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REVIEW

Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis

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SUMMARY Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), a rapidly evolving pandemic worldwide with at least 68 million COVID-19-positive cases and a mortality rate of about 2.2%, as of 10 December 2020. About 20% of COVID-19 patients exhibit moderate to severe symptoms. Severe COVID-19 manifests as acute respiratory distress syndrome (ARDS) with elevated plasma proinflammatory cytokines, including interleukin 1β (IL-1β), IL-6, tumor necrosis factor α (TNF-α), C-X-C motif chemokine ligand 10 (CXCL10/IP10), macrophage inflammatory protein 1 alpha (MIP-1α), and chemokine (C-C motif) ligand 2 (CCL2), with low levels of interferon type I (IFN-I) in the early stage and elevated levels of IFN-I during the advanced stage of COVID-19. Most of the severe and critically ill COVID-19 patients have had preexisting comorbidities, including hypertension,

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diabetes, cardiovascular diseases, and respiratory diseases. These conditions are known to perturb the levels of cytokines, chemokines, and angiotensin-converting enzyme 2 (ACE2), an essential receptor involved in SARS-CoV-2 entry into the host cells. ACE2 downregulation during SARS-CoV-2 infection activates the angiotensin II/angiotensin receptor (AT1R)-mediated hypercytokinemia and hyperinflammatory syndrome. However, several SARS-CoV-2 proteins, including open reading frame 3b (ORF3b), ORF6, ORF7, ORF8, and the nucleocapsid (N) protein, can inhibit IFN type I and II (IFN-I and -II) production. Thus, hyperinflammation, in combination with the lack of IFN responses against SARS-CoV-2 early on during infection, makes the patients succumb rapidly to COVID-19. Therefore, therapeutic approaches involving anti-cytokine/anti-cytokine-signaling and IFN therapy would favor the disease prognosis in COVID-19. This review describes critical host and viral factors underpinning the inflammatory "cytokine storm" induction and IFN antagonism during COVID-19 pathogenesis. Therapeutic approaches to reduce hyperinflammation and their limitations are also discussed.

KEYWORDS SARS-CoV-2, innate immunity, interferon, ACE2, inflammation, comorbidities, proinflammatory cytokines, cell surface receptors, intracellular signaling, antibodies

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative virus of the currently ongoing coronavirus disease 2019 (COVID-19) pandemic, originated in Wuhan, China during late 2019 (1, 2). The rapid increase in morbidity and mortality among COVID-19 cases gained extensive attention from national and federal policymakers in countries worldwide (3). Although controversy exists on the origin of this virus, SARS-CoV-2 is thought to be transmitted from bats to humans, potentially through a yet-to-be-determined intermediate host (4, 5). The World Health Organization (WHO) indicates that COVID-19 is accountable for about 68 million cases and 1.5 million deaths as of 10 December 2020 (6). SARS-CoV-2 infection in people results in a range of clinical outcomes, from asymptomatic to mild, moderate, or severe disease with symptoms that include high fever, cough, fatigue, and dyspnea followed by death due to respiratory failure (7, 8).

The severity of COVID-19 depends on multiple factors, such as host genetic makeup, aging, and preexistence with comorbid health conditions, including cardio-pulmonary diseases, diabetes, and hypertension (7, 9–11). Patients with chronic conditions, such as autoimmune diseases, cancers, and organ transplants undergoing immunosuppressive therapy, have a higher risk of developing severe disease. Host immunosuppression might potentially inhibit neutralizing antibodies in addition to inhibiting the cytokine storm. Therefore, it could delay the virus clearance. Recently, Chang et al. reported that delayed virus clearance increased the risk of death (12, 13). However, glucocorticoid (an immunosuppressive anti-inflammatory agent) administration (1 to 2 mg/kg for 3 to 5 days) to COVID-19 patients did not affect the duration of virus clearance (14); hence, the effect of inhibiting neutralizing antibodies on viral load appears to be dependent on the nature of the immunosuppressant used. ACE2 is part of the renin-angiotensin system (RAS), which acts as the receptor for SARS-CoV-2 (15–17). Although perturbation in ACE2 expression is associated with COVID-19 severity in these comorbidities (18), the causal link between disease severity and the levels of ACE2 remains unknown. It was reported that smoking enhanced ACE2 expression in the human lungs (19). Further clinical evidence shows that patients with severe COVID-19 had elevated proinflammatory cytokines, such as IL-1β, IL-2, IL-6, IL-7, IL-8, TNF-α, CCL2, MIP-1α, and CXCL10 (IP10). This surge in inflammatory molecules is also referred to as the "cytokine storm" in COVID-19 (20–24).

Exacerbated lung inflammation due to cytokine dysregulation is the underlying cause of respiratory failure in SARS-CoV-2-infected individuals. The severely ill patients with COVID-19 showed an impaired IFN (IFN-I and IFN-II) production and downregulation of

IFN-stimulated genes (ISGs) (25, 26). Upon virus interaction with the host cells, the pattern recognition receptors (PRRs), such as the Toll-like receptors (TLR3, TLR7, and TLR8), retinoic acid-inducible gene 1 (RIG-1), melanoma differentiation-associated protein (MDA5), and protein kinase C (PKC), engage virus-associated molecular patterns (27). TLR3, TLR7, and TLR 8 are expressed in the endosomal compartment of immune cells, including macrophages, dendritic cells (DC), natural killer (NK) cells, epithelial cells, and fibroblasts (28), whereas RIG-1 and MDA5 are localized in the cytoplasm (29). TLR7 recognizes SARS-CoV-2 single-stranded RNA, while TLR3, RIG-1, and MDA5 sense double-stranded RNA intermediates formed during viral replication (27, 29, 30). Activation of cellular signaling by these PRRs induces IFN-I production (31, 32). However, pathogenic viral repertoire components, including SARS-CoV proteins (e.g., nonstructural protein 1 (NSP1), NSP13, NSP14, membrane (M) protein, spike (S) protein, and N protein), can evade the innate host immunity by antagonizing the IFN response (33–36). Indeed, the IFN dysregulation by ORF3b, ORF6, ORF8, and N proteins of SARS-CoV-2 has been reported recently (36–38). *In vitro* studies revealed that SARS-CoV-2 was sensitive to IFN-I pretreatment, suggesting that early initiation of IFN-I therapy is essential to combat COVID-19 (39, 40).

The focus of this review is to analyze the cytokine induction and impairment of IFN response during COVID-19. It also discusses how to design potential therapeutic approaches to selectively inhibit inflammatory cytokine induction and enhance IFN-mediated antiviral functions and their potential risk factors during SARS-CoV-2 infection.

SARS-CoV-2 AND COVID-19

SARS-CoV-2 belongs to the genus *Betacoronavirus* (41) under the family *Coronaviridae* and order *Nidovirales* (1). It is an enveloped, spherical-to-pleomorphic virus with a diameter ranging from 60 to 140 nm (41, 42). The virus comprises a single-strand positive-sense RNA genome of about 29.9 kb nucleotides (2). The SARS-CoV-2 genome sequence and phylogenetic analysis revealed that it is more closely related to SARS-like coronaviruses (CoV) of bats than to SARS-CoV and Middle East respiratory coronavirus (MERS-CoV) (43). SARS-CoV-2 shares a nucleotide identity of 96.2% with bat coronavirus, whereas SARS-CoV has 79.5% identity with SARS-CoV-2 (44). This finding suggests that SARS-CoV-2 might have originated in bats. Due to the inherent feature of error-prone viral RNA polymerases, viruses will accumulate mutations during every replication cycle, leading to the formation of a diverse population of viruses in a single infected host (45). This process leads to the evolution of the viruses, contributing to "species-jumping." Indeed, COVID-19 is the third emerging CoV disease that originated from bats in recent years, preceded by SARS in 2002 and MERS in 2012 (46). However, the mode of transmission from bat to human is yet to be determined, although the human-to-human transmission of SARS-CoV-2 occurs primarily through aerosolized droplets generated during sneezing and coughing of patients with COVID-19 (47). According to a New York State Health Department report, about 90% of the case fatalities were associated with at least one of the comorbidities, such as hypertension, obesity, diabetes, hyperlipidemia, dementia, coronary artery disease, renal disease, atrial fibrillation, chronic obstructive pulmonary disease, cancer, and stroke (48).

