., Mack, E., Skevaki, C., Gaik, C., ... & Burchert, A. (2020). Ruxolitinib for the treatment of SARS-CoV-2 induced acute respiratory distress syndrome (ARDS). *Leukemia, 34*(8), 2276-2278.
113. Richardson, P. J., Ottaviani, S., Prelle, A., Stebbing, J., Casalini, G., & Corbellino, M. (2020). CNS penetration of potential anti-COVID-19 drugs. *Journal of Neurology, 267*(7), 1880-1882.
114. Stebbing, J., Sánchez Nievas, G., Falcone, M., Youhanna, S., Richardson, P., Ottaviani, S., ... & Lauschke, V. M. (2021). JAK inhibition reduces SARS-CoV-2 liver infectivity and modulates inflammatory responses to reduce morbidity and mortality. *Science advances, 7*(1), eabe4724.
115. Bronte, V., Ugel, S., Tinazzi, E., Vella, A., De Sanctis, F., Canè, S., ... & Olivieri, O. (2020). Baricitinib restrains the immune dysregulation in patients with severe COVID-19. *The Journal of clinical investigation, 130*(12), 6409-6416.
116. Rodriguez-Garcia, J. L., Sanchez-Nievas, G., Arevalo-Serrano, J., Garcia-Gomez, C., Jimenez-Vizuete, J. M., & Martinez-Alfaro, E. (2021). Baricitinib improves respiratory function in patients treated with corticosteroids for SARS-CoV-2 pneumonia: an observational cohort study. *Rheumatology, 60*(1), 399-407.
117. He, Y., Hara, H., & Núñez, G. (2016). Mechanism and regulation of NLRP3 inflammasome activation. *Trends in biochemical sciences, 41*(12), 1012-1021.
118. Abderrazak, A., Syrovets, T., Couchie, D., El Hadri, K., Friguet, B., Simmet, T., & Rouis, M. (2015). NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. *Redox biology, 4,* 296-307.
119. Tomani, J. C. D., Kagisha, V., Tchinda, A. T., Jansen, O., Ledoux, A., Vanhamme, L., ... & Souopgui, J. (2020). The inhibition of NLRP3 inflammasome and IL-6 production by hibiscus noldeae baker f. Derived constituents provides a link to its anti-Inflammatory therapeutic potentials. *Molecules, 25*(20), 4693.
120. Wen, Y., Liu, Y., Tang, T., Lv, L., Liu, H., Ma, K., & Liu, B. (2016). NLRP3 inflammasome activation is involved in Ang II-induced kidney damage via mitochondrial dysfunction. *Oncotarget, 7*(34), 54290.
121. Van den Berg, D. F., & Te Velde, A. A. (2020). Severe COVID-19: NLRP3 inflammasome dysregulated. *Frontiers in immunology, 11*, 1580.
122. Liang, Y., Zhou, H. F., Tong, M., Chen, L., Ren, K., & Zhao, G. J. (2019). Colchicine inhibits endothelial inflammation via NLRP3/CRP pathway. *International journal of cardiology, 294*, 55.
123. Gandolfini, I., Delsante, M., Fiaccadori, E., Zaza, G., Manenti, L., Degli Antoni, A., ... & Maggiore, U. (2022). COVID-19 in kidney transplant recipients. *American Journal of Transplantation, 20*(7), 1941.
124. Manenti, L., Maggiore, U., Fiaccadori, E., Meschi, T., Antoni, A. D., Nouvenne, A., ... & Peruzzi, L. (2021). Reduced mortality in COVID-19 patients treated with colchicine: Results from a retrospective, observational study. *PLoS One, 16*(3), e0248276.
125. Mareev, V., Orlova, Y., Plisyk, A., Pavlikova, E., Akopyan, Z., Matskeplishvili, S., ... & Kamalov, A. (2021). Proactive anti-inflammatory therapy with colchicine in the treatment of advanced stages of new coronavirus infection. The first results of the COLORIT study.
126. Hariyanto, T. I., Halim, D. A., Jodhinata, C., Yanto, T. A., & Kurniawan, A. (2021). Colchicine treatment can improve outcomes of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. *Clinical and Experimental Pharmacology and Physiology, 48*(6), 823-830.
