Targeting COVID-19 Based on Pathogenesis

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Abstract

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Targeting COVID-19 Based on Pathogenesis

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Abstract

The new coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are causing health crisis of the world. To control the pandemic SARS-CoV-2 infection, world-wide vaccination is under way. However, no specific therapeutic drugs are available in the treatment of COVID-19 yet. To find the effectively 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 the host cells, and induction of cytokine storm and cellular damage. Therefore, the potential targets may include the structural proteins of the virus that play roles in the pathogenesis, viral replication associated enzymes, inflammatory cytokines, and signaling proteins that mediate the induction

of cytokine storm. Further exploring this strategy may benefit us in finding novel therapeutic targets and effective treatment of COVID-19.

Key Words: SARS-CoV-2, Cytokine storm, Toll-like receptors, Janus kinase, NLRP3 inflammasome

INTRODUCTION

Since December 2019, SARS-CoV-2 infection had hit over 134 million people worldwide with 2.66 million cases of death until the date of April 6, 2021. SARS-CoV-2 infection not only causes severe respiratory impairment, but also leads to complications in other systems of the body, including cardiac and nervous system, which contribute to the increased mortality in patients with COVID-19 (Helms et al., 2020; Huang et al., 2020b; Mao et al., 2020; Thepmankorn et al., 2021). Although the efforts are underway to control the pandemic with implementation of vaccination, there is still a lack of specific drugs for the treatment of COVID-19. However, multiple therapeutic targets have been proposed and multiple associated therapeutic agents have been tried in the clinical setting. To explore the pathogenic mechanisms associated with COVID-19 and thereby find more effectively therapeutic targets are the urgent task for the control of COVID-19 and reduction of mortality.

The induction of COVID-19 by SARS-CoV-2 infection involve multiple steps that include viral entry into the host cells, replication of 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 potential target processes and specific protein targets that are involved in these processes.

VIRAL INVASION

Viral entry into the host cells, such as epithelial cells in respiratory duct, is the first 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 play important roles in the viral entry into the host cells.

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 (Chen, Guo, Pan & Zhao, 2020). SARS-CoV-2 can bind to angiotensin converting enzyme 2 (ACE2) through the receptor-binding domain (RBD) of the S-protein (Lan et al., 2020) with a higher affinity relative to SARS-CoV (Chen, Guo, Pan & Zhao, 2020). In addition, SARS-CoV-2 does not use other receptors as other coronavirus, such as aminopeptidase N and dipeptidyl peptidase-4 (Zhou et al., 2020a) .

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 S1 subunit is essential for the virus attachment to host cell receptor ACE2; while S2 is critical for virus entry by regulating viral membrane fusion to host cell membrane (Kirchdoerfer et al., 2016; Wrapp et al., 2020; Yan, Zhang, Li, Xia, Guo & Zhou, 2020). Both S1-RBD and S2 domains represent important potential targets for the development of SARS-CoV-2 vaccine and therapeutic drugs (Huang et al., 2020a). In fact, many of the currently developed vaccines target S-protein of SARS-CoV-2 (Folegatti et al., 2020; Logunov et al., 2020; Smith et al., 2020; Zhu et al., 2020). 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 the RBD and neutralize viral infection in nonhuman primates (Walsh et al., 2020; Yang et al., 2020). The development of monoclonal antibody against or inhibitors of S-protein might hold great promise to find therapeutic candidates for COVID-19.

ACE2

The S-protein of both SARS-CoV and SARS-CoV- has been known to utilize ACE2 as a receptor for host cell entry (Li et al., 2003; Wang et al., 2020a). ACE2 is a metallopeptidase that is expressed on major viral target cells, such as type II pneumocytes and enterocytes (Hamming, Timens, Bulthuis, Lely, Navis & van Goor, 2004; Mossel et al., 2008; To & Lo, 2004). ACE2 is expressed not only in type II alveolar cells (AT2), but also in myocardial cells, proximal tubule cells of the kidney, ileum and esophagus epithelial cells, and bladder urothelial cells, and 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 (Li et al., 2003; Wong, Li, Moore, Choe & Farzan, 2004). 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 at the border between the S1 and S2 subunits for priming to facilitate the viral entry into the cells.

A clinical-grade soluble recombinant human ACE2 protein (rhACE2) 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) (Monteil et al., 2021). However, whether rhACE2 administration *in vivo* can prevent SARS-CoV-2 infection remains unknown. The roles of ACE2 inhibitors, such as nicotinamide (Takahashi, Yoshiya, Yoshizawa-Kumagaye & Sugiyama, 2015), in SARS-CoV-2 infection should be further investigated.

S-protein can also regulate cellular signing pathways. 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 (Chen et al., 2010). ANG II can also reduce the expression of ACE2 mRNA and increase the extracellular signal-regulated kinase (ERK) 1/ERK2 activity, which was prevented by the mitogen-activated protein kinase (MAPK) inhibitor PD98059 in vascular smooth muscle cells (Gallagher, Ferrario & Tallant, 2008). Further study indicates that MAPK kinase (MEK) inhibitors (VS-6766, trametinib and selumetinib) reduced ACE2 expression and attenuated the release of inflammatory cytokines during SARS-Cov-2 infection (Zhou et al., 2020b).

2.3. Transmembrane Serine Protease 2 (TMPRSS2)

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 also express in the heart, digestive tract, liver, kidney, brain, and other organs (Dong et al., 2020).

One key function of TMPRSS2 is to prime the viral S-protein to facilitate the interaction between S-protein and ACE2, which is essential for viral infectivity. Both SARS-CoV and SARS-CoV-2 use the serine protease TMPRSS2 for S protein priming (Hoffmann et al., 2020) . The binding of RBD within the S1 domain to ACE2 could trigger the effects of TMPRSS2 on the cleavage of S-protein at the S1 and S2 border sites and facilitate cell membrane fusion for viral entry (Walls, Park, Tortorici, Wall, McGuire & Veesler, 2020).

Inhibition of TMPRSS2 has been shown to block the entry of SARS-CoV-2 (Hoffmann et al., 2020; Sagar et al., 2021). Inhibition of TMPRSS2 by camostat mesylate in human lung Calu-3 cells significantly reduced infection of the cells by SARS-CoV-2. Nafamostat mesylate, which has been demonstrated to inhibit TMPRSS2-dependent host cell entry of MERS-CoV (Yamamoto et al., 2016), can also prevent SARS-CoV-2 entry into host cells with roughly 15-fold-higher efficiency than camostat mesylate (Hoffmann et al., 2020). Another TMPRSS2 inhibitor, α-1antitrypsin, blocks the SARS-CoV-2 from entering host cells. The clinical efficacy of these inhibitors is under evaluation for COVID-19.

2.4. High-density lipoprotein (HDL) scavenger receptor B type 1 (SR-B1)

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 (Shen, Asthana, Kraemer & Azhar, 2018). Co-expression of SR-B1and ACE2 has been found in human pulmonary and extrapulmonary tissues (Wei et al., 2020).

SR-B1 may be associated with the facilitating 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 (Wei et al., 2020). Although the interaction between HDL and S-protein may not the 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 (RdRp)

SARS-CoV-2 is an RNA virus and its replication is dependent on 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 (Perlman & Netland, 2009; Wu et al., 2020b). In addition, NSP8 functions as a primase that is associated with RNA synthesis (Perlman & Netland, 2009). 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 (Yin et al., 2020).

Since there are 96.35%, 98.8% and 97.5% similarities in NSP12, NSP7 and NSP8 between SARS-CoV and SARS-CoV-2, respectively (Ruan, Yang, Wang, Jiang & Song, 2020), inhibitors of the NSP12–NSP7–NSP8 complex for SARS-CoV may also inhibit the complex of SARS-CoV-2 (Yin et al., 2020). Therefore, the NSP12–NSP7–NSP8 complex and RdRp activity inhibitor remdesivir has also been investigated in SARS-CoV-2 (Yin et al., 2020).