COVID-19 PATHOLOGY

SARS-CoV-2 is commonly known to be transmitted by an aerosol route; however, other unidentified transmission modes should also be considered. The SARS-CoV-2 infection leads to mild/moderate disease symptoms in about 81% of patients with no or mild pneumonia; however, in 14% of cases, the symptoms are severe, including dyspnea and $\leq$93% of blood oxygen saturation. In 5% of COVID-19 cases, the disease symptoms are critical, marked with respiratory failure and multiple organ failure (10). Furthermore, COVID-19 patients with a mild disease show nonspecific symptoms, such as fever and nonproductive cough. In contrast, the moderate-to-severe illness is characterized by pneumonia, requiring hospitalization and ventilation support (49) (Table 1). Like other respiratory infections (e.g., influenza virus), SARS-CoV-2 infection of the lungs can breach

TABLE 1 COVID-19 disease and pathology in humans

COVID-19 symptom class	Clinical manifestations	Lesions/blood parameters	References
Generalized symptoms	Fever[a], anorexia[b], fatigue[a], headache[c], shivering[c], loss of smell and taste	Lymphopenia, leukopenia, elevated C-reactive protein, decreased oxygen saturation, thrombocytopenia, elevated proinflammatory cytokines, increased lactate dehydrogenase, hyponatremia, serum amyloid A, procalcitonin, ferritin, D-dimer and fibrinogen	189–192
Respiratory system	Cough[a], expectoration[a], chest tightness[b], shortness of breath[b], dyspnea[b], runny nose	Ground glass opacities in the lung on CT-scan, patchy consolidation, alveolar exudates, and interlobular involvement, pulmonary embolism/thrombi, alveolar septal vascular congestion, and edema, monocyte and lymphocyte infiltration	190, 191, 193
Gastrointestinal system	Pharyngalgia[c], nausea[c], vomiting[c], diarrhea[c], abdominal pain and discomfort[c]	Elevated AST and ALT[d]	192
Renal system	Proteinuria, hematuria, and acute kidney injury in 19.5 % to 75 % of COVID-19 patients.	Elevated creatinine, acute tubular necrosis, lymphocyte infiltration, $CD68^+$ macrophages in the interstitium, C5b-9 deposition on tubules, luminal brush border sloughing, hyaline casts, microthrombi, and mild interstitial fibrosis	192, 194, 195
Ocular system	Epiphora, conjunctival congestion, foreign body sensation, itching, dry eye	Hemorrhages in retina	196
Musculoskeletal system	Muscle soreness[b], backpain		190
Cardiovascular system	Cardiac arrhythmia, hypovolemia, dehydration	Hypercoagulopathy, myocardial injury	189, 193
Neurological system	Headache, dizziness, loss of taste and smell, ataxia, seizures, confusion, Loss of consciousness in severe cases	Cerebral thrombosis, cerebral hemorrhage	190, 197, 198

[a]Most common symptoms.
[b]Frequently observed symptoms.
[c]Less common symptoms.
[d]AST, aspartate transaminase; ALT, alanine transaminase.

the innate immune barriers, such as epithelial integrity, and make the patient susceptible to secondary infections by opportunistic pathogens residing in the respiratory tract. The severe manifestations of COVID-19 can be complicated by pulmonary secondary bacterial infections and generalized septicemia. However, by including broad-spectrum antibacterial drugs in the COVID-19 treatment regimen, the complications due to secondary bacterial infection in hospitalized patients might be minimized (50, 51).

Though COVID-19-associated lesions can occur in multiple organs, significant and prominent changes in the gross and histopathological features are observed mostly in the lungs. Computed tomography diagnosis with bilateral ground-glass opacities in the lungs is a critical pathognomonic feature in moderate and severe COVID-19 patients (52). In deceased COVID-19 patients, lungs had bilateral pleural effusion, pleural adhesion, multifocal consolidated areas with hemorrhages, and hepatization (53). On histological examinations, the lungs show diffuse alveolar damage with various degrees of hyaline membrane formation, desquamation of alveolar epithelia, extensive infiltration of alveolar macrophages and scattered neutrophils and lymphocytes into alveolar spaces with sero-muco-fibrinoid exudates, mucinous exudates in bronchi and bronchioles, peribronchiolar metaplasia, interstitial fibrous hyperplasia, and focal hemorrhages in alveoli and interstitial tissues (53).

Furthermore, the endothelial cells of small pulmonary arteries are swollen and shed into the lumen, and small- to large-sized thromboemboli are seen in small pulmonary arteries and postcapillary venules (52, 53). In the lungs, alveolar macrophages are either scattered or appear as clusters of giant cells expressing proinflammatory cytokines

(53). The postmortem findings of the heart show multifocal myocardial infarction, myocardial atrophy, and interstitial fibrous hyperplasia. Kidneys show fibrotic glomeruli and edematous tubular epithelium (53). The presence of SARS-CoV-2 has been demonstrated in various organs, including the respiratory tract, kidney, heart, brain, liver, spleen, intestine, brain, and blood (54, 55).

The severity of COVID-19 was reported to be associated with the cytokine storm and impairment of type I IFNs (IFN-α and IFN-β) production. Expression of ACE2, a key SARS-CoV-2 entry receptor, has been reported in various human tissues, including nasal mucosa, olfactory neuroepithelium, larynx, sinuses, bronchi, type II pneumocytes of lungs, endothelial cells of blood vessels, and the intestinal tract (56–58). Prominent expression of ACE2 was also noted in immune cells, such as macrophages of the lungs (alveolar macrophages), lymph nodes and spleen, and blood monocytes of different organs (53, 58). Following infection with SARS-CoV-2, these myeloid cell types show induction of cytokines such as IL-6 and TNF-α expression (53). These proinflammatory cytokines play a primary role in tissue injury and the formation of thromboemboli, acute respiratory failure, and multiorgan failure. Similarly, in severe cases of COVID-19, lack of IFN-I induction and downregulation of ISGs are observed, despite high viral load in the blood (25, 26).

Although the SARS-CoV-2 receptor ACE2 is expressed in multiple organs and cell types at greater levels than in the lungs, COVID-19 severity correlates mainly with the lung pathology. It indicates that the underlying cause for severe COVID-19 might be the activation of inflammatory cells and the release of inflammatory molecules.

CYTOKINE STORM IN COVID-19

Clinical studies indicate that the severity of COVID-19 positively correlates with the levels of inflammatory cytokines, including IL-1β, TNF-α, monocyte chemoattractant protein 1 (MCP-1)/CCL2, IL-2, sIL-2RA, IL-6, IL-7, IL-17, IL-18, granulocyte colony stimulating factor (G-CSF), IP10, macrophage colony stimulating factor (M-CSF), MIP-1α/CCL3, MCP-3, and anti-inflammatory cytokines such as IL-10 in the plasma/serum of patients (25, 59). The key symptoms of COVID-19 cases, such as inflammatory cytokine storm, multiorgan failure, and acute respiratory distress syndrome (ARDS), follow a similar pathological course as hemophagocytic lymphohistiocytosis (HLH), including high fever, dyspnea, lymphopenia, and elevated cytokines, including IL-1β, TNF-α, and IL-6, serum ferritin, D-dimers, and C-reactive protein (CRP) (20, 60, 61). HLH is a life-threatening hyperinflammation (cytokine storm) condition mediated by aberrant activation of NK cells, T cells, and macrophages; it can be genetic or acquired (62). Acquired HLH is common in adults and is induced by external triggers, including viral infections (e.g., Epstein-Barr virus or herpes simplex virus) (60). Therefore, it is possible that SARS-CoV-2 infection might induce HLH that progresses to multiorgan failure and ARDS in some patients (60). Further, pediatric inflammatory, multisystem syndrome temporally associated with COVID-19 (PIMS-TS) in children is a novel condition identified during early 2020 (63, 64). Pediatric patients with unremitting fever, inflammation (elevated CRP, neutrophilia, and lymphopenia), single- to multiorgan failure (cardiac, respiratory, renal, gastrointestinal, and/or neurological), including features of Kawasaki disease (a rare pediatric vasculitis in children), are defined as PIMS-TS patients (63–66). The PIMS-TS patients show either nonspecific symptoms or a Kawasaki disease-like phenotype, including high fever, exanthema, mucosal changes, and swollen extremities (67, 68). PIMS-TS in children is potentially induced through SARS-CoV-2 infection and mediated by excessive cytokine production and associated hyperinflammation (63, 66, 68). This acute surge in several inflammatory cytokines following SARS-CoV-2 infection (cytokine storm/hypercytokinemia) is one of the hallmarks of COVID-19, leading to ARDS, disseminated intravascular coagulation, and multiple organ failure. Among the cytokines, levels of IP-10, MCP-3, IL-2, IL-7, IL-10, G-CSF, MCP-1, MIP-1α, and TNF-α are significantly elevated in severe COVID-19 cases that require intensive care and ventilation for oxygen support (59, 69). In these cases, induction of cytokines is mediated through the following: (i) the angiotensin II/AT1R pathway (16, 70); (ii) the ACE2 signaling pathway (71, 72); and (iii) the innate immune signaling routes, including the PRRs such as TLRs, RIG-1, and MDA5

pathways (36) and nucleotide-binding oligomerization domain (NOD), leucine-rich repeat domain (LRR), and pyrin domain containing protein 3 (Nlrp3) inflammasomes (73, 74).

Modulation of ACE2 Expression by SARS-CoV-2 and Comorbidities

The ACE2 receptor is a critical component of the renin-angiotensin system (RAS), wherein angiotensinogen is cleaved by renin into angiotensin I and is converted into angiotensin II by ACE. Angiotensin II is an active component of RAS; it binds to the AT1R receptor and controls blood pressure and the immune system, leading to vasoconstriction and inflammation, as well as tissue injury. ACE2 converts angiotensin II to angiotensin 1-7, which counterbalances the angiotensin II-mediated effects by exerting vasodilation and anti-inflammation (17). In fact, augmenting ACE2 function or blocking angiotensin II function is beneficial in treating heart diseases (17, 75).