127. Ahmed, S. M. U., Luo, L., Namani, A., Wang, X. J., & Tang, X. (2017). Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochimica et Biophysica Acta (BBA)-Molecular basis of disease, 1863*(2), 585-597.
128. Kobayashi, E. H., Suzuki, T., Funayama, R., Nagashima,

T., Hayashi, M., Sekine, H., ... & Yamamoto, M. (2016). Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. *Nature communications, 7*(1), 11624.

129. Thimmulappa, R. K., Lee, H., Rangasamy, T., Reddy, S. P., Yamamoto, M., Kensler, T. W., & Biswal, S. (2016). Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. *The Journal of clinical investigation, 116*(4), 984-995.
130. Reichard, J. F., Motz, G. T., & Puga, A. (2007). Heme oxygenase-1 induction by NRF2 requires inactivation of the transcriptional repressor BACH1. *Nucleic acids research, 35*(21), 7074-7086.
131. Vijayan, V., Wagener, F. A., & Immenschuh, S. (2018). The macrophage heme-heme oxygenase-1 system and its role in inflammation. *Biochemical pharmacology, 153*, 159-167.
132. Otterbein, L. E., Bach, F. H., Alam, J., Soares, M., Tao Lu, H., Wysk, M., ... & Choi, A. M. (2000). Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nature medicine, 6*(4), 422-428.
133. Kuhn, A. M., Tzieply, N., Schmidt, M. V., Von Knethen, A., Namgaladze, D., Yamamoto, M., & Brüne, B. (2011). Antioxidant signaling via Nrf2 counteracts lipopolysaccharide-mediated inflammatory responses in foam cell macrophages. *Free Radical Biology and Medicine, 50*(10), 1382-1391.
134. Liu, X., Zhang, X., Ding, Y., Zhou, W., Tao, L., Lu, P., ... & Hu, R. (2017). Nuclear factor E2-related factor-2 negatively regulates NLRP3 inflammasome activity by inhibiting reactive oxygen species-induced NLRP3 priming. *Antioxidants & redox signaling, 26*(1), 28-43.
135. Thimmulappa, R. K., Scollick, C., Traore, K., Yates, M., Trush, M. A., Liby, K. T., ... & Biswal, S. (2006). Nrf2-dependent protection from LPS induced inflammatory response and mortality by CDDO-Imidazolide. *Biochemical and biophysical research communications, 351*(4), 883-889.
136. Bellezza, I., Tucci, A., Galli, F., Grottelli, S., Mierla, A. L., Pilolli, F., & Minelli, A. (2012). Inhibition of NF-κB nuclear translocation via HO-1 activation underlies α-tocopheryl succinate toxicity. *The Journal of nutritional biochemistry, 23*(12), 1583-1591.
137. Lawrence, T., & Fong, C. (2010). The resolution of inflammation: anti-inflammatory roles for NF-κB. *The international journal of biochemistry & cell biology, 42*(4), 519-523.
138. Zinovkin, R. A., & Grebenchikov, O. A. (2020). Transcription factor Nrf2 as a potential therapeutic target for prevention of cytokine storm in COVID-19 patients. *Biochemistry (Moscow), 85*, 833-837.
139. Olagnier, D., Farahani, E., Thyrsted, J., Blay-Cadanet, J., Herengt, A., Idorn, M., ... & Holm, C. K. (2020). SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. *Nature communications, 11*(1), 4938.
140. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., ... & Brown, M. S. (1993). SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell, 75*(1), 187-197.
141. Kober, D. L., Xu, S., Li, S., Bajaj, B., Liang, G., Rosenbaum, D. M., & Radhakrishnan, A. (2020). Identification of a degradation signal at the carboxy terminus of SREBP2: A new role for this domain in cholesterol homeostasis. *Proceedings of the National Academy of Sciences, 117*(45), 28080-28091.
142. Xiao, H., Lu, M., Lin, T. Y., Chen, Z., Chen, G., Wang, W. C., ... & Shyy, J. Y. (2013). Sterol regulatory element binding protein 2 activation of NLRP3 inflammasome in endothelium mediates hemodynamic-induced atherosclerosis susceptibility. *Circulation, 128*(6), 632-642.