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 (Yin et al., 2020). 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 shown to reduce the mortality in COVID-19 patients, it appears to accelerate the recovery when its administration was initiated early (Beigel et al., 2020). Remdesivir has been authorized in the USA for patients with severe COVID-19 since a NIH clinical trials demonstrated that it can accelerate the recovery and shorten the hospital stay of severe COVID-19 patients (Beigel et al., 2020). A recent report indicates that remdesivir given within 9 days from symptom-onset was associated with decrease in mortality in moderate-to-severe COVID-19 patients (Mehta, Bansal, Bysani & Kalpakam, 2021).

However, the effectiveness of remdesivir is far from convincing (Brouqui, Giraud-Gatineau & Raoult, 2020). An earlier clinical trial in China did not show significant benefit of remdesivir in severe COVID-19 patients (Wang et al., 2020b). The discrepancy of results between China and the USA has been ascribed to the difference in genetic backgrounds of patients with COVID-19 (Wang, Cui, Ouyang, Zhan, Guo & Yin, 2020). More effective inhibitors of SARS-CoV-1 RdRp are required for better management of COVID-19.

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 the activity of RdRp (Rathnayake et al., 2020). The antiviral and cell protection efficacy of 3CLPro inhibition has been illustrated in simian Vero cells infected by SARS-CoV-2 (Jin et al., 2020).

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 (Bera, 2021). Similarly, HCV protease inhibitors paritaprevir and simeprevir were also identified as potential inhibitors of SARS-CoV-2 3CLPro (Alamri et al., 2020). 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 (Mody et al., 2021).

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 antiviral 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 (Cao et al., 2020a). 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 significant inhibit 3ClPro in an *in vitro* enzymatic assays (Mahdi, Motyan, Szojka, Golda, Miczi & Tozser, 2020). The aforementioned 3CLPro inhibitors may benefit COVID-19 patients, which is needed to be proved in future clinical trials.

In regard of 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 (Rut et al., 2020). Non-covalent small molecule SARS-CoV PLPro inhibitors has been shown to inhibit SARS-CoV-2 PLPro and display antiviral activity in a SARS-CoV-2 infection model (Klemm et al., 2020). 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 bind 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 (Shin et al., 2020).

N-protein

To prevent the replication of SARS-CoV-2 RNA, the N-protein of the virus may also be the 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 plays a major role in packing the viral RNA into viral ribonucleoproteins (McBride, van Zyl & Fielding, 2014). It also helps in viral RNA transcription and replication (Cong et al., 2020). The NTD functions for RNA-binding and the CTD domain plays a role in oligomerization, while the central linker is necessary for primary phosphorylation (Kang et al., 2020). 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 (Batra et al., 2021). But the higher N-protein antibody titer may indicate the higher viral load and more severe disease, which cause the poor outcomes, not the N-protein antibody itself leads 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 have been found by single immuno-informatics and structure-based drug discovery techniques (Kwarteng, Asiedu, Sylverken, Larbi, Sakyi & Asiedu, 2021), suggesting that N-protein might be associated with overactive immune responses and the development of the NTD inhibitors may hold some promises. The role of N-protein inhibition in the treatment of anti-SARS-CoV-2 should be further evaluated.

4. CYTOKINE STORM

The cytokine storm during SARS-CoV-2 infection has been associated with the severity and mortality in COVID-19 patients. The impaired acquired immune responses and uncontrolled inflammatory innate responses to SARS-CoV-2 may cause cytokine storm (Hu, Huang & Yin, 2021; Thepmankorn et al., 2021). During SARS-CoV-2 infection, the interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are the most frequently reported cytokines that are significantly increased (Qin et al., 2020; Thepmankorn et al., 2021). Other cytokines that are increased but not exclusively include granulocyte stimulating factor (G-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein (MCP)-1, MCP-3, macrophage inflammatory protein 1α (MIP-1A), cutaneous T-cell attracting chemokine (CTACK), IFN-γ, and TNF-α, and IL-1˜10 and 18 (Hadjadj et al., 2020). To prevent cytokine storm should be an important strategic measure for the treatment of COVID-19 (Lariccia, Magi, Serfilippi, Toujani, Gratteri & Amoroso, 2020).

Corticosteroids have been used in COVID-19 pneumonia to reduce systemic inflammation; however, they

may increase the risk of delayed viral elimination and secondary infection. Since cytokines are the effectors in the cytokine storm, the specific cytokine antagonists should reduce cytokine induced damage when they are used for the treatment of COVID-19. Either monoclonal antibodies against cytokines or pharmacological inhibitors of cytokines will fall into this category.

4.1. IL-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 (D'Alessandro et al., 2020).

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 intra cellular 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 receptor 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 the activation of gp130 leads to activation of IL-6 trans-signaling pathway (Ebihara, Matsuda, Nakamura, Matsuda & Murakami, 2011). Classic IL-6 signaling is generally regarded as anti-inflammation and protective pathway, while IL-6 trans-signaling is mainly pro-inflammatory pathway (Garbers, Aparicio-Siegmund & Rose-John, 2015). Blockade of IL-6 trans-signaling, not the full blockade of IL-6 signaling, prevents inflammation (Barkhausen et al., 2011).

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) (Wang S, Zhang W 2016), and PI3K/Akt (Zegeye et al., 2018), which are translocated into the nucleus to activate target genes (Figure 1).

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 (Kaman, Azmy, Chichra, Britto-Leon & Price, 2021). The level of IL-6 is valuable as a prognostic parameter for the disease severity in COVID-19 patients (Liu et al., 2020). Increasing IL-6 concentrations are associated with the requirement of ventilatory support, the progression of ARDS, and the risk of death (Herold et al., 2020; Wu et al., 2020a). Although a positive correlation between IL-6 and CRP exists in COVID-19 patients, IL-6 appears to be a better predictor for the disease progression, since IL-6, not CRP, levels were significantly lower in survivors than in non-survivors of all age groups of COVID-19 patients, suggesting that IL-6 is predictive of in-hospital mortality after SARS-CoV-2 infection (Santa Cruz et al., 2021; Zhang et al., 2020).

Since IL-6 functions through its receptor and the monoclonal antibody against IL-6R has been developed to prevent the activation of IL-6R by IL-6. Tocilizumab is a monoclonal antibody against IL-6R that has been approved by the US FDA for the treatment of cytokine release syndrome. It is also rational to expect tocilizumab to be capable of attenuating the cytokine storm induced by SARS-CoV-2 (Pelaia, Calabrese, Garofalo, Bruni, Vatrella & Pelaia, 2021).

Primary data using tocilizumab demonstrated some benefits for critical COVID-19 patients. Tocilizumab can rapidly resolve the symptoms. In severe COVID-19 patients, fever returned to normal on the first day after tocilizumab administration; other symptoms were improved remarkably within a few days: 75.0% of patients reduced their requirement of oxygen intake within 5 d; and the percentage of lymphocytes in peripheral blood returned to normal in 52.6% of patients on the fifth day after treatment (Xu et al., 2020).

In severe to critical COVID-19 disease with hypercytokinemia in ICU, tocilizumab significantly reduced inflammatory response illustrated by the decrease in CRP, decreased the requirement of respiratory sup-

port, and lowered the mortality (Nasa et al., 2020). The cases with unfavorable outcomes after tocilizumab treatment were linked to the failure in reducing the level of CRP (Luo, Liu, Qiu, Liu, Liu & Li, 2020). In addition, since IL-6 is the main inducer of CRP production in the liver during the acute phase response (Sproston, El Mohtadi, Slevin, Gilmore & Ashworth, 2018), tocilizumab seemed to be more effective in patients with markedly elevated CRP (> 200 mg/L) . Systemic review of the online data suggests that addition of tocilizumab to the standard of care might reduce mortality in severe COVID-19; however, the inconsistency in the effectiveness exists among patients with reduced severity (Boregowda, Perisetti, Nanjappa, Gajendran, Kutti Sridharan & Goyal, 2020; Gorgolas Hernandez-Mora et al., 2021).

The severity of COVID-19 is an important parameter that affects the effectiveness of tocilizumab, since tocilizumab given to severe, not critical (requirement of intubation or ICU admission), SARS-CoV-2 pneumonia patients increased the requirement of intubation and elevated the mortality. In contrast, in patients with COVID-19 pneumonia and life-threatening acute respiratory distress syndrome (ARDS) requiring ventilatory support, intravenous administration of tocilizumab significantly improve the patients' respiratory conditions and downregulate the inflammatory markers (Conrozier et al., 2020; Toniati et al., 2020). However, two phase III clinical trials include an earlier one by Roche (Roche, 2020) and a recent report (Rosas et al., 2021), the use of tocilizumab in hospitalized patients with severe COVID-19 pneumonia did not result in significantly better clinical status or lower mortality.