The binding of SARS-CoV-2 to host ACE2 makes the latter molecule unavailable/incapable of converting angiotensin II to host-protective and anti-inflammatory peptide angiotensin 1-7. While ACE2 expression is higher in children, the levels reduce during postnatal life (76), which could be a reason for the low incidence and less severe COVID-19 in children (77). In rats, ACE2 expression decreased with aging, and no significant gender differences exist between young and middle-aged rats in the levels of ACE2, although a higher ACE2 level was noted in the older female rats than in male rats (78). A high ACE2 level in plasma has been associated with a protective effect against influenza virus infection and in COVID-19-mediated lung injury (79). This effect is due to the angiotensin II/AT1R-mediated upregulation of inflammatory components CCL-2, IL-8, and CCL-5 together with reactive oxygen species (ROS) (80). The ROS induce transcription factors, nuclear factor kappa B (NF-κB), and activator protein 1 (AP-1) and contribute to inflammation. Further, angiotensin II induces TLR4 expression and activation, maturation of DCs via NF-κB, extracellular-signal-regulated kinase (ERK) 1/2, and signal transducer and activator of transcription 1 (STAT1) pathways, proliferation, and migration of T cells, causing increased ROS production and immune cell chemotaxis from circulation to the site of inflammation (81). Angiotensin II-stimulated, CCL2/chemokine receptor type 2 (CCR2)-mediated macrophage activation also induces the levels of IL-6, TNF-α, IL-1β, and other cytokines (82, 83). In endothelial cells, angiotensin II increases the expression of cell adhesion molecules, P-selectin, and L-selectin, involved in cellular homeostasis and inflammation (84).

Although treatment with AT1R antagonists is beneficial in protecting against inflammation and tissue injury, results from AT1R knockout (KO) mice showed that this receptor might have an immunomodulatory effect (17, 80). In contrast, mice overexpressing ACE2 were susceptible to SARS-CoV infection (85). Sward and coworkers report that the soluble ACE2 levels in humans were low in both males and females of ~8 to 12 years old and were comparable between the sexes until the age of 12, while with increasing age up to 24 years, the levels were higher in males than in females (86). This study suggests that an increase in soluble ACE2 is indirect evidence for the presence of membrane-bound ACE2 and that SARS-CoV binding to ACE2 might enhance cleaving of the membrane-bound ACE2 into a soluble form by a disintegrin and metallopeptidase domain 17 (ADMA17) protein (85, 86). However, experimental and/or clinical evidence for the causal link between soluble/membrane-bound ACE2 and SARS-CoV infectivity is lacking.

In healthy individuals, ACE2 expression is higher in the colon than other organs, including gallbladder, heart, kidney, epididymis, breast, ovary, lung, prostate, esophagus, tongue, liver, pancreas, and cerebellum (87, 88). However, in severe COVID-19 cases, significant lung injury and respiratory failure are noted (8, 44, 89). Therefore, it appears that the levels of ACE2 expression in a tissue/organ are not directly related to the extent of tissue damage observed during COVID-19. On the contrary, studies have shown increased ACE2 expression, and thus more receptors available for SARS-CoV-2 infection, in individuals with comorbid health conditions who are vulnerable to severe COVID-19 (18, 19). These observations suggest that the outcome of SARS-CoV-2 infection is dependent on factors other than mere ACE2 expression levels and includes the virus inoculum, duration of exposure, and the host immune status (90, 91). The virus does not need to occupy many ACE2

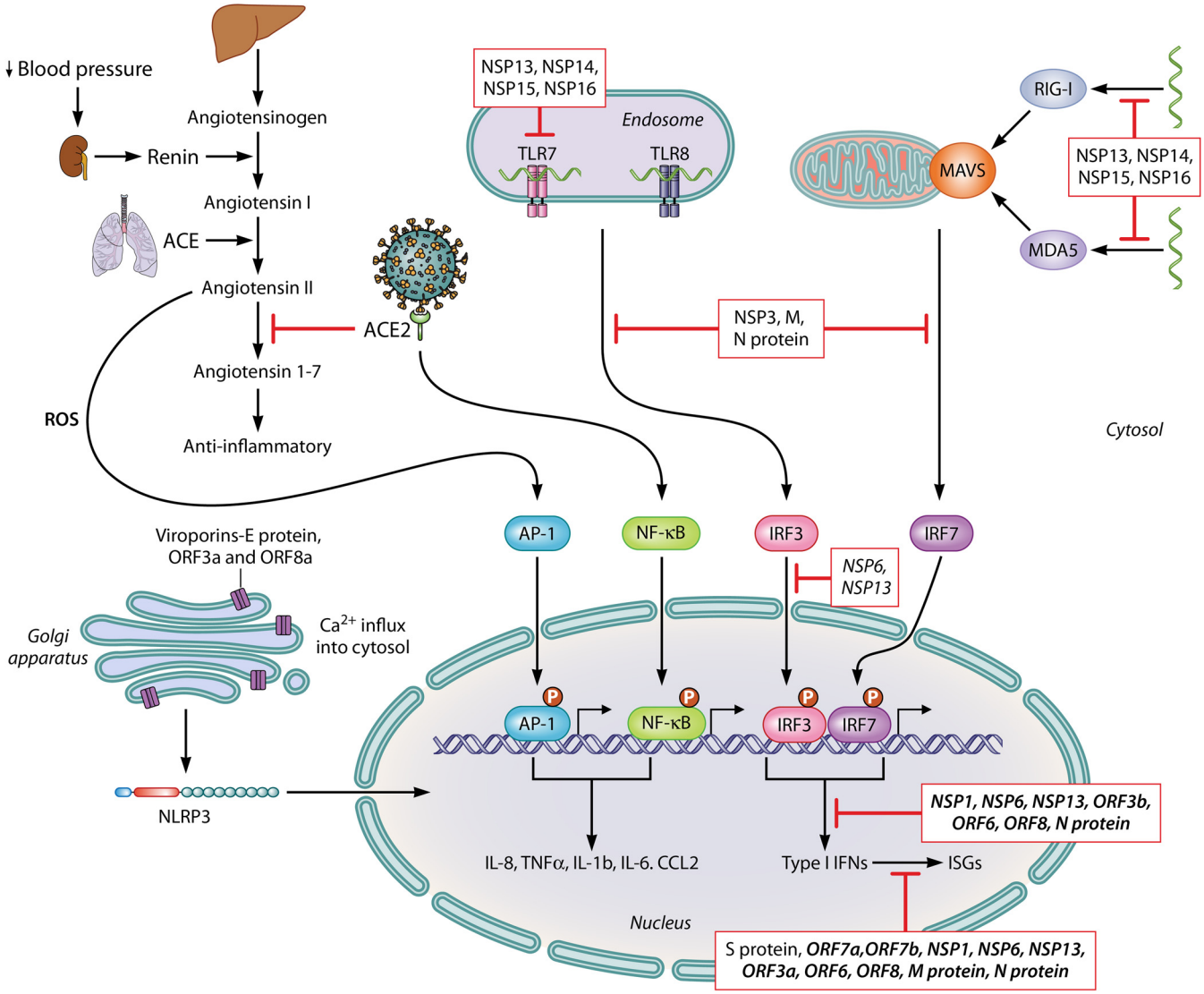

FIG 1 Interaction of cellular pathways and networks for cytokine induction and IFN antagonism during COVID-19. The SARS-CoV-2 proteins that potentially inhibit IFN induction, IFN signaling, and ISGs are indicated in bold italics. NSP, nonstructural proteins; M, matrix protein; N, nucleoprotein; S, spike protein; ORF, open reading frame; inhibitor, blocks the putative pathways and acts as an IFN antagonist.

receptors to mount a successful infection; if it did, then severe COVID-19 complications would involve a high degree of colon and kidney injury rather than lung injury.

Moreover, severe lung pathology in COVID-19 cases potentially involves multifaceted mechanisms, including ACE2, putative receptors such as vimentin, the cluster of differentiation 209 (CD209)/cluster of differentiation 209 ligand (CD209L) virus entry factors, and activation of inflammatory cells. Nonetheless, severe disease pathology and elevated death rates occur among elderly COVID-19 patients and those with comorbidities such as hypertension, cardiovascular disorders, cigarette smoking, diabetes, respiratory infections, and usage of the anti-inflammatory drug ibuprofen (11, 18, 92). These conditions have been reported to decrease or increase ACE2 expression in a context-dependent fashion, as reported in various studies (18, 19, 93). In general, SARS-CoV-2 infection in people with comorbidities showed ACE2 deficiency and increased severity of COVID-19 (81) (Fig. 1).