143. Kusnadi, A., Park, S. H., Yuan, R., Pannellini, T., Giannopoulou, E., Oliver, D., ... & Ivashkiv, L. B. (2019). The cytokine TNF promotes transcription factor SREBP activity and binding to inflammatory genes to activate macrophages and limit tissue repair. *Immunity, 51*(2), 241-257.
144. Lee, W., Ahn, J. H., Park, H. H., Kim, H. N., Kim, H., Yoo, Y., ... & Seo, Y. K. (2020). COVID-19-activated SREBP2 disturbs cholesterol biosynthesis and leads to cytokine storm. *Signal transduction and targeted therapy, 5*(1), 186.
145. Chen, I. Y., Chang, S. C., Wu, H. Y., Yu, T. C., Wei, W. C., Lin, S., ... & Chang, M. F. (2010). Upregulation of the chemokine (CC motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE2 signaling pathway. *Journal of virology, 84*(15), 7703-7712.
146. Ye, Y., & Hogue, B. G. (2007). Role of the coronavirus E viroporin protein transmembrane domain in virus assembly. *Journal of virology, 81*(7), 3597-3607.
147. Xia, B., Shen, X., He, Y., Pan, X., Liu, F. L., Wang, Y., ... & Gao, Z. (2021). SARS-CoV-2 envelope protein causes acute respiratory distress syndrome (ARDS)-like pathological damages and constitutes an antiviral target. *Cell research, 31*(8), 847-860.
148. Zheng, Y., Zhuang, M. W., Han, L., Zhang, J., Nan, M. L., Zhan, P., ... & Wang, P. H. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) protein inhibits type I and III interferon production by targeting RIG-I/MDA-5 signaling. *Signal transduction and targeted therapy, 5*(1), 299.
149. Park, A., & Iwasaki, A. (2020). Type I and type III interferons–induction, signaling, evasion, and application to combat COVID-19. *Cell host & microbe, 27*(6), 870-878.
150. Fu, Y. Z., Wang, S. Y., Zheng, Z. Q., Huang, Y., Li, W. W., Xu, Z. S., & Wang, Y. Y. (2021). SARS-CoV-2 membrane glycoprotein M antagonizes the MAVS-mediated innate antiviral response. *Cellular & molecular immunology, 18*(3), 613-620.
151. Zhao, Y., Sui, L., Wu, P., Wang, W., Wang, Z., Yu, Y., ... & Wang, G. (2021). A dual-role of SARS-CoV-2 nucleocapsid protein in regulating innate immune response. *Signal Transduction and Targeted Therapy, 6*(1), 331.
152. Chen, X., Yang, X., Zheng, Y., Yang, Y., Xing, Y., & Chen, Z. (2014). SARS coronavirus papain-like protease inhibits the type I interferon signaling pathway through interaction with the STING-TRAF3-TBK1 complex. *Protein & cell, 5*(5), 369-381.

Name	Source	Function
Spike protein (S-protein)	Virus	S1 domain regulates the attachment of the virus to host cells. While S2 domain mediates membrane fusion between virus and host cells
Angiotensin converting enzyme 2 (ACE2)	Host cells	Binds to S-protein and triggers conformational rearrangements in S-protein, facilitating priming of S-protein by proteolytic enzymes
Furin	Host cells	Cleaves S-protein at the S1/S2 site into two segments, the S1 (1-685) and the S2 (686-1273) domains.
