Number 54 • Volume 4 October 2020

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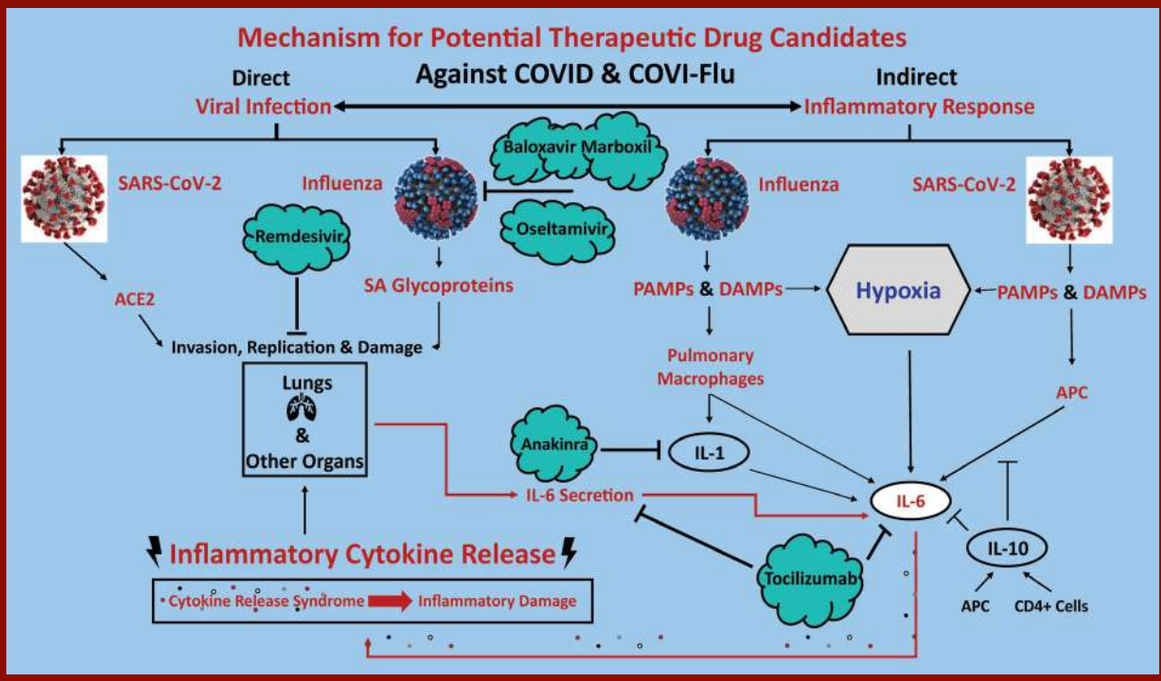
Injury, Inflammation, and Sepsis: Laboratory and Clinical Approaches



Mechanism for Potential Therapeutic Drug Candidates for COVID & Covi-Flu. Known pharmacological agents may act to halt the progress of SARS-CoV-2 and Covi-Flu. This model proposes the use of one or more of the widely available drugs Anakinra, Tocilizumab, Remdesivir, Ibrutinib, Oseltamivir and Baloxavir Marboxil. Their respective mechanistic intervention is provided both here and in the accompanying table.

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SHOCK, Vol. 54, No. 4, pp. 438–450, 2020

Review Article

POTENTIAL IMMUNOTHERAPEUTIC TARGETS FOR HYPOXIA DUE TO COVI-FLU

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Received 31 May 2020; first review completed 13 Jun 2020; accepted in final form 6 Jul 2020

ABSTRACT—The world is currently embroiled in a pandemic of coronavirus disease 2019 (COVID-19), a respiratory illness caused by the novel betacoronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The severity of COVID-19 disease ranges from asymptomatic to fatal acute respiratory distress syndrome. In few patients, the disease undergoes phenotypic differentiation between 7 and 14 days of acute illness, either resulting in full recovery or symptom escalation. However, the mechanism of such variation is not clear, but the facts suggest that patient's immune status, comorbidities, and the systemic effects of the viral infection (potentially depending on the SARS-CoV-2 strain involved) play a key role. Subsequently, patients with the most severe symptoms tend to have poor outcomes, manifest severe hypoxia, and possess elevated levels of pro-inflammatory cytokines (including IL-1*b*, IL-6, IFN-*g*, and TNF-*a*) along with elevated levels of the anti-inflammatory cytokine IL-10, marked lymphopenia, and elevated neutrophil-to-lymphocyte ratios. Based on the available evidence, we propose a mechanism wherein SARS-CoV-2 infection induces direct organ damage while also fueling an IL-6-mediated cytokine release syndrome (CRS) and hypoxia, resulting in escalating systemic inflammation, multi-organ damage, and end-organ failure. Elevated IL-6 and hypoxia together predisposes patients to pulmonary hypertension, and the presence of asymptomatic hypoxia in COVID-19 further compounds this problem. Due to the similar downstream mediators, we discuss the potential synergistic effects and systemic ramifications of SARS-CoV-2 and influenza virus during co-infection, a phenomenon we have termed ''COVI-Flu.'' Additionally, the differences between CRS and cytokine storm are highlighted. Finally, novel management approaches, clinical trials, and therapeutic strategies toward both SARS-CoV-2 and COVI-Flu infection are discussed, highlighting host response optimization and systemic inflammation reduction.

KEYWORDS—COVID-19, cytokine release syndrome (CRS), IL-10, IL-6, mesenchymal stem cell therapy

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel betacoronavirus, first emerged in Wuhan, China in December 2019. SARS-CoV-2 causes a respiratory illness termed coronavirus disease 2019 (COVID-19) (1, 2). With a median incubation period of 4 to 5 days (3 – 5), COVID-19 patients usually present with dry cough, fever, and fatigue. However, a highly heterogenous collection of symptoms has been reported, including respiratory, neurological, gastrointestinal, cardiovascular, and cutaneous manifestations, potentially due to SARS-CoV-2 strain variabilities (3, 6). This disease tends to peak within 6 days of symptom onset, and manifests as atypical pneumonia with diffuse alveolar damage and progressive respiratory failure, which may evolve

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YL: Idea conception; Primary and initial author for design, framework, and refinement of SARS-CoV-2 and COVI-Flu models; Review of literature; Summarizing and organizing of data; Primary and main manuscript writing contribution; Participation in post-peer review manuscript revisions.

TE: Review of literature; Intellectual contribution to the design, framework, and refinement of the proposed SARS-CoV-2 and COVI-Flu models; Writing of the abstract and several manuscript sections.

SSR: Intellectual contribution to the design, framework, and refinement of the SARS-CoV-2 and COVI-Flu models; Creating a digital representation of the proposed model; Critical input into the written work.

SPS: Review of literature; Intellectual contribution to the proposed SARS-CoV-2 model; Addition of substantial, clinically oriented written content; Critical input into the written work; Participation in post-peer review manuscript revisions.

PN: Input into the manuscript.

SD: Input into the manuscript.

SG: Input into the manuscript and intellectual contribution for COVI-Flu and hypoxia.

IC: Input into the manuscript and intellectual contribution for COVI-Flu and hypoxia.