ACE2 Signaling

The attachment of the spike (S) protein of SARS-CoV with the ACE2 receptor activates a downstream signaling cascade that ultimately leads to elevated cytokine levels (71, 72). Chang and coworkers reported that the recombinant baculovirus expressing the SARS-CoV

S protein induced IL-8 production by lung epithelial and fibroblast cell lines, compared to the control virus lacking the S protein. The S protein fragment (amino acids [aa] 324 to 688) is responsible for the IL-8 induction, mediated through the mitogen-activated protein kinase (MAPK) and AP-1 activation (71). The level of CCL2 was upregulated in Vero E6 cells treated with purified S protein or virus-like particles (VLPs) containing the S protein of SARS-CoV (72). Furthermore, S protein attachment activated ACE2 production by casein kinase II-mediated phosphorylation, followed by Ras, c-Raf to extracellular signal-regulated protein kinase 1/2 (ERK1/2), and AP-1 signaling (72). Notably, the induction of inflammatory molecules IL-8 and CCL2 was independent of NF-κB signaling (71, 72) (Fig. 1). However, inflammatory cytokine induction through S protein and VLP-ACE2-mediated signaling has not been reported. Future studies using S protein treatment of ACE2-expressing macrophages, such as lung resident and $CD68^+$ $CD169^+$ macrophages, found in spleen and lymph nodes, and their cytokine profile analysis, are needed for a detailed understanding of ACE2 signaling-mediated cytokine induction during COVID-19 (94, 95). Elucidating the host components of inflammatory cytokine induction pathways might help to develop pathway-specific inhibitors to combat COVID-19.

Innate Immune Activation in Viral Infections

The PRRs, including TLR3, TLR7, TLR8, RIG-1, and MDA5, are present in various immune cells and can bind to various viral components (31). The host cell receptors, including RIG-1 like receptors (RLRs), RIG1, and MDA-5, recognize viral RNAs with 5′ triphosphate or blunt ends, RNAs lacking 2-O' methylation, and double-stranded RNA (dsRNA) intermediates, while TLR7/8 binds to single-stranded RNA (ssRNA), which signals to phosphorylate interferon regulatory factor 3 (IRF3)/IRF7 (32) and activate NF-κB (31), which promotes transcription of cytokines such as TNF-α, IL-1β, and IL-6. Further, the cytosolic NOD-like receptors (NLRs) recognize viral RNAs and other intracellular stimuli and activate inflammasomes. The SARS-CoV-encoded proteins, envelope (E), ORF3a, and ORF8a, can act as viroporins, which form calcium ion channels in the endoplasmic reticulum-Golgi apparatus intermediate in infected cells (73, 96, 97). The change in intracellular calcium homeostasis activates NLRP3-mediated inflammasomes, which induces the cleavage and secretion of IL-1β and IL-18. Then, it will further stimulate the inflammatory cascade by inducing IL-6 and TNF-α production in alveolar macrophages and pulmonary tissues (74) (Fig. 1). These mechanistic pathways are utilized by SARS-CoV-2-encoded E, ORF3a, and ORF8a proteins to activate inflammasomes through NLRP3, which plays a crucial role in cytokine storm and tissue injury.

Role of Transcription Factors in the COVID-19 Cytokine Storm

Studies on SARS-CoV-2-mediated cytokine induction report that the upregulation of the NF-κB signaling pathway is the key to activating cytokine storm and hyperinflammation (98, 99). NF-κB is a family of inducible transcription factors, which are sequestered in the cytoplasm by inhibitory proteins called IκB. The activation of NF-κB and subsequent nuclear translocation are mediated through the degradation of IκB (100). SARS-CoV-2 S protein interaction with ACE2 induces a higher expression of NF-κB than SARS-CoV S protein (101), which could be due to the higher binding affinity of SARS-CoV-2 S with ACE2 (101). The peripheral blood mononuclear cells (PBMCs) from COVID-19 patients showed elevated levels of inflammatory NF-κB pathway signaling mediators, IL1R1, myeloid differentiation factor 88 (MYD88), IL-1R-associated kinase (IRAK1), TNF receptor- associated factor 6 (TRAF6), NFκB1, and Rel-like domain-containing protein A (RELA) (102). Neufeldt et al. reported specific activation of the NF-κB pathway, but not the IRF3 pathway, in SARS-CoV-2-infected A549-ACE2 cells (99). Further, they observed that NF-κB activation was mediated by cyclic CMP-AMP synthase (cGAS)-stimulator of interferon genes (STING, a cytoplasmic DNA sensor of stress) (99). Inhibition of nuclear translocation of NF-κB diminished the virus- or LPS-induced cytokine storm (101). Although SARS-CoV S and N proteins activate the NF-κB pathway and downstream cytokines (103, 104), the S protein was reported to induce CCL2 through the activation of the Ras-ERK-AP-1 pathway (72).

In addition, TLR4, TLR4 ligand, S100 calcium-binding protein A9 (S100A9), an alarmin, and TLR4 signaling mediators CD14, MYD88, IRAK1, TRAF6, Toll-interleukin 1 receptor (TIR) domain-containing adaptor protein (TIRAP), and TIR domain-containing adaptor molecule (TICAM) were also found to be upregulated in the PBMCs of COVID-19 patients (102). In *in vitro* experiments using human PBMCs, the recombinant SARS-CoV-2 S2 and N protein were found to activate the inflammatory cascade, including TLR4 ligand and S100A9, and the activation of TLR4 signaling would potentially amplify NF-κB activation and thereby could aggravate cytokine storm (102). In the PBMCs of COVID-19 patients, NF-κB activation leads to activation of sterol regulatory element-binding protein 2 (STREBP2), a cholesterol synthesis regulator. Induction of STREBP2 was found to enhance cytokine storm and the upregulation of STEBP2 was correlated with severe COVID-19 (105). Thus, it appears that SARS-CoV-2 infection activates multiple transcription factors that regulate the production of inflammatory molecules.

IMPAIRMENT OF IFN PRODUCTION IN COVID-19

During CoV infection, PRRs, including TLR3, TLR7, TLR8, RIG-1, and MDA5, recognize the viral RNA and activate IRF3/IRF7 to induce IFN-I production (21). Though the TLRs, RIG-1, and MDA5 receptors activate the signaling to produce IFN-I and other cytokines, COVID-19 is characterized by elevated cytokine levels and dampened IFN-I response. IFN-I exhibit antiviral functions by inducing the transcription of ISGs, which restrict unique steps of viral replication. Thus, the ability of SARS-CoV-2 to downregulate the host IFN-I response is considered a viral strategy to evade host immunity (21).

In general, type I IFNs, such as IFN-α and IFN-β, are key antiviral factors. Host cells treated with type I IFNs significantly inhibited SARS-CoV replication. In contrast, pretreatment of cells with type II IFN (IFN-γ) did not show any viral inhibition (106). However, Mossel et al. reported that IFN-α and IFN-γ showed a synergistic effect in inhibiting SARS-CoV replication *in vitro* (107). The onset of the IFN-I response differs strikingly, depending on the severity of COVID-19. Thus, in humans, a strong IFN-I response was noted in early stages of infection with mild COVID-19 symptoms, while at later stages of infection in severe cases, delayed onset of IFN-I response has been observed (21, 108). In severe cases of COVID-19, the IFN-I response was undetectable during the early stage of infection. Delayed induction of IFN-I was reported to enhance hyperinflammation, by recruiting monocytes and macrophages in human ACE2 (hACE2)-expressing mouse models of COVID-19 (109, 110). On single-cell RNA analysis of PBMCs from severe COVID-19 patients, IFN-I was upregulated along with other hyperinflammatory cytokines (109). Contrarily, analysis of plasma samples from critically ill COVID-19 patients revealed that a proportion of the samples did not show any IFN-α. Moreover, in a subset of COVID-19 cases with mild symptoms, no IFN-α was detected in the plasma (111). Transcriptomic analysis of SARS-CoV-2-infected A549 cells overexpressing ACE2 exhibited a very low induction of type I and III IFNs and ISGs (26). In the postmortem lung samples of COVID-19 patients, the levels of both IFN-I and IFN-III were undetectable (26). Moreover, only a limited subset of ISGs, including bone marrow stromal antigen 2 (BST-2 or tetherin or CD317), IFI30, and interferon-induced transmembrane protein (IFITM), were upregulated in SARS-CoV-2-infected cells (26, 112). The IFN-I-mediated SARS-CoV-2 restriction might be mediated through BST-2, a type II transmembrane receptor that acts as a defense against enveloped viruses, including retroviruses, filoviruses, influenza virus, SARS-CoV, and human coronavirus 229E (HCoV-229E). BST-2 binds to the viral envelope glycoprotein, tethers the virion on the plasma membrane during assembly, and inhibits virus release from the infected host cell (113, 114). In HeLa cells overexpressing ACE2 transfected with the SARS-CoV-2 matrix (M) gene, BST-2 was found to be colocalized with the M protein and inhibited SARS-CoV-2 virion release (112). BST-2 KD HeLa-ACE2 cells showed an increase in SARS-CoV-2 virus titer over the wild-type HeLa-ACE2 cell line (115). These studies suggest that BST-2 plays a crucial role in the pathogenesis of COVID-19. Interferon inducible transmembrane protein (IFITM) uniquely restricts the cellular entry of several viruses, including influenza virus, Ebola virus, and CoV (116). The cellular entry of SARS-CoV-2 was observed to be inhibited

