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The role of IL-6 and other mediators in the cytokine storm associated with SARS-CoV-2 infection



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1. To define the immunopathogenesis of the cytokine storm associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
2. To explore the role of the IL-6 in the SARS-CoV-2 inflammatory and viral responses.
3. To describe the role of biological immunotherapies targeting the hyperinflammatory immune response in SARS-CoV-2.
4. To outline the therapeutic strategy targeting upstream and downstream immunologic pathways in SARS-CoV-2.

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The coronavirus disease 2019 pandemic caused by severe acute respiratory syndrome coronavirus 2 presents with a spectrum of clinical manifestations from asymptomatic or mild, self-limited constitutional symptoms to a hyperinflammatory state (“cytokine storm”) followed by acute respiratory distress syndrome and death. The objective of this study was to provide an evidence-based review of the associated pathways and potential treatment of the hyperinflammatory state associated with severe acute respiratory syndrome coronavirus 2 infection. Dysregulated immune responses have been reported to occur in a smaller subset of those infected with severe acute respiratory syndrome coronavirus 2, leading to clinical

deterioration 7 to 10 days after initial presentation.

A hyperinflammatory state referred to as cytokine storm in its severest form has been marked by elevation of IL-6, IL-10, TNF- α , and other cytokines and severe CD4⁺ and CD8⁺ T-cell lymphopenia and coagulopathy. Recognition of at-risk patients could permit early institution of aggressive intensive care and antiviral and immune treatment to reduce the complications related to this proinflammatory state. Several reports and ongoing clinical trials provide hope that available immunomodulatory therapies could have therapeutic potential in these severe cases. This review highlights our current state of knowledge of immune mechanisms

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and targeted immunomodulatory treatment options for the current coronavirus disease 2019 pandemic. (J Allergy Clin Immunol 2020;146:518-34.)

Key words: *IL-6, sepsis, cytokine storm, cytokines, COVID-19, SARS-CoV-2, TNF- α , JAK, STING, proinflammatory, hyperinflammatory, hemophagocytic lymphohistiocytosis*

The coronavirus disease 2019 (COVID-19) pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presents an enormous challenge for public health and clinicians globally. Increased understanding of the immunopathogenesis of SARS-CoV-2 infection as well as ongoing clinical trials of host target drugs such as hydroxychloroquine, direct antivirals, convalescent plasma (CP), and other immunomodulatory agents hold promise for future evidence-based and targeted therapies to reduce the morbidity and mortality of the most vulnerable populations.

Although infection is often asymptomatic or associated with mild to moderate self-limiting symptoms such as fever, dry cough, myalgia, and fatigue,¹⁻⁶ a subset of patients with severe SARS-CoV-2 infection develop a clinically severe hyperinflammatory state or cytokine storm (CS) for which pulmonary involvement such as acute respiratory distress syndrome (ARDS) is a cardinal feature.^{1,7} Furthermore, a subgroup of previously healthy children has been diagnosed with a multisystem inflammatory syndrome associated with acute SARS-CoV-2 infection that appears distinct from the adult CS.⁸

Although the individual components of CS are varied, IL-6 has emerged of particular interest in the context of SARS-CoV-2 infection after being identified as the most significant predictor of mortality in recent retrospective studies of patient survival in COVID-19.¹ Herein, we review the current understanding of the origin and mechanisms of CS associated with SARS-CoV-2 infection, with focus on the identification and implication of IL-6 and other proinflammatory cytokines and pathways in CS-driven ARDS, and discuss the potential utility of anti-IL-6 and other cytokine-targeting immunomodulatory biologics for the treatment of this critically ill population.