PS: Review of literature and writing of manuscript; Critical input into the written work.

All authors read, reviewed, and approved the final manuscript.

The authors report no conflicts of interest.

DOI: 10.1097/SHK.0000000000001627

into critical illness involving multi-organ dysfunction and fatality rate of > 50% in approximately 15% of patients (1, 3, 7).

Autopsy findings of pulmonary tissue show bilateral diffuse alveolar injury with cellular fibromyxoid exudates. Interstitial lymphocytic infiltration is also apparent. Most of the infiltrated immune cells within the alveoli are noted to be CD4+ T lymphocytes (7, 8). Of interest, peripheral blood CD4+ and CD8+ T-cell counts tend to be greatly reduced, despite appearing to be in a hyperactive state (9). Moreover, the CD4+ T-cell fraction harbored a substantially increased percentage of pro-inflammatory CCR6+ Th17 cells, and CD8+ T-cells were highly positive for cytotoxic granules. The most severely affected patients also have highly elevated serum levels of IL-2, IL-6, IL-10, IFN-*g*, IFN-*b*, and TNF-*a*, along with decreased CD4+ and CD8+ T-cell counts (9). A lower proportion of regulatory T-cells (Tregs) is usually correlated with more severe disease (10).

Taken together, these findings support the conceptual framework that SARS-CoV-2 infection selectively weakens, hyperactivates, and misdirects the immune response, either directly or indirectly, via an inflammatory cascade with the resultant multi-organ dysfunction. However, it is important to note that this inflammatory process is distinct from the classically reported cytokine storm, characterized by a several-fold increase of systemic symptoms and multi-organ failure. Rather, SARS-CoV-2 infection induces a similar, but less severe, hyperinflammatory state called cytokine release syndrome (CRS) (11). Additionally, mortality from COVID-19-induced CRS is primarily due to respiratory failure, while that in cytokine storm is due to status epilepticus or distributive shock (12). Based on this information, we propose a model for how the SARS-CoV-2-mediated inflammatory response leads to systemic inflammation, multi-organ dysfunction, and subsequent morbidity and mortality.

SARS-CoV-2 INFECTION: DIRECT AND INDIRECT MECHANISMS FOR SYSTEMIC INJURY

SARS-CoV-2 is primarily transmitted through respiratory droplets (7, 13), a route especially relevant in the healthcare setting (5, 14). Upon entering the body, the virus primarily infects epithelial cells in the upper aerodigestive tract (e.g., nasal and oropharyngeal surfaces), where it reproduces asymptomatically for approximately 2 to 4 days (7), followed by clinical manifestations of the viral illness, including systemic inflammatory damage through both direct and indirect pathways (Fig. 1).

Direct pathway for systemic injury in SARS-CoV-2 infection

Once inside the respiratory tract, the spike glycoprotein (SG) on the viral surface binds to angiotensin-converting enzyme 2 (ACE2) on the surface of upper and lower respiratory epithelial cells (15, 16). The type II transmembrane serine protease TMPRSS2 primes SG for entry into the target cells through

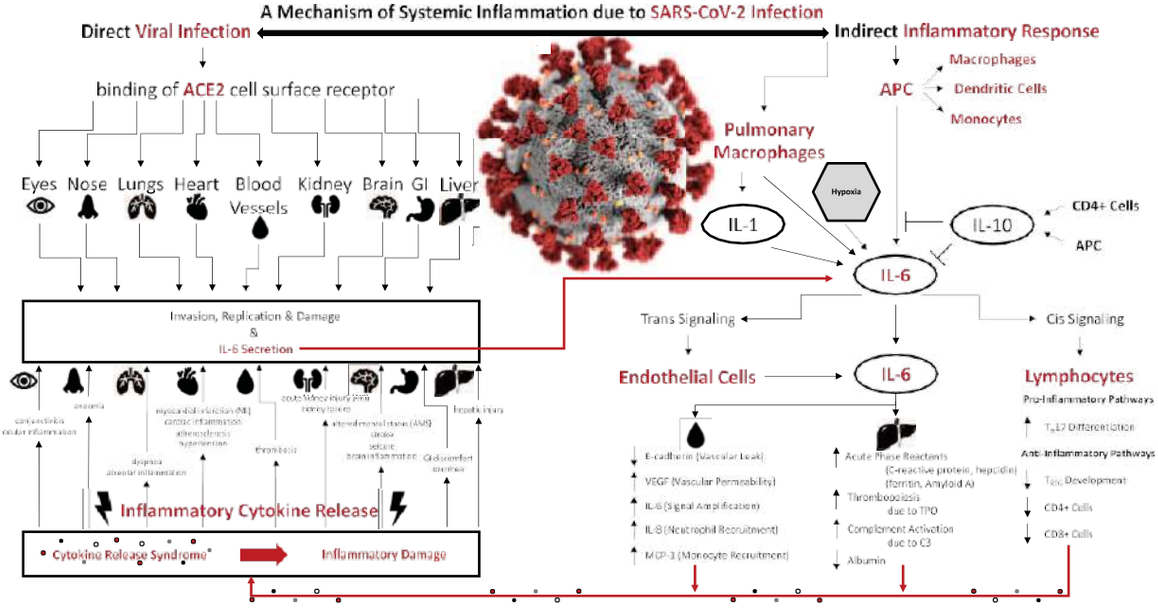


FIG. 1. Mechanism of systemic inflammation due to SARS-CoV-2 infection. The virus binds directly to ACE2 on epithelial cells and undergoes invasion, replication, and causes organ-specific damage. Virally infected cells release PAMPs and DAMPs that recruit APCs and result in the release of IL-6. Pulmonary macrophages release IL-1*b* that further increases downstream release of IL-6. CD4+ T cells and APCs release IL-10 to dampen the hyperactive immune response but is ineffective. IL-6 acts through cis-signaling on lymphocytes to stimulate a pro-inflammatory state and dampen the anti-inflammatory pathways. Through trans-signaling, IL-6 stimulates endothelial cells to release additional IL-6, which can act on the endothelial cells of blood vessels and hepatocytes to enhance the inflammatory response via a positive feedback mechanism. The combination of upregulated pro-inflammatory pathways, downregulated anti-inflammatory pathways and cytokine and chemokine release, results in Cytokine Release Syndrome (CRS), which causes systemic inflammatory damage. The image of SARS-CoV-2 was derived from the Centers for Disease Control and Prevention (CDC) website: https://phil.cdc.gov/Details.aspx?pid=23312. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; APCs, antigen-presenting cells.

endocytosis (17). In addition to the respiratory tract, SARS-CoV-2 binds to epithelial cells on the eyes, heart, vasculature, kidneys, brain, gastrointestinal (GI) tract, pancreas, and liver (7). The cells most susceptible to infection are found in the lungs, nasopharyngeal tract, and intestine, due to higher expression of ACE2 receptor and TMPRSS2 enzyme on cell surfaces (18).