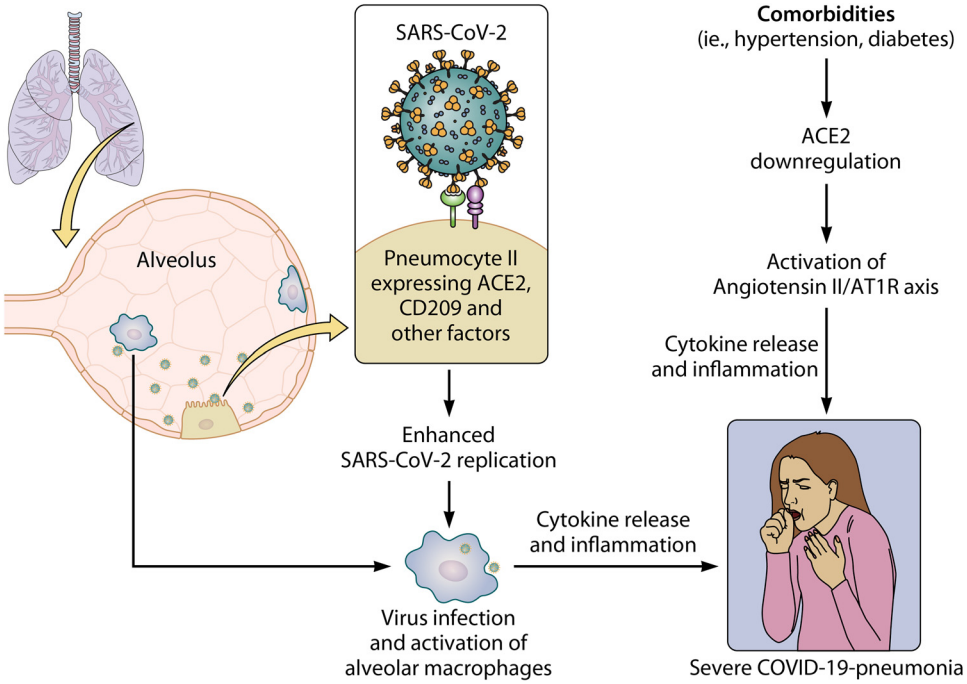

FIG 2 Potential mechanisms that downregulate ACE2 expression and enhance cytokine storm in COVID-cases with comorbidities. Induction of ACE2, CD209, and other host factors enhances SARS-CoV-2 replication and infection, which further activates macrophages to produce inflammatory cytokines. In comorbid conditions, such as hypertension and diabetes, downregulation of ACE2 can activate macrophages and the renin-angiotensin system, leading to cytokine storm and inflammation.

by IFITM1, 2, and 3 (117, 118); their expression is induced by type I and type II interferons. These IFITMs restrict the cathepsin L cleavage of S protein and S protein-mediated entry and replication of SARS-CoV and SARS-CoV-2 (118–120).

IFN ANTAGONISM BY SARS-CoV-2 AND RELATED CoVs

To overcome the IFN-mediated host immune defense, several families of viruses have evolved mechanisms to antagonize either recognition by PRRs, the IFN production pathway, or the functions of ISGs. CoVs, including SARS-CoV, MERS-CoV, and mouse hepatitis virus (MHV), are well known to antagonize IFNs through nonstructural proteins (NSPs) and structural proteins (33, 121, 122). The SARS-CoV-2 proteins, through their IFN-antagonistic activities, contribute to the low IFN-α and IFN-γ levels observed in COVID-19 patient samples (25, 111). Indeed, the administration of IFN-I early during disease leads to a favorable prognosis in COVID-19 patients (36). Like the ORF3b of SARS-CoV, the SARS-CoV-2 ORF3b also has potent anti-IFN-I activity, although the latter ORF is truncated at 22 amino acids, due to the presence of a premature stop codon, compared to the SARS-CoV ORF3b (37). Studies have also shown that ORF6, ORF8, and the N protein of SARS-CoV-2 can inhibit the expression of IFN-β and ISGs (38). Further, screening of overexpression of a panel of 27 SARS-CoV-2 proteins in 293FT cells (human embryonic kidney 293 derived cells) revealed the ORF6 protein strongly inhibited the promoter activity of both type I (IFN-α2 and IFN-β) and type III (IFN-λ) IFNs and ISGs. Other SARS-CoV-2 proteins, including NSP13, NSP14, and NSP15, also showed similar inhibitory effects (123). Moreover, ORF6, NSP13, NSP14, and NSP15 inhibited the nuclear localization of IRF3, and ORF6 inhibited STAT1, a key regulator of IFN signaling, in 293FT cells (123). In human dendritic cells (DCs), SARS-CoV-2 was reported to antagonize the phosphorylation of STAT1 and abolish IFN production (124) (Fig. 2).

The following section summarizes the various mechanism of IFN antagonism by SARS-CoV and MERS-CoV and their relevance to SARS-CoV-2 proteins that have potential IFN antagonism during COVID-19.

Evasion from Cellular Detection

The CoV 5′-capped RNA and NSPs in the replication-transcription complex (RTC) consist of capping enzymes, NSP13 (RNA triphosphatase), NSP14 (N7-methyltransferase), and NSP16 (2′-*O*-MTase) (125, 126). It was reported that the 2′-*O*-methylation by NSP16 contributes to CoV pathogenesis through innate immune evasion. Consistently, 2′-*O*-methyltransferase (MTase)-deficient MHV (a CoV infecting mouse) induces an antiviral type I IFN response (125).

RNA triphosphatase and Cap 1 MTase. NSP13 is a Cap 1 MTase essential for the addition of Cap 1 (127). While Cap 1 is methylated at the 2′ position of the first ribose, Cap 2 is methylated at the 2′ position of the first two riboses in the 5′ end of mRNA/viral RNA (128). The 2′-*O*-methyltransferase function is associated with K-D-K-E (lysine-aspartate-lysine-glutamate) residues in NSP13 (129). Since these residues are conserved among SARS-CoV, MERS-CoV, and SARS-CoV-2, it is logical to assume that the SARS-CoV-2 might exploit the NSP13 to generate a methylated cap on the viral RNA. Furthermore, the NSP13 of SARS-CoV possesses nucleoside triphosphatase (NTPase) and RNA helicase activities (125). Similarly, the SARS-CoV-2 NSP13 protein overexpressed in *Escherichia coli* had NTPase and RNA helicase-unwinding activities in a dose-dependent manner (130). Considering RNA helicase's role in viral pathogenesis, targeting the NSP13 activity might be a useful antiviral target against COVID-19 (131).

The 3′ to 5′ exonuclease activity and N-7 MTase. The NSP14 of CoV possesses exonuclease activity essential for proofreading and replication fidelity of the viral genome. In addition to the proofreading function, the exonuclease enzyme can cleave RNA-PAMPs (pathogen-associated molecular patterns) and result in evasion of recognition by PRRs. MHV with an aspartate-to-alanine mutation at aa 89 (D89A) and glutamate-to-alanine mutation at aa 91 (E91A) showed a lack of exonuclease activity and was more sensitive to IFN pretreatment of infected murine bone marrow-derived macrophages. The MHV without exonuclease activity did not confer resistance to IFN-β over MHV with exonuclease activity (122). However, the MHV NSP14 lacking exoribonuclease did not induce IFN and RNase L expression, which could be due to multiple IFN antagonists encoded by MHV (122). The MHV NSP14 and the SARS-CoV-2 NSP14 share 46% homology at the amino acid level and both have conserved a DE motif, which is responsible for the exonuclease activity. It was recently reported that the SARS-CoV-2 NSP14 inhibits the IFN promoter activity (123).

The 2′-*O*-MTase. The NSP16 of MHV showed 2′-*O*-methyl transferase (2′-*O*-MTase) activity, mediated by the D130 residue. Similar to NSP13, NSP16 of feline CoV also possesses the K-D-K-E tetrad (132). Importantly, SARS-CoV-2 has a conserved D130 residue, which could mediate the formation of a 2′-*O*-methylated cap in the viral RNA. Thus, a SARS-CoV NSP16 D130A mutant, lacking 2′-*O*-MTase activity, was more sensitive to IFN response, particularly for interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) (36, 133).

Endoribonuclease. CoVs infecting vertebrates encode endoribonuclease in NSP15. Overexpression studies using SARS-CoV endoribonucleases suggest that NSP15 can function as an IFN antagonist. NSP15 cleaves the RNA-PAMPs and prevents the activation of host innate immune pathways, similar to pestiviruses' envelope glycoprotein (E^{rns}) RNase activity (123). However, the precise mechanism of action remains to be determined. During CoV replication, the viral RNAs were found in the lumen of a double-membrane vesicle, while NSP3, NSP5, and NSP8 were at the outer membrane of vesicles. This observation suggests that SARS-CoV can prevent recognition by PRRs through NSPs, although this notion needs to be validated through further studies. Further, overexpression studies showed that NSP15 and NSP16 could inhibit IFN promoter activity (123).