In conclusion, the effectiveness of tocilizumab might be subjected to various factors, such as the severity of the disease and the inflammatory status. The value of tocilizumab in the treatment of COVID-19 should be further evaluated. One side effect of tocilizumab is that its administration in COVID-19 patients significantly increased IL-6 levels (Antwi-Amoabeng, Kanji, Ford, Beutler, Riddle & Siddiqui, 2020). Tocilizumab only blocks IL-6R without effects on the production of IL-6. Blockade of IL-6R might reduce the IL-6 consumption that leads to an increase in IL-6 (Nishimoto, Terao, Mima, Nakahara, Takagi & Kakehi, 2008). The concern is whether higher IL-6 levels after tocilizumab treatment in COVID-19 patients would have adverse effects or exacerbate the CNS inflammation due to impermeability of BBB to tocilizumab (Zhang et al., 2020).

Since it is the trans IL-6 pathway that has been associated with inflammation, the specific antagonists for this pathway might increase the efficacy and reduce the side effects. The development of regulators of trans-IL-6 signaling pathway may hold better promise for the treatment of COVID-19.

4.2. IL-1

IL-1 that include IL-1α and IL-1β is also an important inflammatory cytokine. IL-1α is released from dying epithelial and endothelial cells, while IL-1β comes from activated macrophages, monocytes, and neutrophils. IL-1 is primarily 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 (Nieto-Torres et al., 2014).

IL-1 receptor (IL-1R) antagonists have also been used in trial treatment in COVID-19 patients. Anakinra is a protein inhibitor of IL-1R. In COVID-19 patients with respiratory insufficiency and hyperinflammation, pneumonia, or ARDS, application of anakinra significantly reduced the mortality (Cavalli & Dagna, 2021; Franzetti et al., 2021; Pontali et al., 2021). Intravenous administration of anakinra (5 mg/kg twice a day) can significantly decrease serum CRP and improve respiratory function in COVID-19 patients with ARDS (Cauchois et al., 2020; Cavalli et al., 2020). Subcutaneous application of anakinra (100 mg twice a day for 72 h, then 100 mg daily for 7 days) also decreased the mortality in COVID-19 patients with respiratory improvement (Huet et al., 2020).

Similarly, the human IL-1R monoclonal antibody, canakinumab can attenuate the systemic inflammation and improve respiratory function in patients with bilateral pneumonia and hyperinflammation after SARS-CoV-2 infection (Ucciferri et al., 2020).

The development of cytokine antagonists is good strategy, and this type of drugs might benefit patients in reducing the specific cytokine induced inflammatory damage. However, cytokine storm induced by SARS-

CoV-2 involves multiple cytokines, it is impossible for one cytokine antagonist to block all the inflammatory responses. Anyway, cytokine antagonists have shown some benefit in reducing symptoms and are deserved to be evaluated in the treatment of COVID-19.

5. CYTOKINE ASSOCIATED SIGNALING PATHWAYS

5.1. Toll-like Receptors (TLRs)

TLRs are the major pattern recognition receptors (PRRs) that can identify pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs). The function of TLRs is closely related to 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 (Kawasaki & Kawai, 2014). .

TLR 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 (Goulopoulou, McCarthy & Webb, 2016). 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, MAPKs, or interferon-regulatory factor (IRF). Subsequently, the transcription of genes that are responsible for synthesis and the release of proinflammatory cytokines are activated, promoting the release of cytokines, such as including TNF-α, and IL-1β, IL-6, IL-18, chemokines, and type I IFNs (Costa et al., 2018; Vogelpoel et al., 2015) (Figure 2).

In SARS-CoV-2 induced cytokine storm, TLR4 seems to play a role (Aboudounya & Heads, 2021). Recombinant proteins of SARS-CoV-2 has 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 (Sohn et al., 2020). Activation of TLR4 in lung macrophages resulted in a concentration- and time-dependent increase in the production of chemokines and cytokines (Grassin-Delyle, Abrial, Salvator, Brollo, Naline & Devillier, 2020). In patients with COVID-19, the expression of TLR 4 and NF-ϰB target genes was upregulated (Sohn et al., 2020). Among TLRs, TLR4 is most likely to be involved in recognizing MAMP from SARS-CoV-2 to induce inflammatory responses (Grassin-Delyle, Abrial, Salvator, Brollo, Naline & Devillier, 2020). 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 (Choudhury & Mukherjee, 2020). Antagonists of TLR4 may attenuate the cytokine storm and reduce the viral entry in severe COVID-19 patients (Choudhury, Das, Patra & Mukherjee, 2021).

5.2. Angiotensin 2 (Ang II)

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 Ang II. ACE2 functions to converse Ang II to Ang 1-7 (Figure 2). 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, and impaired redox balance, and vasoconstriction (Eguchi, Kawai, Scalia & Rizzo, 2018), while activation of AT2R leads to anti-inflammatory, anti-fibrotic, and vasodilation (D'Ardes et al., 2020). Ang II can also increase the recruitment of immune cells to the injury sites and enhances the release of inflammatory cytokines (Nataraj et al., 1999). Ang II may regulate TLR4 mediated pathway by upregulating the expression of TLR4 and the downstream pathways (Wu et al., 2009).

Ang II induced cell signaling pathway is a potential mechanism underlying the cytokine storm caused by

SARS-CoV-2. The binding of S-protein of SARS-CoV-2 to ACE2 on the cell surface caused downregulation of ACE2 expression. The reduced expression and activity of ACE2 resulted in an increase in Ang II, leading to elevated inflammatory responses (Figure 2). The Ang II regulators to balance the Ang II double edged function might benefit COVID-19.

5.3. Janus-associated kinase (JAK)

The JAK family is one of ten recognized families of non-receptor tyrosine kinases. Mammalian members of Jaks 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 (El Jammal, Gerfaud-Valentin, Seve & Jamilloux, 2020; Jamilloux, El Jammal, Vuitton, Gerfaud-Valentin, Kerever & Seve, 2019). JAK associates with cytokine receptors to mediate cytokines 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 common γ chain (γc) as receptor subunit (Moradian et al., 2020; Pesu, Laurence, Kishore, Zwillich, Chan & O'Shea, 2008). JAK1 also regulate cell signaling downstream of IL-6, which activate their receptor to initiate the dimerization of receptor subunit gp130 (Barkhausen et al., 2011). JAK2 activation is essential for the signaling transduction of erythropoietin and other growth factors (Maiese, Chong, Shang & Wang, 2012).

Since the multiple cytokines are involved in SARS-CoV-2 induced cytokine storm, JAK inhibitors have been therapeutically used in COVID-19 patients (Seif et al., 2020). Ruxolitinib is a potent and selective inhibitor of JAK1/2 with some levels of inhibitory effects on TYK2 and JAK3. COVID-19 patients received ruxolitinib plus standard-of-care treatment 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 (Cao et al., 2020b). 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 (La Rosee & La Rosee, 2020). For an 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 (Neubauer et al., 2020).

Baricitinib is an oral JAK inhibitor. Clinical studies have demonstrated the ability of baricitinib to reduce the viral titers and decrease IL-6 level with resolution of fever and cough symptoms in COVID-19 patients (Richardson, Ottaviani, Prelle, Stebbing, Casalini & Corbellino, 2020; Stebbing et al., 2021). 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 (Bronte et al., 2020). In moderate to severe SARS-CoV-2 pneumonia, baricitinib has add-on effect when combined with corticosteroids in improving pulmonary function (Rodriguez-Garcia, Sanchez-Nievas, Arevalo-Serrano, Garcia-Gomez, Jimenez-Vizuete & Martinez-Alfaro, 2021). Baricitinib exerts both anti-inflammatory and anti-viral activities effects and one additional advantage is its ability to cross BBB into the CNS (Richardson, Ottaviani, Prelle, Stebbing, Casalini & Corbellino, 2020).