Upon cellular entry, SARS-CoV-2 co-opts host cellular machinery to replicate its genome and produce new virions. In addition, viral entry causes release of IL-6, a cytokine involved in immune signal amplification in the lungs and in multiple other organ systems (19). In a study by Renieris et al. (20), elevated levels of IL-6 and low hydrogen sulfide (H_2S) were found in the serum of patients who died of COVID-19. In contrast, low levels of IL-6 and higher levels of H_2S in the serum of COVID-19 survivors support the inflammatory role of IL-6 and the potential of H_2S as an IL-6-targeted treatment for COVID-19-related disease. Initially, the resulting damage to organs and tissues can manifest as anosmia and ageusia (21), then progresses to dyspnea (22), myocardial injury (23), renal failure (24), neurological manifestations (25), and GI symptoms (26) (Fig. 1).

Indirect pathway for systemic injury in SARS-CoV-2 infection

SARS-CoV-2 infected cells likely undergo pyroptosis, releasing damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) (27). These are recognized by antigen-presenting cells (APCs); most prominently by the pulmonary macrophages (28), but also by dendritic cells (DCs) and monocytes (29). In response, the APCs release pro-inflammatory cytokines, including IL-1*b*, IL-6, IFN-*g*, MCP1, and IP-10 to recruit additional monocytes, macrophages, and T-cells to the site of infection (1, 27) (Table 1). Early secretion of IL-1*b* acts as a driver of the pro-inflammatory pathway, resulting in recruitment of neutrophils and cytotoxic T-cells, as well as an upregulation of other pro-inflammatory cytokines, including IL-6 (30).

In the context of SARS-CoV-2, dysregulation of the innate immune response leads to progressive inflammation and end-organ damage. Brief periods of viremia may also contribute to ''metastatic'' viral infections across various ACE2 rich tissues (7). When combined, these mechanisms of SARS-CoV-2 may act synergistically, further amplifying the severity of the systemic response.

Role of IL-6 and IL-10 in SARS-CoV-2 infection

Studies show that elevated IL-6 and IL-10 levels are associated with more severe SARS-CoV-2 infections (1). The interaction between IL-6 and IL-10 is evident in IL-6 knockout mice, with decreased IL-10 production by macrophages compared with their normal counterparts (31). IL-6 also induces the expression of multiple anti-inflammatory factors, including IL-10, suggesting a dual role of IL-6 in the regulation of the inflammatory response. In addition, IL-10 is also likely upregulated to counter the overwhelming SARS-CoV-2 infection, but may also be involved in the infiltration of inflammatory cells and lung fibrosis (32). Innate lymphocytes, APCs, and CD4βT-cells secrete the anti-inflammatory cytokine IL-10 to attenuate the inflammatory process and prevent immune-mediated damage to the lungs and other tissues (33) (Fig. 1). However, in severe COVID-19 cases with CRS, IL-10 is likely unsuccessful in dampening the runaway pro-inflammatory response (34). The combination of the pro-inflammatory pathway upregulation, coupled with downregulation of the anti-inflammatory pathway and increased cytokine production, leads to the emergence of CRS, which can result in diffuse systemic inflammation and injury (35) (Fig. 1).

Effects of SARS-CoV-2 infection on immune cells

IL-6 acts primarily in signal amplification, with both anti-inflammatory and pro-inflammatory effects (11). IL-6 binds to lymphocytes via cis-signaling and to endothelial cells via trans-signaling (Fig. 1). Acting as a ligand, it activates the JAK-STAT (STAT1, STAT3 and, to a lesser extent, STAT5) pathway (36), which further amplifies intracellular cascades converging on SRC-YAP-NOTCH (37), RAS-RAF (38), and AKT/PI3K (39) pathways downstream (Fig. 2). When IL-6 acts as a ligand for IL-6R on lymphocytes, cytoplasmic pro-inflammatory pathways are upregulated and anti-inflammatory pathways are downregulated (Fig. 1) (40).

Effects of SARS-CoV-2 on vascular endothelial cells

Endothelial cells do not express IL-6R; instead, they respond to IL-6 through trans-binding to gp130 (41) (Fig. 2). In the vasculature, IL-6 increases permeability through downregulation of E-cadherin and upregulation of vascular endothelial

TABLE 1. Pro-inflammatory cytokines and their respective function

Pro-inflammatory cytokine	Function
IL-1*b*	Mediator of inflammatory response; mediates lymphocyte growth and differentiation; costimulator of T-cell function; involved in autoimmune inflammation; involved in cellular proliferation, differentiation, and apoptosis
IL-6	Acute phase reactant; mediator of fever, stimulates neutrophil production in the bone marrow; supports B-cell growth; antagonist to Tregs
IFN-*g*	Inhibitor of viral replication, immune modulator; antitumor properties; increases antigen presentation and macrophage lysosomal activity; primes alveolar macrophages against secondary bacterial infections; promotes adhesion and binding for leukocyte migration; promotes Th1 and suppresses Th2 immune response
MCP-1	APC recruitment to sites of inflammation; monocyte infiltration
IP-10	Chemoattraction of immune cells (APCs, NK cells, and T-cells); promotes T-cell adhesion to endothelial cells

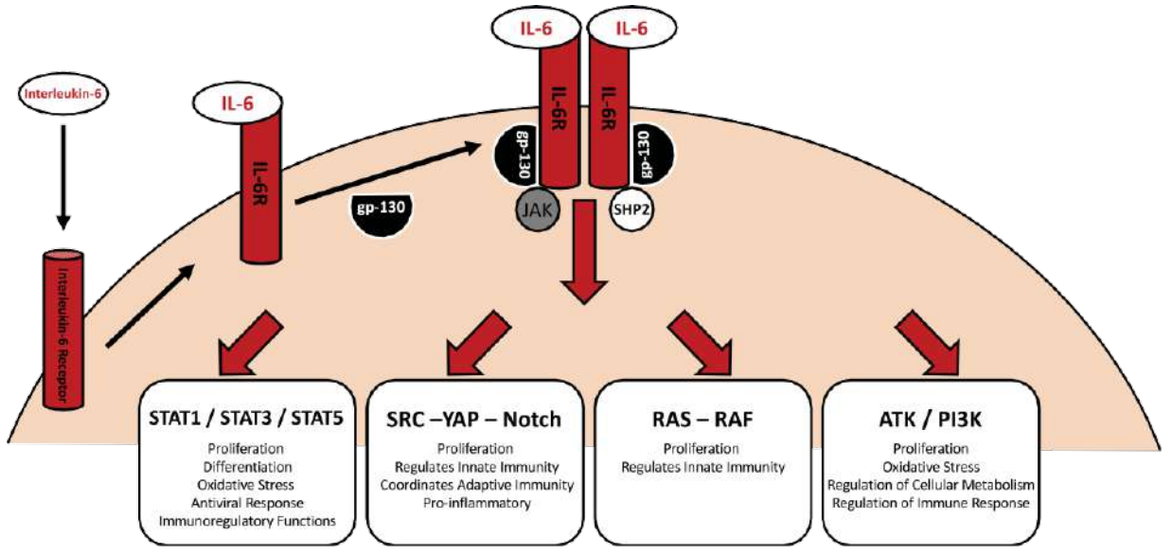


Fig. 2. Downstream pathways post-IL-6 binding. Via a cis-signaling type mechanism, the extracellular ligand interleukin-6 (IL-6) binds to the transmembrane receptor, IL-6R, and activates JAK/STAT pathways. Via a trans-signaling mechanism, IL6 binds to IL-6R activating JAK/STAT, and gp130. Both pathways converge onto one of four downstream cell signaling pathways through a signal transduction cascade. The activated pathways and their respective functions are stated here.

growth factor (42) resulting in edema (42). Hypoalbuminemia secondary to the hepatic effects of IL-6 further worsens the edema (43). Furthermore, endothelial cells secrete MCP-1 to recruit monocytes (44) and IL-8 to promote neutrophil chemotaxis and wound healing in severe inflammatory states (45). The effect of SARS-CoV-2 on vasculature is also corroborated by the evidence of post-COVID-19 vasculitis, an immune Kawasaki-like disorder recently identified in the pediatric population (46). This is generally consistent with the tendency of COVID-19 patients to present, either immediately or in a delayed fashion, with thrombotic and thromboembolic findings (7) (Fig. 1).