Inhibition of IFN Gene Expression

The NSP1 of SARS-CoV suppresses host gene expression and mRNA degradation and inhibits the translation of several proteins, including IFN, in infected cells. *In vitro* assays using cell culture showed the NSP1 protein binds to the 40S ribosome subunit and blocks the capped mRNA- and internal ribosomal entry site (IRES)-dependent mRNA translation. The K164A and H165A mutations abolished the functions of NSP1, such as in mRNA degradation and translation inhibition (134). The NSP1 proteins of of SARS-CoV and SARS-CoV-2 share

84% amino acid homology (135) and the K164 and H165 residues are conserved between both viruses. Similar to SARS-CoV, the NSP1 of SARS-CoV-2 binds to the 40S ribosome and shuts down mRNA translation in cell culture systems (136). Further, SARS-CoV-2 NSP1 was found to inhibit the nuclear RNA export factor 1 (NXF1)-mediated nuclear export of cellular mRNA by interfering with the interaction of NXF1 with mRNA adapters and the nuclear pore complex (137). Thus, NSP1 of SARS-CoV-2 acts at multiple levels and could potentially contribute to the downregulation of IFN production (136, 137).

Inhibition of PRR-Mediated Signaling Pathways

The papain-like protease (PLpro) or NSP3 of CoVs cleaves the viral polyprotein to produce NSP1 and NSP4 (138). In addition to the protease activity, PLpro of SARS-CoV has IFN-antagonizing activity independent of protease domains. *In vitro* studies using cell culture showed the PLpro core domain inhibits the Sendai virus- and poly(I·C)-mediated IFN induction (33). Specifically, PLpro inhibited the TLR3/RIG-1/MDA5 signaling by inhibiting the phosphorylation, dimerization, and nuclear localization of IRF3 (139). Similarly, SARS-CoV-2 PLpro was reported to inhibit the phosphorylation of IRF3, nuclear localization of IRF3, and to cleave interferon-stimulated gene 15 protein (ISG15) from IRF3 (140). PLpro of SARS-CoV inhibits the stimulator of interferon gene (STING)-mediated RIG-I signaling. On encountering dsRNA, RIG-I signals through TRAF3, which stimulates the inhibitor of nuclear factor kappa-B kinase/IκB kinase (IKK) dependent kinase TBK1, resulting in phosphorylation and dimerization of IRF3 required for nuclear translocation (32). The interaction of TRAF3 with STING is essential for TRAF family member-associated NFKB activator (TANK)-binding kinase 1 (TBK1)-mediated activation of IRF3 (141, 142). The PLpro of SARS-CoV binds to TRAF3, STING, and TBK1 and inhibits the polyubiquitination of RIG-I, TRAF3, STING, TBK1, and IRF3, which is required for downstream signaling, thereby affecting the formation of the TRAF3-STING-TBK1 complex. Further, SARS-CoV-2 PLpro also inhibits the phosphorylation of TBK1 (140). Hence, SARS-CoV-2 PLpro inhibits IRF3 activation and antagonizes the type I IFN response (140, 141). The PLpro core protein domains of SARS-CoV and SARS-CoV-2, excluding the protease domain, share 82% amino acid homology (140). Interestingly, overexpression of SARS-CoV-2 PLpro in 293FT cells did not affect the IFN promoter activity; further, these overexpressing cells lacked deubiquitination (123).

In SARS-CoV-infected cells, the M protein is predominantly localized in the Golgi complex and aids in virus assembly. Among various proteins of SARS-CoV that inhibit IFN production, the M protein contributes significantly. Specifically, the M protein inhibits dsRNA-induced IFN production in virus-infected host cells. Overexpression studies of the M and transducer proteins of the IFN production pathway in HEK293 cells revealed that M protein associates with RIG1, TBK1, inhibitor of nuclear factor kappa-B kinase subunit ε (IKKε), and TRAF3, and suppresses the transcriptional activities induced by RIG1, MDA5, TBK1, and IKKε (143). The TRAF3-TANK-TBK1-IKKε complex formation is essential for the downstream activation of IRF3/IRF7. However, physical interaction between the M protein and TRAF3, TBK1, and IKKε inhibits the TRAF3-TANK-TBK1-IKKε complex formation by abolishing the binding between TBK1 and TRAF3, and TRAF3 and IKKε. Thus, neither activated IRF3 nor IFN transcripts were observed in SARS-CoV-infected cells (143).

In vitro studies show that overexpression of SARS-CoV-2 proteins, including NSP1, NSP6, NSP13, and ORF6, can inhibit IFN-β induction (144). In similar experiments, SARS-CoV-2 proteins NSP1, NSP6, NSP7, NSP13, ORF3a, M, ORF6, ORF7a, and ORF7b inhibit IFN-I signaling. Further, SARS-CoV-2 NSP6 and NSP13 proteins bind the host TBK1 and inhibit the phosphorylation of IRF3, while the viral ORF3 binds the karyopherin α2 and inhibits IRF3 nuclear translocation (144). Proteome analysis on A549 cells expressing ORF9c indicated the downregulation of IFN signaling, cytosolic PRRs, antigen presentation, and complement activation (145).

The N protein is another viral protein found in abundance in CoV-infected host cells. The PRRs, such as TLR3 and cytosolic RIG-1 like receptors (RLRs), recognize and respond to RNA viruses. The cytoplasmic receptor RIG-1 binds to 5′-ppp RNA and short dsRNA in RNA virus-infected cells via its helicase and repressor domain. Following recognition, a tripartite-motif-family protein 25 (TRIM25) E3 ligase ubiquitinylates the caspase recruitment domain (CARD) of RIG-1. The ubiquitinated RIG-1 further activates the signaling cascade through virus-

induced signaling adaptor (VISA), resulting in type I IFN production. In SARS-CoV-infected cells, the viral N protein interacts with TRIM25 and inhibits the ubiquitination of RIG-1, which abolishes the downstream signaling, and type I IFN production is dampened (146). Unlike the SARS-CoV N protein, both the N terminus and the C terminus of the MERS-CoV N protein inhibit IFN-I production. Further, the N proteins of SARS-CoV and MHV (another member of the *Betacoronavirus* genus) interact with the protein activator of protein kinase R (PACT). This recently identified signaling molecule activates the viral RNA-recognizing PRRs RIG-1 and MDA5, leading to a downstream signaling cascade that induces IFN-I production (121). PACT binds to RIG-1 and activates RIG-1 by stimulating its ATPase activity. The N-PACT complex abolishes PACT-mediated activation of RIG-1/MDA5, thereby inhibiting IFN-I production, transcription of ISG, and antiviral defenses (121).

Inhibition of ISGs

Bone marrow stromal antigen 2 (BST-2) is a lipid raft-associated protein, expressed in B cells, plasma, and a subset of DCs; however, BST-2 is also inducible in different cell types in response to IFN-I. BST-2 inhibits the HCoV-229E and SARS-CoV-2 VLP release from the cell membrane. However, several pathogenic viruses evade the inhibitory effect of BST-2 via their proteins, such as the glycoprotein (GP) of ebolaviruses and the S protein of CoVs (113). *In vitro* experiments with cultured cells show that BST-2 colocalizes with the SARS-CoV S protein and tethers the virus from budding and release. Further, the SARS-CoV S protein alleviates BST-2-mediated human immunodeficiency virus 1 (HIV-1) restriction by dampening BST-2 via the lysosomal degradation pathway (113, 114).

The SARS-CoV ORF7a is a type I transmembrane protein localized in the Golgi apparatus and cell membranes. However, when coexpressed with BST-2, ORF7a interacts with BST-2 and is mostly found on the cell surface (147). Stewart et al. reported that HeLa cells transfected with SARS-CoV-2 S protein showed downregulation of BST-2 in S-expressing cells (115). Coexpression analysis showed that ORF7a reduces BST-2 expression, although it did not affect the surface localization of BST-2. Furthermore, ORF7a binds to unglycosylated BST-2 and prevents it from getting glycosylated. Importantly, this N-linked glycosylation is necessary for BST-2-mediated restriction of SARS-CoV (147). The release of a SARS-CoV-2 ORF7a deletion mutant in infected cells was lower in BST-2-expressing cells than in wild-type SARS-CoV-2. This result indicates that the SARS-CoV-2 ORF7a protein antagonizes the BST-2 inhibition of virus release (112). However, the specific residues in ORF7a responsible for viral release through bone marrow stromal antigen 2 (BST-2) evasion are yet to be identified.

IMMUNOTHERAPY TO DAMPEN CYTOKINE STORM IN COVID-19

Immunotherapy involves tweaking host immunity through treatment with small molecule immunomodulatory agents to treat a disease. It has been used to treat autoimmune diseases, hyperinflammatory diseases, and cancer (148, 149). The overt induction of inflammatory cytokines that lead to inflammation and tissue injury during COVID-19 suggests that the suppression of inflammatory cytokines or their signaling pathway could help recovery and reduce mortality (23, 53) (Fig. 3A and B). Therefore, specific inflammatory cytokine(s) and/or their signaling pathway inhibitors have been tested as an adjunct to the standard-of-care treatment regimen for COVID-19 in various clinical studies.

Anti-IL-6 Receptor Antibodies

IL-6 is essential for the maturation and differentiation of cytotoxic T cells, monocyte function, and differentiation of B cells to plasma cells (150, 151). Therefore, inhibition of IL-6 could dampen the inflammatory responses elicited by innate and adaptive immune cells.