5.4. 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 cleave the cytokine precursor pro-IL-1β into mature IL-1β and then of pro-IL-18 into IL-18 (He, Hara & Nunez, 2016). The following release of other inflammatory cytokines, such as IL-6, TNF- α, prostaglandins and leukotrienes (Abderrazak et al., 2015) contributes to the induction

of cytokine storm. Inhibition of NLRP3 has been shown to attenuate the severity of inflammatory diseases (Tomani et al., 2020).

SARS-CoV-2, similar to SARS-CoV, encodes viroporins that have been shown to activate NLRP3 (Chen, Moriyama, Chang & Ichinohe, 2019). Ang II, which can be reduced by ACE2, has been shown to induce the activation of NLRP3 activation in renal tubular epithelial cells (Wen et al., 2016). 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. Downregulation of ACE2 after SARS-CoV-2 infection has been observed (Zhang, Penninger, Li, Zhong & Slutsky, 2020). The different scenarios of NLRP3 activation in COVID-19 may reflect different level of immune responses (van den Berg & Te Velde, 2020). However, the role of NLRP3 in inflammatory response and effectiveness of NLRP3 inhibitors in COVID-19 patients with hyperinflammation remains to be elucidated.

Colchicine, a currently used anti-gout drug, has anti-inflammatory effect by inhibiting neutrophil chemotaxis and inflammasome. The anti-inflammasome function of colchicine is mediated through its inhibiting activity of NLRP3 inflammasome (Liang, Zhou, Tong, Chen, Ren & Zhao, 2019). Colchicine has been shown to not only reduce the inflammatory cytokines, but also decrease the expression of NLRP3 inflammasome (Fujisue et al., 2017).

Treatment with colchicine reduced cytokines and lowered the rate of clinical deterioration in COVID-19 patients (Deftereos et al., 2020; Gandolfini et al., 2020). In hospitalized COVID-19 patients with pneumonia, treatment with colchicine was associated with reduced mortality and accelerated recovery (Manenti et al., 2021). A recent randomized trial indicated that colchicine 1 mg for 1-3 days followed by 0.5 mg/day for 14 days is effective as a proactive anti-inflammatory therapy in hospitalized COVID-19 patients and viral pneumonia (Mareev et al., 2021). The systemic review of total of eight studies with 5778 COVID-19 patients demonstrated that the administration of colchicine was associated with improved outcomes of COVID-19 (Hariyanto, Halim, Jodhinata, Yanto & Kurniawan, 2021).

5.5. The nuclear Factor Erythroid 2-Related Factor 2 (NRF2)

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 (Ahmed, Luo, Namani, Wang & Tang, 2017). 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 (Kobayashi et al., 2016). In contrast, NRF2 knockout mice showed increased levels of proinflammatory cytokines in response to lipopolysaccharide stimulation (Thimmulappa et al., 2006a). In addition, NRF2 induces the expression of heme oxgenase-1 (HO-1) and increases the activity of HO-1 (Reichard, Motz & Puga, 2007). 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 (Vijayan, Wagener & Immenschuh, 2018). CO can inhibit the production of proinflammatory cytokines, such as TNF-α and IL-1β, through mediating p38MPAK pathway (Otterbein et al., 2000). Increased NRF2-dependent HO-1 expression has been associated with anti-inflammatory activity (Kuhn et al., 2011). Moreover, NRF2 has been shown to induce the quinone oxidoreductase (NQO1) expression and thereby inhibit NLRP3 inflammasome activation (Liu et al., 2017). NRF2 also inhibits NF-B transcriptional activity, since NRF2 knockdown significantly increases NF-B-dependent gene transcription (Thimmulappa et al., 2006b). The downstream target of NRF2, HO-1 can also inhibit NF-B activity (Bellezza et al., 2012). In response to oxidative stress, activated IB kinase (IKK) promotes the phosphorylation and degradation of IB. In normal condition, NF-B is trapped in the cytoplasm by IB binding. The loss of IB frees NF-B, which is translocated to nucleus to promote the gene transcription of pro-inflammatory cytokines, such as IL-6, TNF-α, an IL-1 (Lawrence & Fong, 2010)(Figure 3).

As a result, proposed use of NRF2 inducers to prevent development of an excessive inflammatory response in COVID-19 patients is rationale (Zinovkin & Grebenchikov, 2020). Recently, NRF2 agonist 4-octyl-itaconate and dimethyl fumarate has been demonstrated to attenuate inflammatory responses to SARS-CoV2 infection (Olagnier et al., 2020). NRF2 agonists may be potentially valuable candidates in the treatment of SARS-CoV-2 infection.

6. CONCLUSION

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. Further elucidation of these processes and underlying mechanisms may find novel therapeutic targets, which is a critical strategy for developing treatments of COVID-19.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Abderrazak A, Syrovets T, Couchie D, El Hadri K, Friguet B, Simmet T*, et al.* (2015). NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. Redox Biol 4**:** 296-307.

Aboudounya MM, & Heads RJ (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 Inflamm 2021**:** 8874339.

Ahmed SM, Luo L, Namani A, Wang XJ, & Tang X (2017). Nrf2 signaling pathway: Pivotal roles in inflammation. Biochim Biophys Acta Mol Basis Dis 1863**:** 585-597.

Alamri MA, Tahir Ul Qamar M, Mirza MU, Bhadane R, Alqahtani SM, Muneer I*, et al.* (2020). Pharmacoinformatics and molecular dynamics simulation studies reveal potential covalent and FDA-approved inhibitors of SARS-CoV-2 main protease 3CL(pro). J Biomol Struct Dyn**:**1-13.

Antwi-Amoabeng D, Kanji Z, Ford B, Beutler BD, Riddle MS, & Siddiqui F (2020). Clinical outcomes in COVID-19 patients treated with tocilizumab: An individual patient data systematic review. J Med Virol 92**:**2516-2522.

Barkhausen T, Tschernig T, Rosenstiel P, van Griensven M, Vonberg RP, Dorsch M*, et al.* (2011). Selective blockade of interleukin-6 trans-signaling improves survival in a murine polymicrobial sepsis model. Crit Care Med 39**:** 1407-1413.

Batra M, Tian R, Zhang C, Clarence E, Sacher CS, Miranda JN*, et al.* (2021). Role of IgG against N-protein of SARS-CoV2 in COVID19 clinical outcomes. Sci Rep 11**:** 3455.

Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC*, et al.* (2020). Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med 383**:** 1813-1826.

Bellezza I, Tucci A, Galli F, Grottelli S, Mierla AL, Pilolli F*, et al.* (2012). Inhibition of NF-kappaB nuclear translocation via HO-1 activation underlies alpha-tocopheryl succinate toxicity. J Nutr Biochem 23**:** 1583-1591.

Bera K (2021). Binding and inhibitory effect of ravidasvir on 3CL(pro) of SARS-CoV-2: a molecular docking, molecular dynamics and MM/PBSA approach. J Biomol Struct Dyn**:** 1-8.

Boregowda U, Perisetti A, Nanjappa A, Gajendran M, Kutti Sridharan G, & Goyal H (2020). Addition of Tocilizumab to the Standard of Care Reduces Mortality in Severe COVID-19: A Systematic Review and Meta-Analysis. Front Med (Lausanne) 7**:** 586221.

Bronte V, Ugel S, Tinazzi E, Vella A, De Sanctis F, Cane S*, et al.* (2020). Baricitinib restrains the immune dysregulation in patients with severe COVID-19. J Clin Invest 130**:** 6409-6416.

Brouqui P, Giraud-Gatineau A, & Raoult D (2020). Remdesivir investigational trials in COVID-19: a critical reappraisal. New Microbes New Infect**:** 100707.

Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G*, et al.* (2020a). A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med 382**:** 1787-1799.

Cao Y, Wei J, Zou L, Jiang T, Wang G, Chen L*, et al.* (2020b). Ruxolitinib in treatment of severe coronavirus disease 2019 (COVID-19): A multicenter, single-blind, randomized controlled trial. J Allergy Clin Immunol 146**:** 137-146 e133.