SEARCH STRATEGY AND SELECTION CRITERIA

We searched PubMed for peer-reviewed articles published between January 1, 2000, and April 18, 2020 (date of last search), with the terms (“IL-6” OR “interleukin-6” OR “cytokine”) AND (“sepsis” OR “SIRS” OR “systemic inflammatory response syndrome” OR “non-infectious systemic inflammatory response syndrome” OR “ARDS” OR “acute respiratory distress syndrome” OR “cytokine storm” OR “inflammatory response” OR “septic shock” OR “critically ill” OR “organ dysfunction” OR “infection”) AND (“ICU” OR “intensive care unit” OR “ED” OR “emergency department”). A second search was oriented on treatment, with the terms (“IL-6” OR “interleukin-6” OR “IL-1” OR “interleukin-1” OR “TNF” OR “tumor necrosis factor” OR “interferon gamma” OR “STING” OR “interferon pathway”) AND (“sepsis” OR “SIRS” OR “systemic inflammatory response syndrome” OR “ARDS” OR “acute respiratory distress syndrome” OR “cytokine storm” OR “inflammatory response” OR “septic shock” OR “critically ill” OR “organ dysfunction” OR “infection”) AND (“IL-6 inhibitor” OR “interleukin-6 inhibitor” OR “JAK-STAT” OR “tocilizumab” OR “humanized IL-6R antibody” OR “anakinra” OR “IL-1 inhibitor”). Please refer to [Fig E1](#) in this article’s Online

Abbreviations used

ACE2:	Angiotensin-converting enzyme 2
ARDS:	Acute respiratory distress syndrome
BTK:	Bruton’s tyrosine kinase
CAR:	Chimeric antigen receptor
CP:	Convalescent plasma
COVID-19:	Coronavirus disease 2019
CRP:	C-reactive protein
CRS:	Cytokine release syndrome
CS:	Cytokine storm
FDA:	Food and Drug Administration
HLH:	Hemophagocytic lymphohistiocytosis
JAK:	Janus-associated kinase
MAS:	Macrophage activation syndrome
MERS:	Middle East respiratory syndrome
MERS-CoV:	MERS coronavirus
SARS-CoV:	SARS coronavirus
SARS-CoV-2:	Severe acute respiratory syndrome coronavirus 2
STING:	Stimulator of IFN genes

Repository at www.jacionline.org for details concerning the number of articles entered in PubMed with the “cytokine storm” keywords.

All recent articles on COVID-19/SARS-CoV-2 were reviewed including preprints from *bioRxiv* and *medRxiv* as a more real-time resource, but realizing the lack of peer review limitation. To carefully include the proposed trials for COVID-19, we researched the *ClinicalTrials.gov/trials* website.

Articles published in English were selected and reviewed. There was a focus on clinical trials, meta-analysis, randomized controlled trials, and systematic reviews as well as novel and significant studies. Finally, we also identified several new references from those listed in the reviewed articles. Please note that although there is increasing information about SARS-CoV-2 and its immune consequences, most of the literature available on COVID-19 and SARS-CoV-2 infection originates from the onset of the pandemic, in China, with various publications from disease phenotype to immunopathogenesis and follow-up.

OVERVIEW OF SEPSIS AND IMMUNE DYSREGULATION

The release of large quantities of proinflammatory cytokines is termed CS and is associated with various infective precipitants and other hyperinflammatory states.^{9,10} Virally associated causes of particular relevance are the 2003 SARS coronavirus (SARS-CoV) infection that infected more than 8000 globally, primarily in Asia and Canada (Toronto) with an 11% mortality rate,¹¹⁻¹⁴ and the 2012 Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) with a reported case-fatality rate of 35%.¹⁵⁻¹⁷ Although SARS-CoV-2 belongs to the same *Betacoronavirus* genus as SARS-CoV and MERS-CoV, the case fatality associated with both SARS-CoV and MERS-CoV significantly exceeds that of SARS-CoV-2 but the number of cases worldwide associated with SARS-CoV and MERS-CoV is much lower.¹⁸ Genomic evidence suggests that SARS-CoV and SARS-CoV-2 share the same human cell receptor for host entry, the angiotensin-converting enzyme 2 (ACE2).¹⁹ SARS-CoV-2 binds with increased affinity to the ACE2 receptor compared with SARS-CoV, a possible

explanation for the widely spread community transmission from asymptomatic hosts.¹⁸

The clinical correlates of increased inflammation or CS are persistent hypotension, hypothermia or hyperthermia, and end-organ dysfunction, while the laboratory features include hematological anomalies (leukocytosis or leukopenia, thrombocytopenia, and disseminated intravascular coagulation with high fibrinogen, triglycerides, and ferritin), elevated IL-2R (CD25), elevated IL-6, and general markers of end-organ dysfunction (such as elevated creatinine and deranged liver function tests).¹⁰ The CS clinical phenotype is variable and regroups systemic signs of inflammation, multiorgan failure, and mortality.²⁰