Transmembrane serine protease 2 (TMPRSS2)	Host cells	Cleaves S-protein at the S2′ site to facilitate cell membrane fusion between virus and host cells
CD147	Host cells	Functions as a viral receptor that binds to S-protein to mediate viral invasion
Tyrosine-protein kinase receptor UFO (AXL)	Host cells	Interacts with N-terminal domain of S-protein of SARS-CoV-2
Neuropilin 1 (NRP1)	Host cells	NRP1 binds to the C-terminal RRAR motif of the S1 domain and functions to assist the detachment of S2 domain and subsequent vial fusion with host cells
Glucose regulated protein 78 (GRP78)	Host cells	Binds S-protein via its substrate binding domain β (SBDβ) to recognize and mediate entry of viruses
High-density lipoprotein (HDL) scavenger receptor B type 1 (SR-B1)	Host cells	Promotes the attachment of SARS-CoV-2 on ACE2-expressing cells
RNA dependent RNA polymerase (RdRp)	Virus	A major enzyme for viral replication
The 3-chymotrypsin-like protease (3CLPro)	Virus	Processes (cleaves the polyprotein at 11 sites) the generation of non-structural proteins of the virus and assists the replication of the virus
Papain-like protease (PLPro)	Virus	Processes (cleaves the polyprotein at 3 sites) the generation of non-structural proteins of the virus and assists the replication of the virus

Table 1. Important Target Proteins that Function in Viral Entry



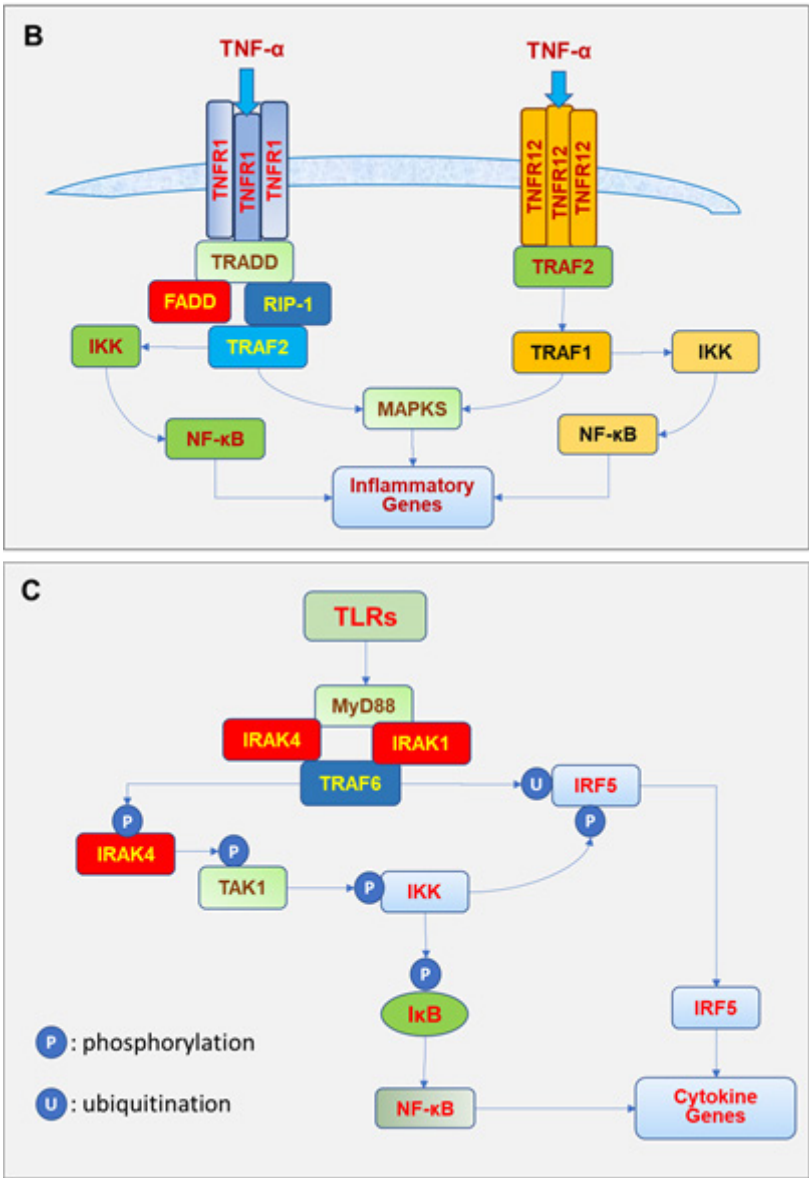


Figure 1: Some Cell Signaling Pathways for Cytokine Release. (A) IL-6 Binds to the Membrane IL-6 Receptor (mIL-6R) and Soluble IL-6 Receptor (sIL-6R). The Binding of IL-6 to both Receptors Results In the Dimerization and Activation of the Glycosylated Type I Membrane Protein of 130–150 kDa (gp130). The Dimerization of Signaling Receptor gp130 Mediates the Activation of Janus