Cauchois R, Koubi M, Delarbre D, Manet C, Carvelli J, Blasco VB*, et al.* (2020). Early IL-1 receptor blockade in severe inflammatory respiratory failure complicating COVID-19. Proc Natl Acad Sci U S A 117**:** 18951-18953.

Cavalli G, & Dagna L (2021). The right place for IL-1 inhibition in COVID-19. Lancet Respir Med 9**:** 223-224.

Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D*, et al.* (2020). Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol 2**:** e325-e331.

Chen IY, Chang SC, Wu HY, Yu TC, Wei WC, Lin S*, et al.* (2010). Upregulation of the chemokine (C-C motif) ligand 2 via a severe acute respiratory syndrome coronavirus spike-ACE2 signaling pathway. J Virol 84**:** 7703-7712.

Chen IY, Moriyama M, Chang MF, & Ichinohe T (2019). Severe Acute Respiratory Syndrome Coronavirus Viroporin 3a Activates the NLRP3 Inflammasome. Front Microbiol 10**:** 50.

Chen Y, Guo Y, Pan Y, & Zhao ZJ (2020). Structure analysis of the receptor binding of 2019-nCoV. Biochem Biophys Res Commun.

Choudhury A, Das NC, Patra R, & Mukherjee S (2021). In silico analyses on the comparative sensing of SARS-CoV-2 mRNA by the intracellular TLRs of humans. J Med Virol 93**:** 2476-2486.

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. J Med Virol 92**:**2105-2113.

Cong Y, Ulasli M, Schepers H, Mauthe M, V'Kovski P, Kriegenburg F*, et al.* (2020). Nucleocapsid Protein Recruitment to Replication-Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. J Virol 94.

Conrozier T, Lohse A, Balblanc JC, Dussert P, Royer PY, Bossert M*, et al.* (2020). Biomarker variation in patients successfully treated with tocilizumab for severe coronavirus disease 2019 (COVID-19): results of a multidisciplinary collaboration. Clin Exp Rheumatol 38**:**742-747.

Costa AG, Ramasawmy R, Val FFA, Ibiapina HNS, Oliveira AC, Tarrago AM*, et al.* (2018). Polymorphisms in TLRs influence circulating cytokines production in Plasmodium vivax malaria: TLR polymorphisms influence cytokine productions in malaria-vivax. Cytokine 110**:**374-380.

D'Alessandro A, Thomas T, Dzieciatkowska M, Hill RC, Francis RO, Hudson KE*, et al.* (2020). Serum Proteomics in COVID-19 Patients: Altered Coagulation and Complement Status as a Function of IL-6 Level. J Proteome Res 19**:** 4417-4427.

D'Ardes D, Boccatonda A, Rossi I, Guagnano MT, Santilli F, Cipollone F*, et al.* (2020). COVID-19 and RAS: Unravelling an Unclear Relationship. Int J Mol Sci 21.

Deftereos S, Giannopoulos G, Vrachatis DA, Siasos G, Giotaki SG, Cleman M*, et al.* (2020). Colchicine as a potent anti-inflammatory treatment in COVID-19: can we teach an old dog new tricks? Eur Heart J Cardiovasc Pharmacother 6**:** 255.

Dong M, Zhang J, Ma X, Tan J, Chen L, Liu S*, et al.* (2020). ACE2, TMPRSS2 distribution and extrapulmonary organ injury in patients with COVID-19. Biomed Pharmacother 131**:** 110678.

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. Invest Ophthalmol Vis Sci 52**:**8549-8557.

Eguchi S, Kawai T, Scalia R, & Rizzo V (2018). Understanding Angiotensin II Type 1 Receptor Signaling in Vascular Pathophysiology. Hypertension 71**:** 804-810.

El Jammal T, Gerfaud-Valentin M, Seve P, & Jamilloux Y (2020). Inhibition of JAK/STAT signaling in rheumatologic disorders: The expanding spectrum. Joint Bone Spine 87**:** 119-129.

Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S*, et al.* (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. Lancet 396**:** 467-478.

Franzetti M, Forastieri A, Borsa N, Pandolfo A, Molteni C, Borghesi L*, et al.* (2021). IL-1 Receptor Antagonist Anakinra in the Treatment of COVID-19 Acute Respiratory Distress Syndrome: A Retrospective, Observational Study. J Immunol 206**:** 1569-1575.

Fujisue K, Sugamura K, Kurokawa H, Matsubara J, Ishii M, Izumiya Y*, et al.* (2017). Colchicine Improves Survival, Left Ventricular Remodeling, and Chronic Cardiac Function After Acute Myocardial Infarction. Circ J 81**:** 1174-1182.

Gallagher PE, Ferrario CM, & Tallant EA (2008). MAP kinase/phosphatase pathway mediates the regulation of ACE2 by angiotensin peptides. Am J Physiol Cell Physiol 295**:** C1169-1174.

Gandolfini I, Delsante M, Fiaccadori E, Zaza G, Manenti L, Degli Antoni A*, et al.* (2020). COVID-19 in kidney transplant recipients. Am J Transplant 20**:** 1941-1943.

Garbers C, Aparicio-Siegmund S, & Rose-John S (2015). The IL-6/gp130/STAT3 signaling axis: recent advances towards specific inhibition. Curr Opin Immunol 34**:** 75-82.

Gorgolas Hernandez-Mora M, Cabello Ubeda A, Prieto-Perez L, Villar Alvarez F, Alvarez Alvarez B, Rodriguez Nieto MJ*, et al.* (2021). Compassionate use of tocilizumab in severe SARS-CoV2 pneumonia. Int J Infect Dis 102**:** 303-309.

Goulopoulou S, McCarthy CG, & Webb RC (2016). Toll-like Receptors in the Vascular System: Sensing the Dangers Within. Pharmacol Rev 68**:** 142-167.

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. J Innate Immun 12**:** 63-73.

Hadjadj J, Castro CN, Tusseau M, Stolzenberg MC, Mazerolles F, Aladjidi N*, et al.* (2020). Early-onset autoimmunity associated with SOCS1 haploinsufficiency. Nat Commun 11**:** 5341.

Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, & van Goor H (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203**:** 631-637.

Hariyanto TI, Halim DA, Jodhinata C, Yanto TA, & Kurniawan A (2021). Colchicine treatment can improve outcomes of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. Clin Exp Pharmacol Physiol.

He Y, Hara H, & Nunez G (2016). Mechanism and Regulation of NLRP3 Inflammasome Activation. Trends Biochem Sci 41**:** 1012-1021.

Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C*, et al.* (2020). Neurologic Features in Severe SARS-CoV-2 Infection. N Engl J Med 382**:** 2268-2270.

Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M*, et al.* (2020). Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J Allergy Clin Immunol 146**:** 128-136 e124.

Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S*, et al.* (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 181**:** 271-280 e278.

Hu B, Huang S, & Yin L (2021). The cytokine storm and COVID-19. J Med Virol 93**:** 250-256.

Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick LC, Rattigan SM*, et al.* (2020a). A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. Nat Commun 11**:** 4704.

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y*, et al.* (2020b). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395**:** 497-506.

Huet T, Beaussier H, Voisin O, Jouveshomme S, Dauriat G, Lazareth I*, et al.* (2020). Anakinra for severe forms of COVID-19: a cohort study. Lancet Rheumatol 2**:** e393-e400.

Jamilloux Y, El Jammal T, Vuitton L, Gerfaud-Valentin M, Kerever S, & Seve P (2019). JAK inhibitors for the treatment of autoimmune and inflammatory diseases. Autoimmun Rev 18**:** 102390.

Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y*, et al.* (2020). Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors. Nature 582**:** 289-293.

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. Respir Med Case Rep 33**:** 101376.

Kang S, Yang M, Hong Z, Zhang L, Huang Z, Chen X*, et al.* (2020). Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. Acta Pharm Sin B 10**:** 1228-1238.

Kawasaki T, & Kawai T (2014). Toll-like receptor signaling pathways. Front Immunol 5**:** 461.

Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, Turner HL*, et al.* (2016). Pre-fusion structure of a human coronavirus spike protein. Nature 531**:** 118-121.

Klemm T, Ebert G, Calleja DJ, Allison CC, Richardson LW, Bernardini JP*, et al.* (2020). Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. EMBO J 39**:** e106275.

Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H*, et al.* (2016). Nrf2 suppresses macrophage inflammatory response by blocking proinflammatory cytokine transcription. Nat Commun 7**:** 11624.

Kuhn AM, Tzieply N, Schmidt MV, von Knethen A, Namgaladze D, Yamamoto M*, et al.* (2011). Antioxidant signaling via Nrf2 counteracts lipopolysaccharide-mediated inflammatory responses in foam cell macrophages. Free Radic Biol Med 50**:** 1382-1391.

Kwarteng A, Asiedu E, Sylverken AA, Larbi A, Sakyi SA, & Asiedu SO (2021). Molecular characterization of interactions between the D614G variant of SARS-CoV-2 S-protein and neutralizing antibodies: A computational approach. Infect Genet Evol 91**:** 104815.

La Rosee F, & La Rosee P (2020). Ruxolitinib in COVID-19 Hyperinflammation and Haematologic Malignancies. Acta Haematol**:**1-3.

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S*, et al.* (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 581**:** 215-220.

Lariccia V, Magi S, Serfilippi T, Toujani M, Gratteri S, & Amoroso S (2020). Challenges and Opportunities from Targeting Inflammatory Responses to SARS-CoV-2 Infection: A Narrative Review. J Clin Med 9.

Lawrence T, & Fong C (2010). The resolution of inflammation: anti-inflammatory roles for NF-kappaB. Int J Biochem Cell Biol 42**:** 519-523.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA*, et al.*(2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426**:** 450-454.

Liang Y, Zhou HF, Tong M, Chen L, Ren K, & Zhao GJ (2019). Colchicine inhibits endothelial inflammation via NLRP3/CRP pathway. Int J Cardiol 294**:** 55.

Liu F, Li L, Xu M, Wu J, Luo D, Zhu Y*, et al.* (2020). Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. J Clin Virol 127**:** 104370.

Liu X, Zhang X, Ding Y, Zhou W, Tao L, Lu P*, et al.* (2017). Nuclear Factor E2-Related Factor-2 Negatively Regulates NLRP3 Inflammasome Activity by Inhibiting Reactive Oxygen Species-Induced NLRP3 Priming. Antioxid Redox Signal 26**:** 28-43.

Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatulin AI, Shcheblyakov DV, Dzharullaeva AS*, et al.* (2020). Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. Lancet 396**:** 887-897.

Luo P, Liu Y, Qiu L, Liu X, Liu D, & Li J (2020). Tocilizumab treatment in COVID-19: A single center experience. J Med Virol 92**:**814-818.

Mahdi M, Motyan JA, Szojka ZI, Golda M, Miczi M, & Tozser J (2020). Analysis of the efficacy of HIV protease inhibitors against SARS-CoV-2's main protease. Virol J 17**:** 190.

Maiese K, Chong ZZ, Shang YC, & Wang S (2012). Erythropoietin: new directions for the nervous system. Int J Mol Sci 13**:**11102-11129.

Manenti L, Maggiore U, Fiaccadori E, Meschi T, Antoni AD, Nouvenne A*, et al.* (2021). Reduced mortality in COVID-19 patients treated with colchicine: Results from a retrospective, observational study. PLoS One 16**:** e0248276.

Mao L, Jin H, Wang M, Hu Y, Chen S, He Q*, et al.* (2020). Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol 77**:** 683-690.

Mareev VY, Orlova YA, Plisyk AG, Pavlikova EP, Akopyan ZA, Matskeplishvili ST*, et al.* (2021). Proactive anti-inflammatory therapy with colchicine in the treatment of advanced stages of new coronavirus infection. The first results of the COLORIT study. Kardiologiia 61**:** 15-27.

McBride R, van Zyl M, & Fielding BC (2014). The coronavirus nucleocapsid is a multifunctional protein. Viruses 6**:**2991-3018.

Mehta RM, 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. Int J Infect Dis 106**:** 71-77.

Mody V, Ho J, Wills S, Mawri A, Lawson L, Ebert M*, et al.* (2021). Identification of 3-chymotrypsin like protease (3CLPro) inhibitors as potential anti-SARS-CoV-2 agents. Commun Biol 4**:** 93.

Monteil V, Dyczynski M, Lauschke VM, Kwon H, Wirnsberger G, Youhanna S*, et al.* (2021). Human soluble ACE2 improves the effect of remdesivir in SARS-CoV-2 infection. EMBO Mol Med 13**:** e13426.

Moradian N, Gouravani M, Salehi MA, Heidari A, Shafeghat M, Hamblin MR*, et al.* (2020). Cytokine release syndrome: inhibition of pro-inflammatory cytokines as a solution for reducing COVID-19 mortality. Eur Cytokine Netw 31**:** 81-93.

Mossel EC, Wang J, Jeffers S, Edeen KE, Wang S, Cosgrove GP*, et al.* (2008). SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. Virology 372**:** 127-135.

Nasa P, Singh A, Upadhyay S, Bagadia S, Polumuru S, Shrivastava PK*, et al.* (2020). Tocilizumab Use in COVID-19 Cytokine-release Syndrome: Retrospective Study of Two Centers. Indian J Crit Care Med 24**:** 771-776.

Nataraj C, Oliverio MI, Mannon RB, Mannon PJ, Audoly LP, Amuchastegui CS*, et al.* (1999). Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway. J Clin Invest 104**:** 1693-1701.

Neubauer A, Wiesmann T, Vogelmeier CF, Mack E, Skevaki C, Gaik C*, et al.* (2020). Ruxolitinib for the treatment of SARS-CoV-2 induced acute respiratory distress syndrome (ARDS). Leukemia 34**:**2276-2278.

Nieto-Torres JL, DeDiego ML, Verdia-Baguena C, Jimenez-Guardeno JM, Regla-Nava JA, Fernandez-Delgado R*, et al.* (2014). Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog 10**:**e1004077.

Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, & Kakehi T (2008). Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. Blood 112**:** 3959-3964.

Olagnier D, Farahani E, Thyrsted J, Blay-Cadanet J, Herengt A, Idorn M*, et al.* (2020). SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. Nat Commun 11**:**4938.

Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M*, et al.*(2000). Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. Nat Med 6**:** 422-428.

Pelaia C, Calabrese C, Garofalo E, Bruni A, Vatrella A, & Pelaia G (2021). Therapeutic Role of Tocilizumab in SARS-CoV-2-Induced Cytokine Storm: Rationale and Current Evidence. Int J Mol Sci 22.

Perlman S, & Netland J (2009). Coronaviruses post-SARS: update on replication and pathogenesis. Nat Rev Microbiol 7**:** 439-450.

Pesu M, Laurence A, Kishore N, Zwillich SH, Chan G, & O'Shea JJ (2008). Therapeutic targeting of Janus kinases. Immunol Rev 223**:**132-142.

Pontali E, Volpi S, Signori A, Antonucci G, Castellaneta M, Buzzi D*, et al.* (2021). Efficacy of early anti-inflammatory treatment with high doses of intravenous anakinra with or without glucocorticoids in patients with severe COVID-19 pneumonia. J Allergy Clin Immunol 147**:** 1217-1225.

Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y*, et al.* (2020). Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis 71**:** 762-768.

Rathnayake AD, Zheng J, Kim Y, Perera KD, Mackin S, Meyerholz DK*, et al.* (2020). 3C-like protease inhibitors block coronavirus replication in vitro and improve survival in MERS-CoV-infected mice. Sci Transl Med 12.

Reichard JF, Motz GT, & Puga A (2007). Heme oxygenase-1 induction by NRF2 requires inactivation of the transcriptional repressor BACH1. Nucleic Acids Res 35**:** 7074-7086.

Richardson PJ, Ottaviani S, Prelle A, Stebbing J, Casalini G, & Corbellino M (2020). CNS penetration of potential anti-COVID-19 drugs. J Neurol 267**:** 1880-1882.

Roche (2020). An update on the phase III COVACTA trial of

Actemra/RoActemra in hospitalised patients with severe COVID-19 associated

pneumonia.