Effects of SARS-CoV-2 on hepatocytes

Recent clinical studies have recognized hepatocyte damage as an important surrogate for severe illness and respiratory deterioration (47). In hepatocytes, IL-6 binds to IL-6R and forms a complex that binds to the membrane protein gp130 to initiate intracellular signal transduction (48) (Fig. 2) and induce the synthesis of acute inflammatory reactants. These include C-reactive protein (CRP), serum amyloid A protein, and hepcidin, amongst others, which propagate the inflammatory response systemically, resulting in further damage (Fig. 1). However, there is reduction in the synthesis of albumin and cytochrome P450 (43). CRP can function as an opsonin and activate the classical complement pathway, contributing to further systemic inflammatory damage (49). Serum amyloid A protein can propagate chronic inflammation and deposit in various tissues resulting in damage, including renal failure (50) as well as heart failure and arrythmias (51). Sustained hepcidin due to chronic inflammation results in anemia due to lack of iron availability for erythropoiesis (52).

Effects of SARS-CoV-2 on the cardiovascular system

Underlying cardiovascular disease is one of the most common risk factors for COVID-19-related deaths (53). Studies have shown that about 10% of COVID-19 patients with pre-existing cardiovascular conditions, specifically those with hypertension and coronary artery disease, will die compared with 1% of COVID-19 patients without underlying medical conditions (54). Other potential risks include older age, high SOFA score, elevated troponin, and elevated d-dimer (>1 *m*g/mL) (23). The most common cardiovascular complications include arrythmias (atrial or ventricular fibrillation, ventricular tachyarrhythmia), cardiac injury, cardiac arrest, heart failure, fulminant myocarditis, pulmonary embolism, and disseminated intravascular coagulation (1, 55). Although the direct mechanism is unknown, many myocardial infarctions experienced by COVID-19 patients appeared to be associated with myocarditis (54). It is presently unclear whether the myocardial injury is due to direct damage, systemic inflammation, or the combined effects of both (23).

COVI-FLU: A BURGEONING CRISIS

The COVID-19 pandemic has radically altered the medical landscape worldwide. As a new semblance of normalcy emerges, it is important to realize that flu season is nearing. Influenza viruses are evolutionarily distant from betacoronaviruses, and prior research has shown that influenza virus infection does not provide durable cross-protection against subsequent infection with coronaviruses (56). In fact, limited reports have already revealed that co-infections with influenza A virus and SARS-CoV-2 have occurred (57). In the proposed

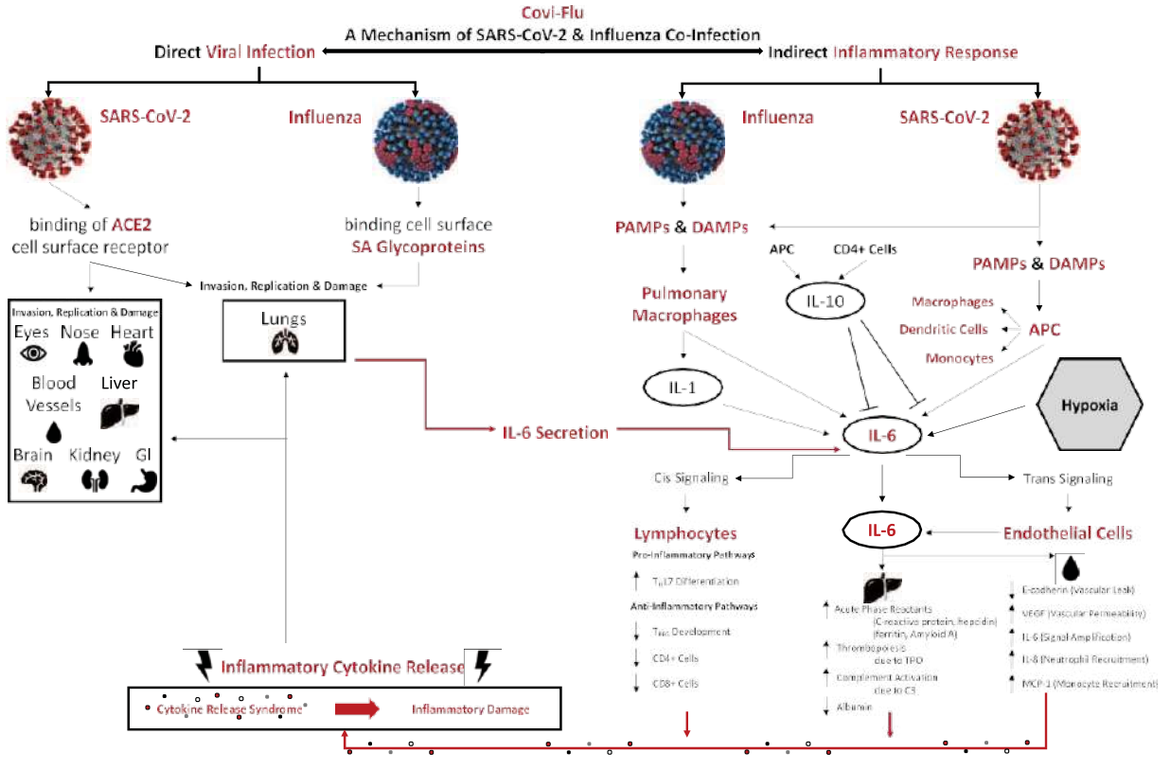


Fig. 3. Model for COVI-Flu (SARS-CoV-2 and influenza co-infection). This is a proposed synergistic interaction between SARS-CoV-2 and influenza viruses resulting in CRS and systemic inflammatory injury. The hypoxic state induced by SARS-CoV-2 exacerbates IL-6 production and secretion creating a positive feedback loop that drives both the direct and indirect pathways of COVI-Flu. The image of SARS-CoV-2 was derived from the Centers for Disease Control and Prevention (CDC) website: https://phil.cdc.gov/Details.aspx?pid=23312. The image of influenza virus was derived from the CDC website: https://www.cdc.gov/flu/images/h1n1/3D_Influenza_transparent_no_key_full_lrg.gif. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; CRS, cytokine release syndrome.

model, which we've termed ''COVI-Flu'' (Fig. 3), both viruses can simultaneously cause direct and indirect damage to the host. This synergistic infection leads to further widespread organ damage by CRS.