Kinases (JAKs) and Subsequent Activation of Phosphatase Src Homology Domains Containing Tyrosine Phosphatase-2 (SHP-2), the Ras/Raf/Mitogen-Activated Protein Kinase (MAPK) Pathway, Signal Transducer and Activator of Transcription Factor-3 (STAT-3), and PI3K/Akt, which are Translocated into the Nucleus to Activate Target Genes. IL-6/mIL-6R Medicated Activation of gp130 Induces IL-6 Classic Signaling Pathway, Leading to Anti-Inflammatory Biological Activities; while IL-6/sIL-6R Induced the Activation of gp130 Leads to Activation of IL-6 Trans-Signaling Pathway that Results in Pro-Inflammatory Responses. (B) Tumor Necrosis Factor -α (TNF-α) Induces Cell Signaling Pathways Through its Receptors (TNFRs). TNF-α Binds to and Activates TNFR1, Promoting the Interaction Between the Intracellular Domains of TNFR1 with TNFR1-Associated Death Domain Protein (TRADD), which Recruits Receptor Interacting Protein-1 (RIP-1) and TNF Receptor-Associated Factor-2 (TRAF-2) to Activate the IκB Kinase (IKK) Followed by Activation of NF-κB, and Mitogen-Activated Protein Kinase (MAPKs), Leading to the Transcriptions of Many Different Genes Including Genes of Inflammatory Cytokines. Binding of TNF-α to TNFR2 Induces Inflammation Through the Direct Recruitment of TRAF2, which in turn Recruits TRAF1, Leading to Activation of IKK and MAPKs. (C) In Response to the Activation of Toll-Like Receptors (TLRs), Myeloid Differentiation Primary-Response 88 (MyD88) is Recruited Along with Interleukin-1 Receptor–Associated Kinase 1/4 (IRAK1/4) and TNF Receptor Associated Factor 6 (TRAF6), which Leads to the Autophosphorylation of IRAK4 and Ubiquitination of Nuclear Translocation of Interferon Regulatory Factor 5 (IRF5). IRAK4 then Phosphorylates and Activates Transforming Growth