Tocilizumab. Tocilizumab is a monoclonal antibody targeting the IL-6 receptor (IL-6R), which exists in both soluble and membrane-bound forms and blocks the binding and signaling of IL-6 (152) (Fig. 3). Tocilizumab has been used to treat rheumatoid arthritis (RA) and other inflammatory disorders (152). However, tocilizumab usage in RA patients is associated with severe herpesvirus (cytomegalovirus and varicella-zoster virus) infections (153). Recent clinical and radiological evidence shows an improvement in disease pathology among

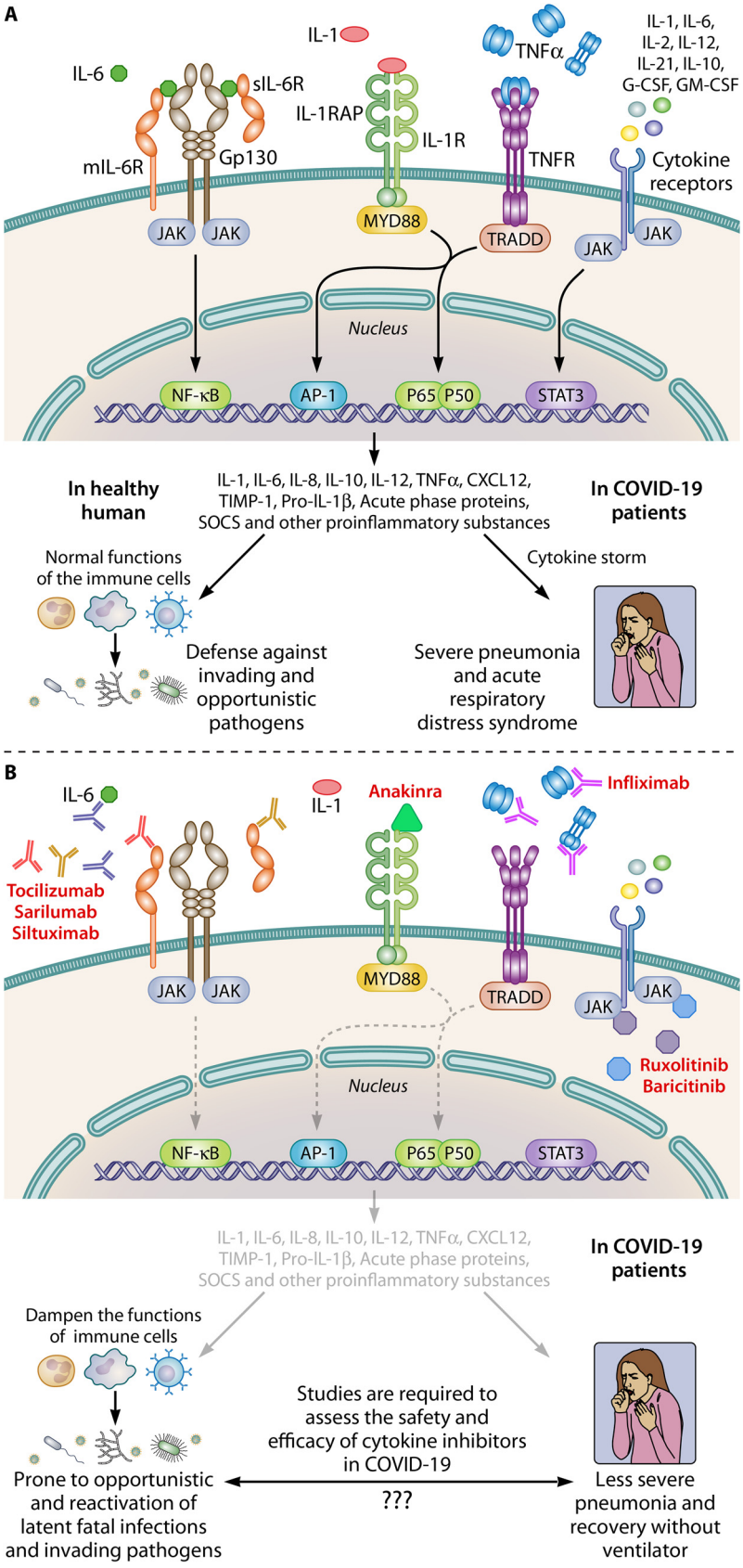

FIG 3 Cytokine storm-mediated hyperinflammation and therapeutic strategies during COVID-19. (A) The proinflammatory cytokines, such as IL-1, IL-6, and TNF-α, mediate the signaling to induce

(Continued on next page)

severe COVID-19 patients treated with tocilizumab. Furthermore, tocilizumab therapy did not affect the antiviral antibody production in treated COVID-19 patients, but it delayed the viral clearance compared to the control/placebo-treated group (153). In this study, however, the initial viral load in the COVID-19 patients in the tocilizumab treatment group was higher than the control/placebo group (153). Thus, the delayed viral clearance in the former group might be due to increased viral load at the beginning of treatment.

The Roche COVACTA phase III clinical trial on tocilizumab (trade name Actemra/RoActemra) reported no significant change in the clinical improvement, percentage of mortality, or ventilator requirement for COVID-19 patients between treated and untreated control groups (154). Clinical investigations reported high incidences of bacterial pneumonia, visceral aspergillosis, hepatitis B reactivation, and herpes simplex virus I reactivation among COVID-19 cases treated with tocilizumab, compared to the control group (155). A patient was reported dead due to liver failure caused by herpes simplex virus reactivation following tocilizumab treatment (155). These adverse effects are also of significant concern in using IL-6 monoclonal antibodies to treat COVID-19 cases.

A polymorphism in the IL-6R (174G/C) allele leads to elevated IL-6 production and severe pneumonia, impacting the clinical presentation and treatment of COVID-19 cases (156). However, the causal role of this polymorphism on COVID-19 severity is yet to be determined. Therefore, future studies in preclinical models are warranted to understand the mechanistic link between IL-6R and disease severity and evaluate the beneficial-versus-adverse effects of targeting IL-6R for COVID-19 treatment.

Sarilumab. Sarilumab, trade name Kevzara, is a human monoclonal antibody against IL-6R. It possesses a high-affinity binding ability to soluble and membrane-bound forms of IL-6R and is approved for RA treatment (157, 158) (Fig. 3). However, in phase III clinical trials conducted by Sanofi and Regeneron, sarilumab did not show any significant beneficial effects on COVID-19 patients. Furthermore, the frequency of multiorgan dysfunction and hypotension was higher in the treated COVID-19 cases than in patients who did not receive sarilumab (159).

Anti-IL-6 Monoclonal Antibody

Siltuximab. Siltuximab is an IL-6-neutralizing monoclonal antibody that inhibits the IL-6 signaling cascade (Fig. 3). In a clinical observational study, siltuximab treatment of COVID-19 patients reduced plasma CRP and IL-6 levels and reduced mortality compared to patients in the placebo group (160). However, due to fewer patients recruited in that study, the results need to be interpreted cautiously. Additional, extensive clinical studies with a large patient cohort are required to assess siltuximab's efficacy and safety to use in COVID-19 patients.

IL-1 Receptor Antagonist

Anakinra. Early during SARS-CoV-2 infection, activation of the NLRP3 inflammasome induces the IL-1β signaling cascade that elevates IL-6 production, a critical inflammatory marker of the cytokine storm in COVID-19 cases (74, 161). A recombinant IL-1 receptor antagonist (IL-1-RA) called anakinra blocks binding of proinflammatory cytokines, IL-1α, and IL-1β to their cognitive receptors (161–163) (Fig. 3). The FDA has approved anakinra for the treatment of RA and for cryopyrin-associated periodic syndromes (162). In general, blockade of proinflammatory cytokines has a risk of elevating opportunistic and secondary bacterial and fungal infections due to immune suppres-

FIG 3 Legend (Continued)
several inflammatory substances and cytokines, including IL-1, IL-6, TNF-α, IL-8, IL-10, and IL-12, which are essential for the functions of the immune system in healthy humans. (B) Overt induction of these cytokines in COVID-19 and their association with hyperinflammation suggest that the use of cytokine signaling blockers, such as IL-6R antibodies (tocilizumab, sarilumab), anti-IL-6 antibody (siltuximab), IL-1R antagonist (anakinra), or JAK1/2 inhibitors (ruxolitinib, baricitinib), might reduce cytokine induction and the severity of COVID-19. On the other hand, inhibition of cytokines would cause immunosuppression and make the patient susceptible to opportunistic infections and reactivation of latent life-threatening infections. Further studies are required to recommend/block these agents as COVID-19 therapeutics.

sion (164). However, episodes of such opportunistic infections, including reactivation of latent *Mycobacterium tuberculosis* infection (LTBI), were rare in RA patients treated with anakinra; furthermore, this drug was beneficial in reducing the bacterial inflammatory diseases (162, 163, 165, 166). In a clinical study, treatment with anakinra improved the survival rate of COVID-19 patients from 56% to 90% (163). However, in that small cohort study, patients also received hydroxychloroquine, ritonavir, lopinavir, and noninvasive ventilation (163). Another study reported improved respiratory function and no death among 22 COVID-19 patients administered with anakinra (161). Other clinical studies have also shown that treatment with anakinra reduced fever and plasma CRP levels and improved the respiratory function of COVID-19 cases (161, 163, 166, 167). These observations suggest that early initiation of anakinra therapy would help reduce inflammation and the requirement for mechanical ventilation, and improve respiratory functions.