Direct pathway for pulmonary and systemic injury in COVI-FLU

Both influenza virus and SARS-CoV-2 infect respiratory epithelial cells, but through different mechanisms. Hemagglutinin proteins on influenza virus bind to sialylated glycoproteins on the surface of ciliated respiratory epithelial cells (58); in contrast, SARS-CoV-2 binds to ACE2 proteins to mediate ingress (17) (Fig. 3). Upon infection, both viruses cause pulmonary damage and IL-6 release (59). Because of the presence of ACE2 receptors on other organ systems, SARS-CoV-2 can also cause systemic damage (Fig. 1).

Indirect pathway for pulmonary and systemic injury COVI-FLU

In the lungs, pulmonary macrophages are the predominant APC (28). Influenza virus and SARS-CoV-2 can both cause release of PAMPs and DAMPs, which are recognized by pulmonary macrophages. This results in the secretion of IL-1*b*, which can induce the recruitment of neutrophils and cytotoxic T-cells, as well as an upregulation of other pro-inflammatory cytokines, including IL-6 (60). Pulmonary macrophages can also release IL-6 directly after recognizing PAMPs and DAMPs (61). Because SARS-CoV-2 is a systemic infection, it can also cause the production of PAMPs and DAMPs in other organ systems, resulting in recognition by APCs and increased IL-6 release (62) (Fig. 3).

IL-6, CRS, and systemic inflammatory damage in COVI-FLU

Since both viruses can cause aberrant over-expression of IL-6, this will result in upregulation of the pro-inflammatory pathway, downregulation of the anti-inflammatory pathway, increased vascular permeability, and release of acute phase reactants, cytokines, and chemokines, resulting in immune-mediated tissue damage (59, 63). This synergistic activation of IL-6 downstream mediators will result in a more robust CRS response, with a magnitude potentially comparable to CAR T-cell cytokine storm, and the possibility for amplified systemic injury compared with infection with either virus alone (64). If we apply the IL-6/IL-10 cytokine model discussed previously to COVI-Flu, we anticipate a higher ratio of IL-6/IL-10 with an unfavorable prognosis (23, 65).

IL-6, hypoxia, and potential therapies in COVI-FLU

Many cases of SARS-CoV-2 present with ''silent hypoxia,'' wherein patients present with oxygen saturations in the low 80s but appear asymptomatic. During this hypoxic state, the blood

is still capable of extracting oxygen from the damaged alveoli, but the process is inefficient due to poor gas exchange. If oxygen saturations continue to fall, it portends a poor prognosis with early warning signs to seek medical intervention. Currently, inhaled oxygen, nitric oxide (NO), and prostaglandins are used in the treatment of primary pulmonary hypertension and oxygen therapy in the management of hypoxia (66, 67). From an immunological perspective, hypoxia can further exacerbate inflammation through the activation of pro-inflammatory pathways. In chronically hypoxic animals, elevated pulmonary IL-6 levels corroborate its damaging effects on lung vascular permeability, and the early inflammatory response to hypoxia (68, 69). Increased IL-6 at the early stages of hypoxia may be interpreted as part of an initial lung inflammatory response to hypoxia (Figs. 1 and 3). The mechanism by which IL-6 contributes to pulmonary vascular remodeling during hypoxia remains unclear. IL-6R is present on multiple cell types, including immune and endothelial cells such as those in the lung.

It is imperative that we anticipate this intersection between SARS-CoV-2 and influenza virus and start to devise effective therapies to combat this potential future pandemic. Fortunately, influenza A virus and SARS-CoV-2 both require TMPRSS2 to facilitate cellular entry (70). Targeting this host protease might provide a means of inhibiting or limiting co-infection. In addition, there are known and effective orally available therapies for influenza, making it possible to avert the full extent of COVI-Flu in patients who present early and are treated promptly (71, 72).

THERAPEUTIC INTERVENTIONS

Here, we present individual therapeutic options for SARS-CoV-2 and influenza. Further clinical investigation is necessary to determine the ideal combination of therapies.

SARS-CoV-2

Recent studies using single-agent therapies have demonstrated minimal effects in improving mortality, especially in patients with severe disease. Of importance, evidence has emerged against the clinical effectiveness of hydroxychloroquine, with ongoing studies examining other therapeutic agents (73). New research suggests—in line with our general hypothesis—that anti-inflammatory effects of dexamethasone may result in favorable immunomodulatory changes and thus lower morbidity and mortality in severely affected COVID-19 patients (74).

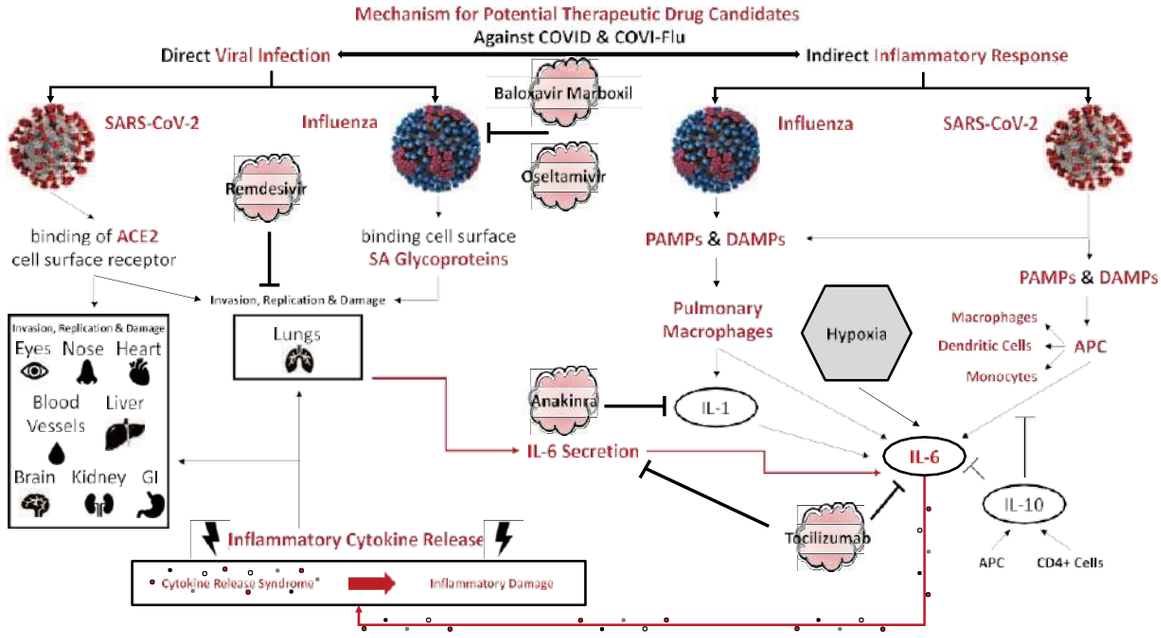
Remdesivir

Remdesivir is a monophosphate prodrug analog of adenosine nucleotide, resulting in inhibition of RNA synthesis (Fig. 4). Because this medication prevented lung hemorrhage and reduced viral titers in MERS-CoV, it became a potential anti-COVID-19 agent (75). A small clinical trial of 12 COVID-19 patients showed that three patients with severe COVID-19 improved after Remdesivir treatment (76). In a study of 1,063 hospitalized patients with advanced COVID-19 and lung involvement, patients who were treated with Remdesivir had a 31% faster recovery time (11 days) than those receiving placebo (15 days). The treatment group also demonstrated a mortality rate of 8%, compared with 11.6% for the placebo group (77). In an Italian study of 1,591 critically ill SARS-CoV-2 ICU patients treated with Remdesivir, only 16% were discharged as a result of treatment (78), thus showing the limited efficacy that this medication has in critically ill COVID-19 patients. Additional studies have shown the potential for resistance to this pro-drug, whether by cellular exocytosis or exoribonuclease mutations that remove the medication (79). However, the utility of Remdesivir is not entirely exhausted, as it may demonstrate greater efficacy in combination drug studies (80).