Factor-β-Activated Kinase 1 (TAK1), which then Phosphorylates IκB Kinase (IKK). The Activated IKK Phosphorylates IRF5 and IκB, Resulting in Nuclear Translocation of IRF5 and Nuclear Factor-Kappa B (NF-κB) Respectively, Leading to Transcription of Inflammatory Cytokine Genes.

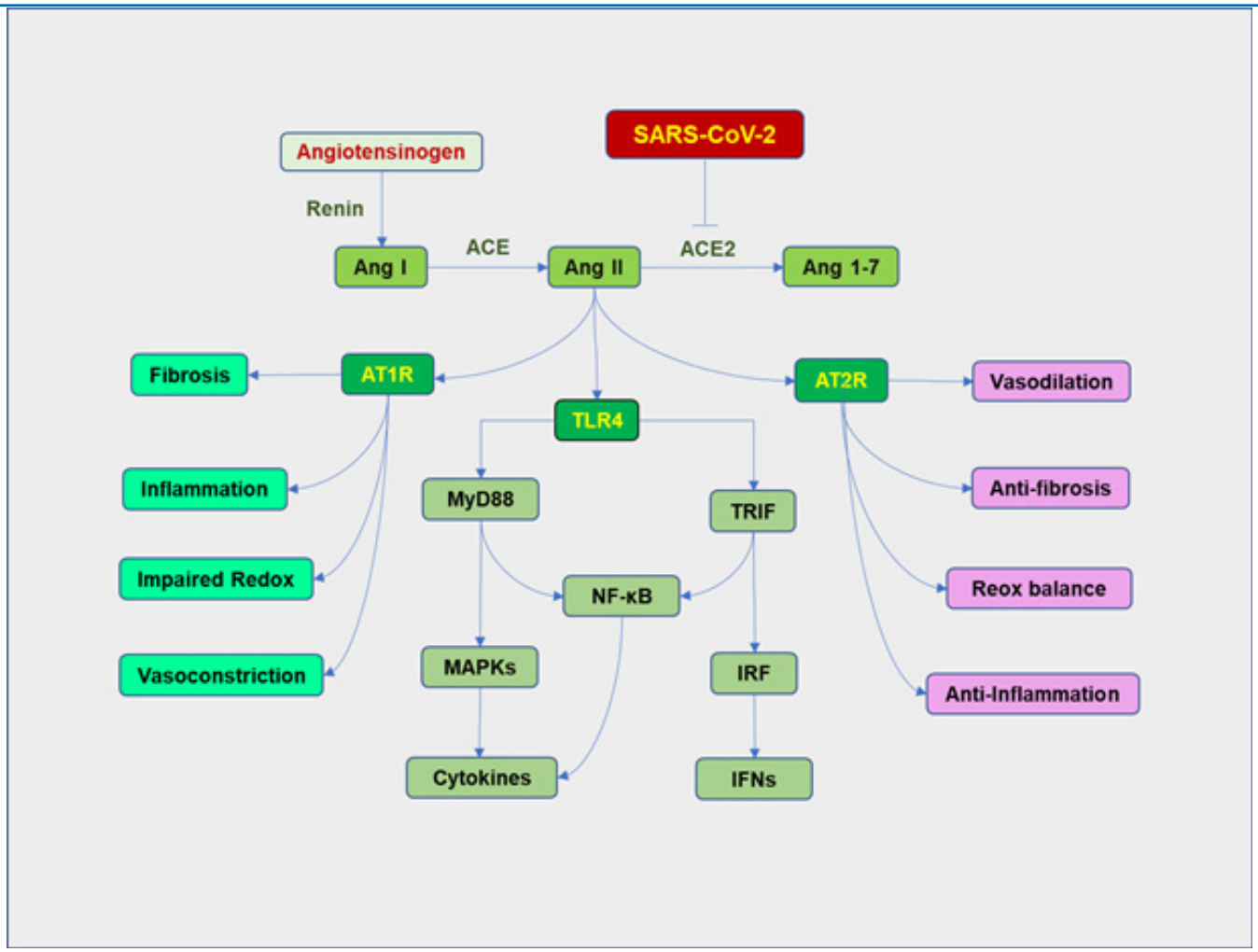


Figure 2: Angiotensin II (Ang II) is Involved in Cytokine Storm During SARS-CoV-2 Infection. In the Renin-Angiotensin System, Renin Cleaves Angiotensinogen into Angiotensin I (Ang I), which is Converted into Ang II by the Angiotensin-Converting Enzyme (ACE). While ACE2 Functions to Converse Ang II to Angiotensin 1-7 (Ang 1-7). The Binding of S-Protein of SARS-CoV-2 to ACE2 Causes the Downregulation of ACE2 Expression, Resulting in an Increase in Ang II Expression. Ang II Binds to and Activates Ang II Receptor Type 1 (AT1R) and Ang II Receptor Type 2 (AT2R). Activation of At1r Induces the Release of Cytokines and Induction of Inflammation, Promotes Fibrosis, Impairs Redox Balance, and Induces Vasoconstriction. In Contrast, Activation of AT2R leads to Anti-Inflammatory, Anti-Fibrotic, Redox Balance, and Vasodilation. In Addition, Ang II Upregulates the Expression of TLR4 and the Downstream Pathways. After Activation, TLRs Recruit Cytoplasmic TIR Domain-Containing Adaptor Proteins such as Myeloid Differentiation Primary-Response 88 (MyD88) and TIR-Containing Adapter-Inducing Interferon-β (TRIF), Leading to the Activation of Nuclear Factor (NF)-κB, Mitogen-Activated Protein Kinases (MAPKs), or Interferon-Regulatory Factor (IRF). Subsequently, the Transcription of Genes that are Responsible for the Synthesis and the Release of Proinflammatory Cytokines are Activated, Promoting the Release of Cytokines and type I Interferons (IFNs).