Anti-TNF-α Antibody

Infliximab. A therapeutic drug capable of inhibiting multiple inflammatory mediators is of great importance to control "cytokine storm" during COVID-19. TNF-α is one of the most prominent cytokines responsible for hyperinflammation during several noninfectious and infectious diseases, including COVID-19. The FDA approved the use of TNF-α inhibitors, including infliximab, an anti-TNF-a monoclonal antibody, to treat autoimmune and inflammatory conditions, such as RA. Usage of TNF-α inhibitor during RA resulted in inhibition of IL-1, IL-6, and other inflammatory mediators (168). A preliminary clinical observation study on infliximab usage showed a reduction in IL-6, CRP, and a favorable prognosis of treated COVID-19 patients (168, 169). However, additional and more extensive clinical data and randomized studies are needed before recommending infliximab for COVID-19 treatment. Another concern is that TNF-α inhibitors can reactivate LTBI in a vulnerable population by their immunosuppressive nature (170, 171). Furthermore, a polymorphism in TNF-α (G308A) was shown to be associated with increased susceptibility to SARS-CoV-2 infection and severe disease symptoms among COVID-19 cases (172). Therefore, it is crucial to screen the COVID-19 patients for LTBI and additional factors, such as genetic predisposition, before starting anti-TNF-α antibody therapy for COVID-19 patients (Fig. 3).

Janus-Kinase Inhibitors

Ruxolitinib. Ruxolitinib is a selective inhibitor of Janus kinase (JAK) 1 and 2, where JAK signaling plays a pivotal role in proinflammatory cytokine-mediated host inflammatory response during infection/disease (173) (Fig. 3). Ruxolitinib-mediated inhibition of JAK reduces the activity of multiple cytokines and chemokines, including TNF-α, IL-1, IL-6, IL-8, IL-12, IFN-γ, GM-CSF, G-CSF, and platelet-derived growth factor (PDGF). Ruxolitinib is approved to treat hyperinflammatory conditions, such as polycythemia vera and primary myelofibrosis. However, ruxolitinib's broad-spectrum anti-inflammatory effects can potentially reduce viral clearance and potentially induce reactivation of LTBI in COVID-19 cases. In a limited number of severe COVID-19 patients, treatment with ruxolitinib decreased the level plasma levels of IL-6, CRP, and ferritin and showed clinical improvement (174). However, a recent phase III clinical trial on ruxolitinib's use for COVID-19 cases showed disappointing results (175). There was no significant difference observed in the rate of respiratory failure, ventilator requirement, and death rate of COVID-19 patients between the ruxolitinib and control treatment group (175). Further, ruxolitinib would cause serious side effects, such as reactivation of LTBI and other serious infections, skin cancers, and diffuse erythematous skin eruptions (176).

Baricitinib. Baricitinib is a potent inhibitor of JAK 1, 2, and AAK1, a numb-associated kinase (NAK) that regulates clathrin-mediated endocytosis of cells (177). Thus, baricitinib could potentially inhibit both the SARS-CoV-2 entry and dampen the host proinflammatory cytokine production (178, 179). However, there is no experimental evidence on baricitinib-mediated inhibition of SARS-CoV-2 endocytosis and entry into host cells. Baricitinib showed a reduction in ACE2 expression in primary liver cell culture and reduced the viral load (178). Whether the inhibition of ACE2 expression

involves JAK signaling-mediated transcription is yet to be studied. In COVID-19 patients with moderate pneumonia, baricitinib showed a reduction in inflammatory markers (IL-6 and CRP) and nasopharyngeal viral load and mortality rate (180). Although the adverse effects of immunosuppression in COVID-19 patients are not fully known, baricitinib treatment was associated with hepatitis B virus reactivation (181).

INTERFERONS AS THERAPEUTICS FOR COVID-19

The type I IFNs (IFN-α and IFN-β) play a crucial role in COVID-19 pathogenesis. Dysfunction of type I IFN (IFN-I) signaling is associated with severe COVID-19, suggesting that IFN therapy could favor virus clearance. Moreover, downregulation of IFN-I was reported in patients with severe COVID-19 (108). In experimental animal studies, delayed induction of IFN response during SARS infection resulted in the accumulation of monocytes, macrophages, secretion of inflammatory cytokines, vascular leakage, and immunopathology, leading to a severe form of the disease (36, 108). Therefore, early administration of IFN-I might potentially enhance virus clearance.

In COVID-19 clinical trials, daily subcutaneous injection of IFN-α2a did not significantly change the number of hospitalizations and ventilation supports compared to the control group. In contrast, faster recovery from disease was observed in COVID-19 cases treated with intranasal IFN-α2a or IFN-α2b administration by nebulization (182–184). In a randomized trial with a limited number of patients, IFN-1β was administered subcutaneously to SARS-CoV-2-infected patients hospitalized within 7 days of onset of symptoms and every other day for 7 days in addition to lopinavir/ritonavir and ribavirin. The study showed the IFN-1β therapy group achieved virus clearance within significantly shorter duration and shorter hospital stays compared to the patients treated with lopinavir/ritonavir and ribavirin alone (185). However, these studies were not well controlled and did not include critically ill patients, and further research is necessary to assess the efficacy and safety of IFN therapy for COVID-19. A randomized controlled trial is ongoing to determine the effectiveness of IFN-β1b compared to IFN-β1a in moderate-to-severe COVID-19 patients, and the outcome is yet to be available (186). A detailed report on the advantages and disadvantages of IFN-I therapy and the status of ongoing clinical trials to evaluate various IFN-I molecules for COVID-19 therapy has recently been published (187).

POTENTIAL ADVERSE EFFECTS OF IMMUNOTHERAPY FOR COVID-19

The human respiratory system harbors numerous bacterial (e.g., *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp.) and fungal (e.g., *Aspergillus* spp., *Cryptococcus* spp.) species as commensals that have the potential to be opportunistic pathogens (188). As the proinflammatory cytokines play a crucial host-protective role in immune cells, any significant inhibition of these molecules would make the host susceptible to opportunistic pathogens. Indeed, respiratory syncytial virus, influenza virus, pneumovirus, and herpes simplex virus infections are common in individuals receiving immunosuppression therapy for other diseases, such as cancer (188). Moreover, inhibition of TNF-α and IL-6 by corresponding antibody therapy has the potential for reactivation of LTBI, hepatitis B, and herpes simplex virus in a vulnerable population (155, 170, 171). Thus, using immunotherapeutic approaches to inhibit the "cytokine storm" in COVID-19 should be considered very carefully (Fig. 3A and B). The effectiveness of tocilizumab, anakinra, and infliximab for COVID-19 therapy needs extensive clinical investigation since these therapeutics are associated with bacterial pneumonia, visceral aspergillosis, hepatitis B reactivation, and herpes simplex virus I reactivation (155). The experimental studies using preclinical models on these immunotherapeutic agents would facilitate a better understanding of the effective dose, duration, initiation of treatment, and potential side effects. Data from such studies would help fine-tune the therapeutic effect of those drugs, without immunosuppression, for COVID-19. Further, screening of COVID-19 patients for coexisting latent infections should be considered a prerequisite to initiating immunotherapy. At present, findings from experimental and clinical studies are

yet to reveal whether the benefits of inhibiting proinflammatory cytokine(s) outweigh the potential side effects mediated through immunosuppression.

CONCLUSIONS

The pathology of severe COVID-19 involves multifaceted immune mechanisms, including ACE2 downregulation and IFN antagonism. The immune evasion properties of SARS-CoV-2 and its proteins are actively involved in inhibiting the host restriction factors, including IFN signaling and ISG induction. Although treatment with recombinant IFN-α, -β, -γ, and ACE2 have indicated some beneficial results against COVID-19 in limited pilot studies, additional and extensive clinical data are necessary to optimize immunotherapy as efficient disease management for COVID-19 (36). Additionally, downregulation of ACE2 expression can activate the inflammatory "cytokine storm" signaling, leading to enhanced severity in COVID-19 cases. Therefore, blocking angiotensin II activity during ACE2 deficiency (AT1R axis-mediated induction of cytokine storm) by AT1R blocker (losartan), or ACE inhibitor, could help control hyperinflammation. These drugs have been prescribed as anti-hypertensive agents for several decades and could be used safely for COVID-19 treatment. A better understanding of the molecular events involved in host-SARS-CoV-2 interactions would shed light on developing improved therapeutic agents to selectively inhibit the hyperinflammation signaling pathway, enhance early IFN response, and aid in rapid viral clearance and better COVID-19 management.

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Santhamani Ramasamy (S.R.) and Selvakumar Subbian (S.S.) conceived the concept and designed the manuscript outline. S.R. drafted the manuscript and prepared the figures and S.S. subsequently edited the manuscript. Both authors have read and approved the manuscript.

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