Indirect pathway inhibitors for SARS-CoV-2 and COVI-Flu

Rationale for systemic therapies and potential therapeutic candidates—The limited efficacy of these single-agent medications is largely due to their inhibition of SARS-CoV-2 solely through the direct pathway. Because SARS-CoV-2 also acts through the indirect pathway, systemic inflammation is largely uninhibited, resulting in profound inflammatory damage. With both SARS-CoV-2 and severe influenza resulting in increased IL-6 cytokine release, it is important to consider the use of combination therapies that can also target the indirect pathway to dampen the CRS.

Since single-agent IL-6 inhibitors have had limited efficacy, we propose the use of combination therapies to inhibit the pro-inflammatory pathway at multiple points in the indirect pathway (81). An example of this is mesenchymal stem cells (MSCs), which act as immunomodulators through stimulation of the Th2 pathway (anti-inflammatory) and downregulation of the Th1 (pro-inflammatory) pathway (Fig. 5) (82). Additionally, these agents induce the proliferation of Tregs, which attenuate inflammation (83). Although MSCs enhance B-cell viability, they also inhibit their proliferation, antibody production, and ability to secrete costimulatory molecules (84). Similarly, MSCs decrease CD8$^+$ T-cell proliferation and cytotoxicity (85) and inhibit the activation and maturation of APCs, including macrophages and DCs (86). By downregulating M1 macrophages, MSCs decrease IL-6 signaling and NO, and increase IL-10 release through upregulation of M2 macrophages. MSCs also decrease natural killer (NK) cell activation and cytotoxicity (87). When applied to our proposed models for both SARS-CoV-2 and COVI-Flu, MSCs should inhibit IL-6 at multiple points within the indirect pathway by decreasing IL-1 and increasing IL-10, which can further act to dampen IL-6 expression, decreasing the pro-inflammatory pathway and increasing Tregs (Fig. 6) (88). This approach was already shown to be safe in a small Phase I clinical trial by Pluristem Therapeutics, yielding an 87.5% survival rate in eight patients with severe COVID-19 with multi-organ dysfunction after a 28-day follow-up (89). The company recently received FDA approval for a Phase II trial for the treatment of 140 patients with severe COVID-19 and acute respiratory distress syndrome (ClinicalTrials.gov Identifier: NCT01679990). Similar results were demonstrated by Mesoblast's MSC trial, which showed an 83% survival in a cohort of 12 patients with severe COVID-19 (90). Accordingly, Mesoblast is slated to begin a Phase II/III



DRUG	MECHANISM
SARS-CoV-2 & Influenza	
Anakinra	recombinant, non-glycosylated human interleukin-1 receptor antagonist (IL-1Ra).
Tocilizumab	IL-6R antagonist.
Remdesivir	monophosphate prodrug analog of adenosine nucleotide; inhibition of RNA synthesis.
Ibrutinib	Bruton's Tyrosine Kinase (BTK) inhibitor; decreases IL-6; increases IL-10.
Influenza	
Oseltamivir	competitive neuraminidase inhibitor.
Baloxavir Marboxil	inhibitor of viral mRNA transcription.

Fig. 4. A, Mechanism for potential therapeutic drug candidates for SARS-CoV-2 and COVI-Flu. Known pharmacological agents may act to halt the progress of SARS-CoV-2 and COVI-Flu. This model proposes the use of one or more of the widely available drugs, including Anakinra, Tocilizumab, Remdesivir, Ibrutinib, Oseltamivir, and Baloxavir Marboxil. Their respective mechanistic intervention is provided both here and in the accompanying table. The image of SARS-CoV-2 was derived from the Centers for Disease Control and Prevention (CDC) website: https://phil.cdc.gov/Details.aspx?pid=23312. The image of influenza virus was derived from the CDC website: https://www.cdc.gov/flu/images/h1n1/3D_Influenza_transparent_no_key_full_lrg.gif. B, Potential therapeutic drug candidates for SARS-CoV-2 and COVI-Flu. The widely available drugs, Anakinra, Tocilizumab, Remdesivir, Ibrutinib, Oseltamivir, and Baloxavir Marboxil may act to halt the progress of SARS-CoV-2 and COVI-Flu. Their respective mechanistic intervention is detailed here in table form. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2.

clinical trial (ClinicalTrials.gov Identifier: NCT04371393). Most recently, Baptist Health demonstrated similar efficacy in three critically ill patients using Restem umbilical cord MSCs, and have received FDA approval for a Phase I clinical trial (91). Although time will tell about the long-term efficacy of these therapies, the initial safety data have demonstrated some promise. Furthermore, although early studies of MSC therapy seem promising, caution must be exercised in their use, as studies concerning the potential adverse effects of MSCs in inhibiting antibody production have not yet been conducted.

Due to the heterogeneity of viral strains with varying mutations that can predispose to treatment resistance, a strategy moving forward could utilize combination therapies that work on the direct and indirect pathways simultaneously to achieve