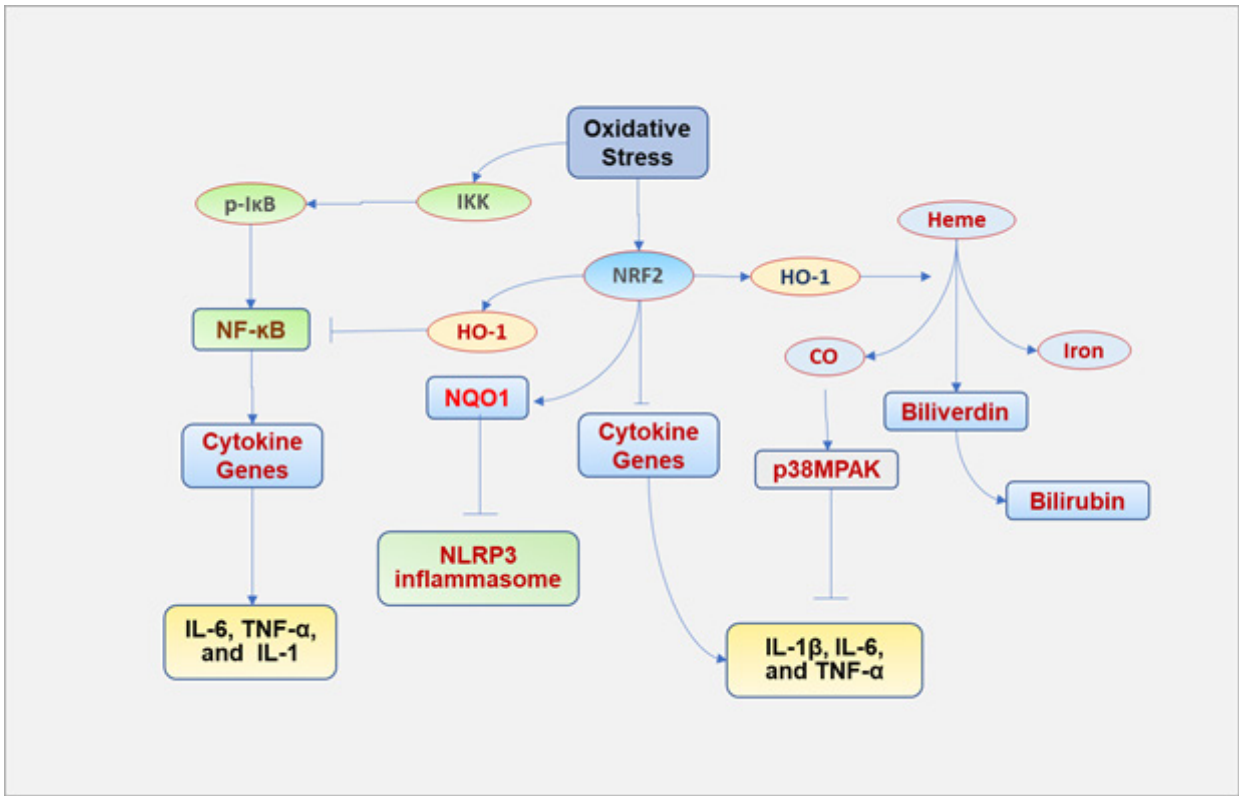


Figure 3: The Crosstalk Between Oxidative Stress and Inflammatory Response. In Response to Oxidative Stress, Activated Nuclear Factor Erythroid 2-Related Factor 2 (NRF2) Represses The Genes that are Associated with the Transcription of Cytokine Genes, Resulting in a Decrease in the Expression of the Inflammatory Cytokines IL-1β, IL-6, and TNF-α. In Addition, NRF2 Induces the Expression of Heme Oxygenase-1 (HO-1) and Increases the Activity of HO-1. HO-1 Functions to Catalyze the Degradation of Heme into Carbon Monoxide (CO), Free Iron, and Biliverdin, which then is Converted to Bilirubin by Biliverdin Reductase. Free Heme is Pro-Inflammatory, while CO, Bilirubin, and HO-1 itself have Significant Anti-Inflammatory Effects. CO can Inhibit the Production of Proinflammatory Cytokines, such as TNF-α and IL-1β, through Mediating p38MPAK Pathway. Moreover, NRF2 can Induce the Expression of Quinone Oxidoreductase (NQO1) that inhibits NLRP3 Inflammasome Ativation. In Response to Oxidative Stress, Activated IκB kinase (IKK) Promotes the Phosphorylation of IκB, Resulting in the Release and Nuclear Translocation of NF-κB, which then Promote the Gene Transcription of Pro-Inflammatory Cytokines, such as IL-6, TNF-α, and IL-1. NRF2 can Inhibit NF-κB Transcriptional Activity Directly or through Activating HO-1.