Rodriguez-Garcia JL, Sanchez-Nievas G, Arevalo-Serrano J, Garcia-Gomez C, Jimenez-Vizuete JM, & Martinez-Alfaro E (2021). Baricitinib improves respiratory function in patients treated with corticosteroids for SARS-CoV-2 pneumonia: an observational cohort study. Rheumatology (Oxford) 60**:** 399-407.

Rosas IO, Brau N, Waters M, Go RC, Hunter BD, Bhagani S*, et al.*(2021). Tocilizumab in Hospitalized Patients with Severe Covid-19 Pneumonia. N Engl J Med.

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 Med 46**:** 846-848.

Rut W, Lv Z, Zmudzinski M, Patchett S, Nayak D, Snipas SJ*, et al.*(2020). Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design. Sci Adv 6.

Sagar S, Rathinavel AK, Lutz WE, Struble LR, Khurana S, Schnaubelt AT*, et al.* (2021). Bromelain inhibits SARS-CoV-2 infection via targeting ACE-2, TMPRSS2, and spike protein. Clin Transl Med 11**:** e281.

Santa Cruz A, Mendes-Frias A, Oliveira AI, Dias L, Matos AR, Carvalho A*, et al.* (2021). Interleukin-6 Is a Biomarker for the Development of Fatal Severe Acute Respiratory Syndrome Coronavirus 2 Pneumonia. Front Immunol 12**:** 613422.

Seif F, Aazami H, Khoshmirsafa M, Kamali M, Mohsenzadegan M, Pornour M*, et al.* (2020). JAK Inhibition as a New Treatment Strategy for Patients with COVID-19. Int Arch Allergy Immunol 181**:** 467-475.

Shen WJ, Asthana S, Kraemer FB, & Azhar S (2018). Scavenger receptor B type 1: expression, molecular regulation, and cholesterol transport function. J Lipid Res 59**:** 1114-1131.

Shin D, Mukherjee R, Grewe D, Bojkova D, Baek K, Bhattacharya A*, et al.* (2020). Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. Nature 587**:** 657-662.

Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J*, et al.*(2020). Immunogenicity of a DNA vaccine candidate for COVID-19. Nat Commun 11**:** 2601.

Sohn KM, Lee SG, Kim HJ, Cheon S, Jeong H, Lee J*, et al.* (2020). COVID-19 Patients Upregulate Toll-like Receptor 4-mediated Inflammatory Signaling That Mimics Bacterial Sepsis. J Korean Med Sci 35**:**e343.

Sproston NR, El Mohtadi M, Slevin M, Gilmore W, & Ashworth JJ (2018). The Effect of C-Reactive Protein Isoforms on Nitric Oxide Production by U937 Monocytes/Macrophages. Front Immunol 9**:** 1500.

Stebbing J, Sanchez Nievas G, Falcone M, Youhanna S, Richardson P, Ottaviani S*, et al.* (2021). JAK inhibition reduces SARS-CoV-2 liver infectivity and modulates inflammatory responses to reduce morbidity and mortality. Sci Adv 7**:** eabe4724.

Takahashi S, Yoshiya T, Yoshizawa-Kumagaye K, & Sugiyama T (2015). Nicotianamine is a novel angiotensin-converting enzyme 2 inhibitor in soybean. Biomed Res 36**:** 219-224.

Thepmankorn P, Bach J, Lasfar A, Zhao X, Souayah S, Chong ZZ*, et al.* (2021). Cytokine storm induced by SARS-CoV-2 infection: The spectrum of its neurological manifestations. Cytokine 138**:**155404.

Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW*, et al.* (2006a). Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis. J Clin Invest 116**:** 984-995.

Thimmulappa RK, Scollick C, Traore K, Yates M, Trush MA, Liby KT*, et al.* (2006b). Nrf2-dependent protection from LPS induced inflammatory response and mortality by CDDO-Imidazolide. Biochem Biophys Res Commun 351**:** 883-889.

To KF, & Lo AW (2004). Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2). J Pathol 203**:** 740-743.

Tomani JCD, Kagisha V, Tchinda AT, Jansen O, Ledoux A, Vanhamme L*, et al.* (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**:** in press.

Toniati P, Piva S, Cattalini M, Garrafa E, Regola F, Castelli F*, et al.* (2020). Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of 100 patients in Brescia, Italy. Autoimmun Rev 19**:** 102568.

Ucciferri C, Auricchio A, Di Nicola M, Potere N, Abbate A, Cipollone F*, et al.* (2020). Canakinumab in a subgroup of patients with COVID-19. Lancet Rheumatol 2**:** e457-ee458.

van den Berg DF, & Te Velde AA (2020). Severe COVID-19: NLRP3 Inflammasome Dysregulated. Front Immunol 11**:** 1580.

Vijayan V, Wagener F, & Immenschuh S (2018). The macrophage heme-heme oxygenase-1 system and its role in inflammation. Biochem Pharmacol 153**:** 159-167.

Vogelpoel LT, Hansen IS, Visser MW, Nagelkerke SQ, Kuijpers TW, Kapsenberg ML*, et al.* (2015). FcgammaRIIa cross-talk with TLRs, IL-1R, and IFNgammaR selectively modulates cytokine production in human myeloid cells. Immunobiology 220**:** 193-199.

Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, & Veesler D (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 183**:** 1735.

Walsh EE, Frenck R, Falsey AR, Kitchin N, Absalon J, Gurtman A*, et al.* (2020). RNA-Based COVID-19 Vaccine BNT162b2 Selected for a Pivotal Efficacy Study. medRxiv.

Wang LY, Cui JJ, Ouyang QY, Zhan Y, Guo CX, & Yin JY (2020). Remdesivir and COVID-19. Lancet 396**:** 953-954.

Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z*, et al.* (2020a). Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell 181**:** 894-904 e899.

Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y*, et al.* (2020b). Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 395**:** 1569-1578.

Wei C, Wan L, Yan Q, Wang X, Zhang J, Yang X*, et al.* (2020). HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. Nat Metab 2**:** 1391-1400.

Wen Y, Liu Y, Tang T, Lv L, Liu H, Ma K*, et al.* (2016). NLRP3 inflammasome activation is involved in Ang II-induced kidney damage via mitochondrial dysfunction. Oncotarget 7**:** 54290-54302.

Wong SK, Li W, Moore MJ, Choe H, & Farzan M (2004). A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 279**:** 3197-3201.

Wrapp D, De Vlieger D, Corbett KS, Torres GM, Wang N, Van Breedam W*, et al.* (2020). Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies. Cell 181**:** 1004-1015 e1015.

Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S*, et al.* (2020a). Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med 180**:** 934-943.

Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y*, et al.* (2020b). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B 10**:**766-788.

Wu J, Yang X, Zhang YF, Zhou SF, Zhang R, Dong XQ*, et al.* (2009). Angiotensin II upregulates Toll-like receptor 4 and enhances lipopolysaccharide-induced CD40 expression in rat peritoneal mesothelial cells. Inflamm Res 58**:** 473-482.

Xu X, Han M, Li T, Sun W, Wang D, Fu B*, et al.* (2020). Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A 117**:** 10970-10975.

Yamamoto M, Matsuyama S, Li X, Takeda M, Kawaguchi Y, Inoue JI*, et al.* (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. Antimicrob Agents Chemother 60**:** 6532-6539.

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**:** 1444-1448.

Yang J, Wang W, Chen Z, Lu S, Yang F, Bi Z*, et al.* (2020). A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. Nature 586**:** 572-577.

Yin W, Mao C, Luan X, Shen DD, Shen Q, Su H*, et al.* (2020). Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. Science 368**:** 1499-1504.

Zegeye MM, Lindkvist M, Falker K, Kumawat AK, Paramel G, Grenegard M*, et al.* (2018). Activation of the JAK/STAT3 and PI3K/AKT pathways are crucial for IL-6 trans-signaling-mediated pro-inflammatory response in human vascular endothelial cells. Cell Commun Signal 16**:** 55.

Zhang H, Penninger JM, Li Y, Zhong N, & Slutsky AS (2020). Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 46**:** 586-590.

Zhang J, Hao Y, Ou W, Ming F, Liang G, Qian Y*, et al.* (2020). Serum interleukin-6 is an indicator for severity in 901 patients with SARS-CoV-2 infection: a cohort study. J Transl Med 18**:** 406.