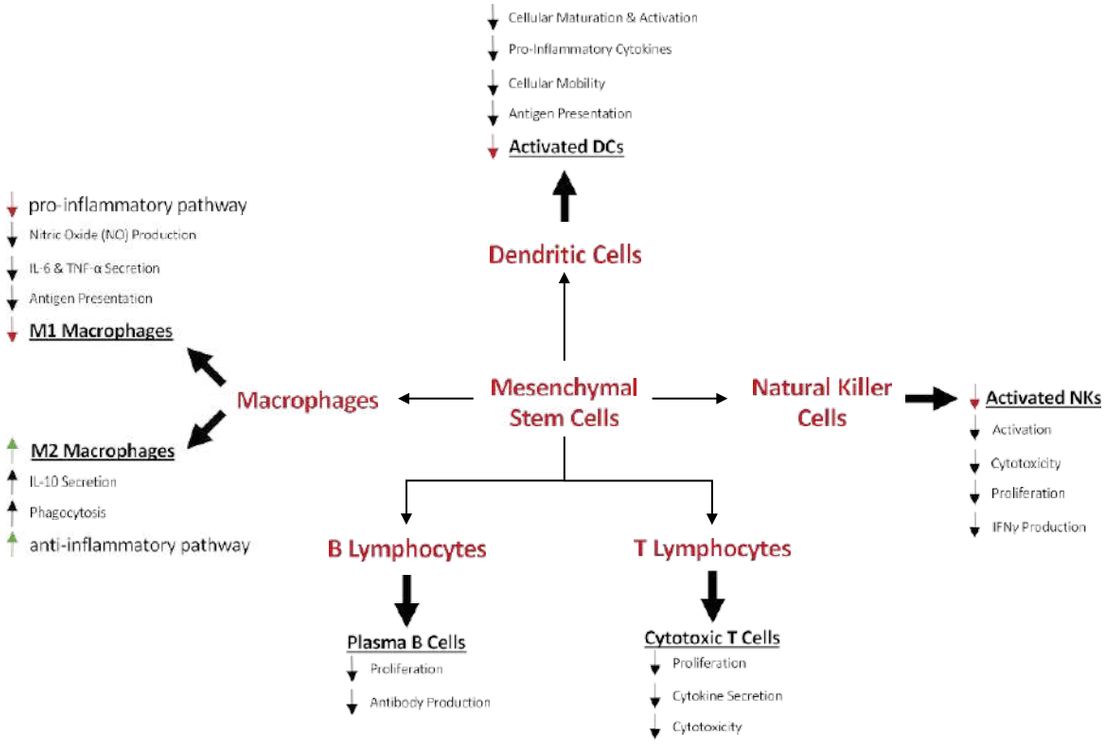


Fig. 5. Effects of mesenchymal stem cell therapy in COVI-Flu. This figure illustrates the proposed therapeutic activity of mesenchymal stem cells (MSCs) within the model of COVI-Flu. MSCs stimulate anti-inflammatory pathways, inhibit pro-inflammatory pathways, inhibit ILs -1 and -6 as well as stimulate IL-10. Increased IL-10 expression can further dampen the effects of IL-6 via a negative feedback mechanism.

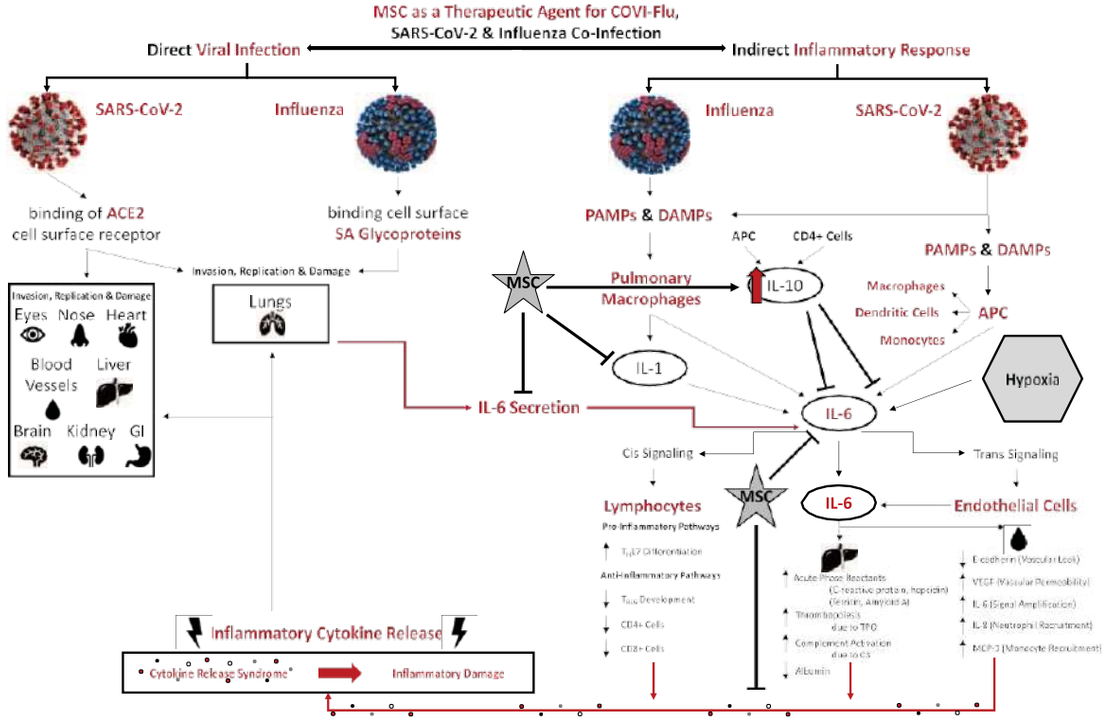


Fig. 6. Mesenchymal stem cells and their effects on immune cells. Mesenchymal stem cells (MSCs) have a variety of profound effects on the gene expression and cellular function of particular classes of cells within the immune system. This therapy can mitigate CRS onset at several stages along the pathway, thereby reducing the development of systemic injury and organ damage. The image of SARS-CoV-2 was derived from the Centers for Disease Control and Prevention (CDC) website: https://phil.cdc.gov/Details.aspx?pid=23312. The image of influenza virus was derived from the CDC website: https://www.cdc.gov/flu/images/h1n1/3D_Influenza_transparent_no_key_full_lrg.gif. CRS indicates cytokine release syndrome.

improved viral control and minimize systemic damage. Although the CRS will likely be of higher grade in COVI-Flu due to the synergistic activation of the downstream IL-6 pathway, we propose using MSCs to dampen the hyperactive immune response (Fig. 6). Following treatment, levels of IL-1, IL-6, and IL-10 should be assessed to determine whether additional cytokine inhibitors, like Anakinra (IL-1) or Tocilizumab (IL-6R blocker), should be added to achieve optimal control of systemic inflammation (Fig. 4A), like in the management of CAR T-cell cytokine storm (11, 92). In a recent study, high-dose Anakinra was associated with improved respiratory symptoms and a 72% reduction of CRS (93). Although Tocilizumab decreases the onset of CRS through competitive inhibition of IL-6 with IL-6R, it has demonstrated mixed efficacy in clinical trials, where repeat doses (two—three) had to be administered in a cohort of 15 critically ill patients to yield such effects, with four patients experiencing rebound IL-6 effects (94). Sarilumab, another IL-6 receptor inhibitor, failed to provide noticeable clinical outcomes in patients with severe and critical COVID-19 infection in a Phase II trial of 126 patients (95). Similarly, Situximab, a chimeric monoclonal antibody against IL-6, failed to produce significant clinical changes in severe COVID-19 patients, where only 33% demonstrated improvement in clinical condition in a cohort of 21 patients (96). Thus, this illustrates the need for combination therapies to better control inflammation.

Ibrutinib

Bruton tyrosine kinase (BTK) inhibitors like Ibrutinib are largely used to manage the inflammatory response in graft-versus-host disease and indolent B-cell malignancies. However, BTK is a key regulator of chemokine and cytokine production in pulmonary macrophages of IL-6, IL-10, TNF*a*, and MCP-1, the same cytokines responsible for CRS and systemic inflammatory damage (97, 98) (Fig. 4B). Having shown promise in downregulating CAR T-cell cytokine storm, it has been suggested to apply this medication to COVID-19 CRS (99) (Fig. 4A). In a recent study, five Waldenstrom's macroglobulinemia patients managed with full dose Ibrutinib were diagnosed with COVID-19 and failed to develop progressive symptoms of dyspnea nor require hospitalization. All patients experienced resolution of symptoms (100). Due to the small sample size, further studies will be required before conclusions can be extended to the general population. AstraZeneca has recently started a Phase I clinical trial (CALAVI) using Acalabrutinib, a highly selective BTK inhibitor normally used to treat mantle cell lymphoma, CLL, and SLL, to reduce systemic inflammation and severity of COVID-19-induced respiratory distress (ClinicalTrials.gov Identifier: NCT04346199).

Potential direct pathway inhibitors in SARS-CoV-2

Although many drug candidates are being tested for COVID-19, most have so far failed due to efficacy and/or safety concerns (101). However, several potential therapies are currently in development (Fig. 4).

Triple antiviral therapy with interferon b*-1b in SARS-CoV-2*

Applying the principle of combination antiretroviral therapy from HIV, a group in China performed an open-label, randomized Phase II clinical trial on 86 patients with mild-to-moderate COVID-19 infections. The patients were treated with either a 14-day course of 400 mg lopinavir (HIV protease inhibitor) and 100 mg ritonavir (HIV protease inhibitor) every 12 h, 400 mg ribavirin (RNA nucleoside inhibitor of replication) every 12 h and three doses of 8 million international units of interferon beta-1b (anti-inflammatory agent) on alternate days (combination group), or 14 days of lopinavir 400 mg and ritonavir 100 mg every 12 h (control group). The combination group demonstrated shorter times to negative nasopharyngeal swab (7 days) compared with 12 days for patients treated with lopinavir/ritonavir alone. This triple-therapy also decreased IL-6 levels, with a favorable morbidity and mortality profile (102). Although the preliminary data was promising, the sample size would have to be expanded with critically ill patients included as well.

Anti-ACE2 receptor human neutralizing antibodies in SARS-CoV-2

An experimental strategy currently in development utilizes antibodies to compete with the virus for the ACE2 receptor. Two human neutralizing antibodies—B38 and H4— that bind to the glycoprotein spike on the virus were isolated from a recovered COVID-19 patient. Through various *in vitro* and *in vivo* studies, scientists confirmed the close homogenous binding between the antibodies and ACE2 receptor (103). Although a promising theory, clinical studies have yet to be performed in a large cohort to verify efficacy.

INFLUENZA

Influenza has been around for decades, yet we still lack effective antiviral therapies (104). Although this illness is largely preventable with vaccines, the rapid mutation rates and strain heterogeneity largely dictate their effectiveness (105). Thus, it is critical that we start to identify viable antiviral candidates.

Oseltamivir in influenza viral infection

Oseltamivir is a competitive neuraminidase inhibitor that cleaves sialic acid on the surface of human cells, preventing virion release (106) (Fig. 4). However, studies have shown that Oseltamivir only offers symptomatic improvement with a shortened recovery time by 2 days in patients with high-grade influenza if treated within the first 48 h of symptom onset (107).

Baloxavir marboxil in influenza viral infection

In October 2018, the FDA approved Baloxavir Marboxil, which represents a new antiviral influenza drug class that targets the endosomal function of viral PA polymerase and inhibits the transcription of viral mRNA (108) (Fig. 4). This medication has demonstrated efficacy against influenza viruses A and B with a single dose. This agent was superior in reducing viral loads, with a faster median duration of viral detection of 24 h compared with 72 h for Oseltamivir and fever resolution (109). In summary, having an alternative antiviral candidate to Oseltamivir is promising, especially if patients experience high grade CRS.

Natural killer cell therapy

Natural Killer (NK) cells are innate lymphocytes that play a central role in the killing of virally infected cells and modulating the innate and adaptive immune response through the secretion of IFN-*g*, TNF-*a*, GM-CSF, and chemokines (110). In severe SARS-CoV-2 (111) and influenza infections (112), there is impaired NK cell function and overall decrease in the NK population. The FDA has recently approved a Phase I/II clinical trial of up to 86 patients diagnosed with COVID-19 to receive an infusion of cryopreserved allogenic, off-the-shelf NK cells developed from placental hematopoietic stem cells to limit SARS-CoV-2 replication and disease progression (ClinicalTrials.gov Identifier: NCT04365101).

PREVENTIVE INTERVENTION FOR COVI-Flu

Based on the presentation of our COVI-Flu model, it is critical to consider the impact of COVI-Flu on the general population, and particularly on patients with pre-existing conditions. Since both SARS-CoV-2 and influenza have a strong similarity between their inflammatory mechanisms of cell injury and multi-organ dysfunction, we submit that an early start of a seasonal flu vaccination program with priority to the older and vulnerable population be carried out to avoid the concurrent devastating effects of SARS-CoV-2 and influenza. Early flu vaccination should prime the patient with cytokine release event memory and thus dampen the future SARS-CoV-2-induced inflammatory response reducing the severity of concomitant infections.

CONCLUSION

SARS-CoV-2 is a highly transmissible betacoronavirus that has caused a global pandemic that has rapidly and drastically altered the face of healthcare and daily life. However, this tragedy has helped to forge novel collaborations and has fast-tracked the drug discovery process, which has resulted in remarkable productivity in a relatively short time. Herein, we presented a model system for direct and systemic damage for SARS-CoV-2 (Fig. 1) and propose a cytokine-based assessment of severity and prognosis for COVID-19. Although IL-6 activates downstream pro-inflammatory pathways and down-regulates anti-inflammatory mechanisms, the resulting CRS is of lower grade than CAR T-cell-mediated cytokine storm. Because both influenza virus and SARS-CoV-2 utilize IL-6 signaling (Fig. 2), we proposed a model system for a synergistic response due to both viruses (Fig. 3). Irrespective of whether the patient is infected with SARS-CoV-2, influenza, or both, severe infection will result in higher grade CRS that will require systemic anti-inflammatory therapies. As of this writing, mesenchymal stem cell therapy has shown great potential in clinical trials and mechanistically offers an effective mode of protection despite the various viral strains and potential for mutation that can promote resistance (Fig. 4, A and B). Adopting treatment strategies from anticancer therapies and HIV antiretroviral therapy, we feel that systemic combination therapies offer the ideal means of curbing systemic inflammation and viral progression with protection against treatment resistance (Fig. 4). Although the optimal direct pathway treatment strategy is currently uncertain, we have highlighted some unique approaches that are currently being studied at the bench and in clinical trials. Because several studies have suggested that COVID-induced CRS is due to a biochemical pathway mutation, perhaps gene therapy can provide future solutions (113, 114). Additional studies are necessary to identify the ideal combination therapy to manage both severe SARS-CoV-2 and COVI-Flu, especially with the potential challenges that this synergism may pose to global health security.

ACKNOWLEDGMENTS

The authors thank the WACEM-ACAIM Joint Global COVID-19 Taskforce for discussions and input on the manuscript.

YL: I would like to thank my parents for their unconditional love and support. I am grateful to Dr SG for the opportunity to contribute to the Taskforce. I would also like to acknowledge the mentors who have played a profound role in my career development and to the patients that I help comanage who inspire me to strive for greatness daily.

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