Zhou JH, Wu B, Wang WX, Lei F, Cheng X, Qin JJ*, et al.* (2020a). No significant association between dipeptidyl peptidase-4 inhibitors and adverse outcomes of COVID-19. World J Clin Cases 8**:** 5576-5588.

Zhou L, Huntington K, Zhang S, Carlsen L, So EY, Parker C*, et al.*(2020b). MEK inhibitors reduce cellular expression of ACE2, pERK, pRb while stimulating NK-mediated cytotoxicity and attenuating inflammatory cytokines relevant to SARS-CoV-2 infection. Oncotarget 11**:**4201-4223.

Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou LH*, et al.* (2020). Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 396**:**479-488.

Zinovkin RA, & Grebenchikov OA (2020). Transcription Factor Nrf2 as a Potential Therapeutic Target for Prevention of Cytokine Storm in COVID-19 Patients. Biochemistry (Mosc) 85**:** 833-837.

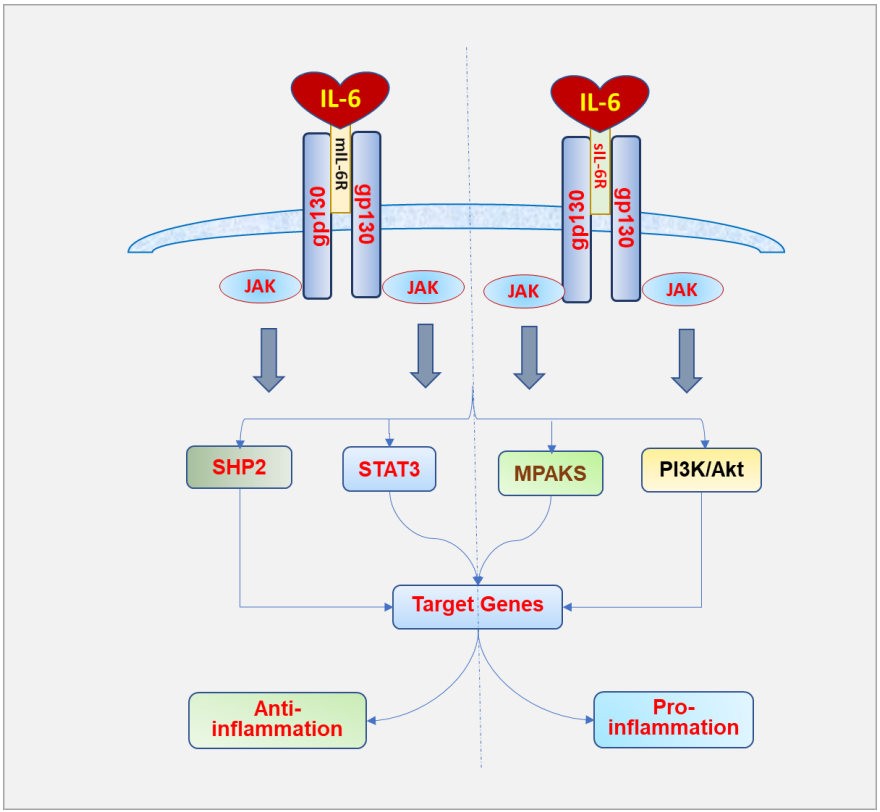

Figure 1. IL-6 mediates a series of cell signaling pathways. IL-6 binds to the membrane IL-6 receptor (mIL-6R) and soluble IL-6 receptor (sIL-6R). The binding of IL-6 to both receptors results in the dimerization and activation of the glycosylated type I membrane protein of 130–150 kDa (gp130). The dimerization 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.

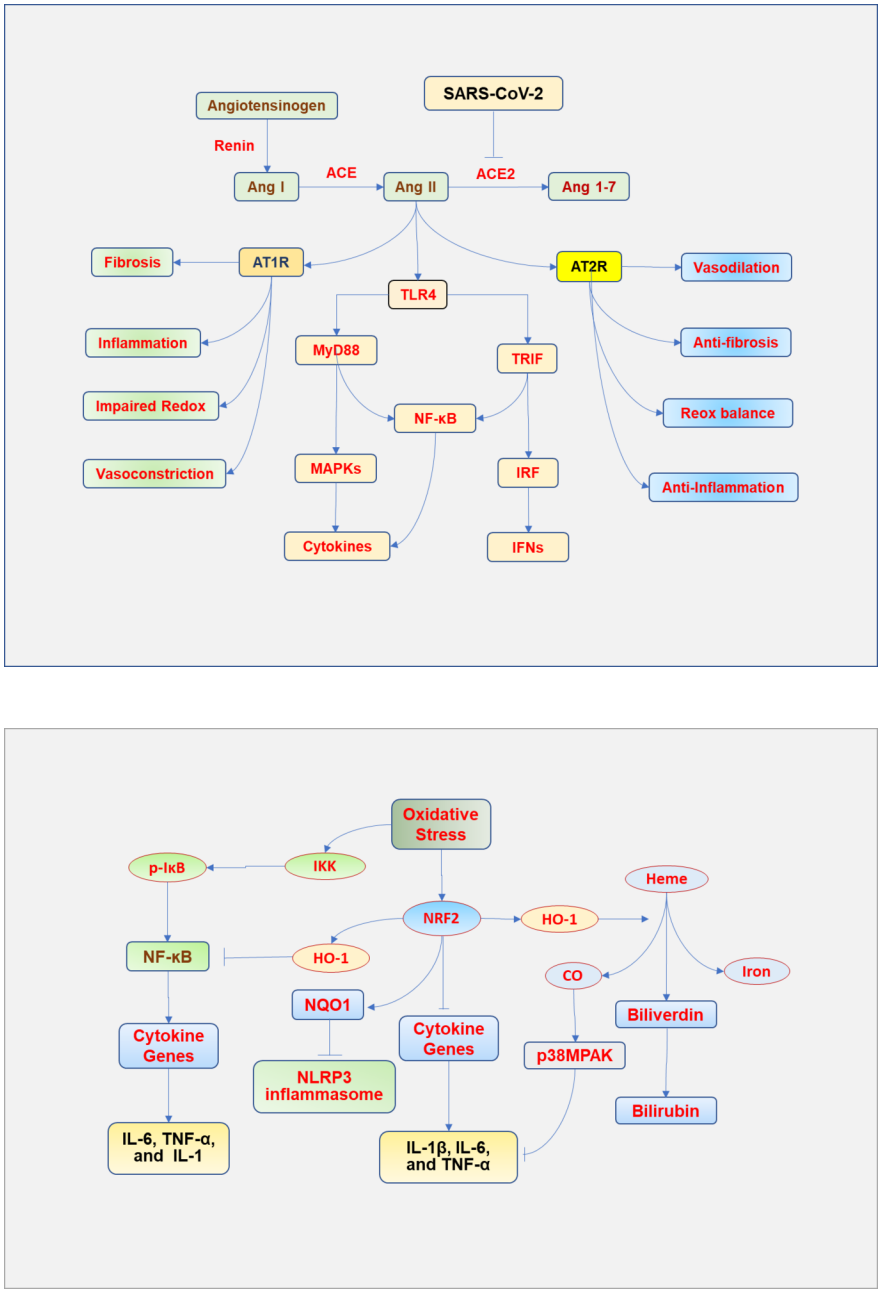

Figure 2. Angiotensin II (Ang II) is involved in the diction of cytokine storm during SARS-CoV-2 infection. In the renin-angiotensin system, renin cleaves angiotensinogen into angiotensin I (Ang I), which is converted into Ang II by the angiotensin-converting enzyme (ACE). While ACE2 functions to converse Ang II to Angiotensin 1-7 (Ang 1-7). The binding of the S-protein of SARS-CoV-2 to ACE2 on the cell surface 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, and 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 role of nuclear factor erythroid 2-related factor 2 (NRF2) induced cell signaling. In response to oxidative stress, activated 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 activation. In response to oxidative stress, activated IB kinase (IKK) promotes the phosphorylation of IB, resulting in the release and nuclear translocation of NF-B, which then promote the gene transcription of pro-inflammatory cytokines, such as IL-6, TNF-α, and IL-1. NRF2 can inhibit NF-B transcriptional activity directly or through activating HO-1.