The hyperinflammatory state associated with severe viral infection shares significant features with CS, and genetic factors associated with hemophagocytic lymphohistiocytosis (HLH) have been identified, suggesting that these conditions may in fact be part of the same spectrum.²¹ HLH is caused by an abnormal regulation of activated macrophages and lymphocytes, leading to a hyperinflammatory state.²¹ IFN- γ is an important cytokine responsible for the organ damage leading to mortality in HLH.²⁰ Other cytokines such as IL-6, TNF- α , IL-1, and IL-18 are also increased during HLH.²² Several genes that play a role in the pathway of natural killer cells and CD8⁺ T cells have been described as causal for the development of the familial form of HLH. Some examples include the lysosomal trafficking regulator mutation described in the Chediak-Higashi syndrome, the RAB27A mutation in the Griscelli syndrome as well as other mutations in perforin (PRF1). The main clinical and laboratory manifestations of HLH are fever, cytopenia, coagulopathy, liver dysfunction, hyperferritinemia, decreased or absent natural killer-cell function, increased soluble CD25 (IL-2R), and presence of hemophagocytosis on bone marrow biopsy or other organs.²¹ The secondary form of HLH has been described in influenza during the 2009 H1N1 pandemic, the largest cohort describing 9 cases with a mortality rate of 89% (8 of 9 cases).²³ The macrophage activation syndrome (MAS) has similar clinical features to HLH and is considered a related disorder that occurs primarily in patients with rheumatologic diseases.²⁴ The excessive inflammatory response described in MAS is characterized by an increased IL-1 β , with successful reports on the use of anakinra, an IL-1 receptor antagonist.^{25,26} In a cohort of 19 adult patients with secondary HLH/MAS, 63.1% (12 of 19) had systemic infections as precipitating causes.²⁷ This group used anakinra in 12 of 19 patients and reported 4 deaths among those patients treated with anakinra.²⁷

The concept of shared immunologic features between HLH, MAS, and CS has been entertained with extensive discussion that these diseases could be part of an overlapping immunopathology with shared but also distinctive triggers and genetic features.²⁰ To assess the possible genetic predisposition of individuals with no past medical history to develop HLH secondary to severe H1N1 influenza, Schulert et al²⁷ performed whole-exome sequencing in 14 patients, 13 of whom had evidence of hemophagocytosis at autopsy, and detected 5 heterozygous variants in lysosomal trafficking regulator (2 of whom also had a heterozygous PRF1 mutation).²⁷

Pediatric multisystem inflammatory syndrome

Children appear protected from SARS-CoV-2 infection and are more likely to be asymptomatic or have less severe

infection compared with their adult counterparts.^{28,29} However, more recently, a pediatric multisystem inflammatory syndrome has been described that appears distinct from the adult CS associated with acute SARS-CoV-2 infection and may follow mild or asymptomatic SARS-CoV-2 infection in previously healthy children. This illness has been reported in children aged from early childhood to adolescence, has Kawasaki-like features, and has been reported in areas with a high SARS-CoV-2 prevalence in children exposed to positive COVID-19 family members. In multiple cases, the child had positive SARS-CoV-2 serology but negative SARS-CoV-2 PCR result. Deaths of at least 3 children associated with this syndrome have been reported in the United States and the United Kingdom.⁸ Children with this syndrome have presented with similar features to Kawasaki disease,^{29,30} with high fever, rash, conjunctivitis, and diarrhea, and shown evidence of myocardial involvement, with elevated troponins, arrhythmia, shock, abnormal echocardiographic vascular findings, and coronary artery aneurysms. They have shown evidence of a hyperinflammatory state with elevated ferritin, triglycerides, and D-dimers. Treatments have included high-dose intravenous immunoglobulin and aspirin, as suggested for Kawasaki disease.³⁰ Currently, the specific immunopathogenic link to SARS-CoV-2 infection and the role of adjunctive immunomodulatory and anticytokine therapy in this syndrome is unknown.

The immunopathogenesis of CS in the setting of a viral respiratory tract infection

The airway epithelium is part of the first line of defense in presence of an airborne viral pathogen recognized as a pathogen-associated molecular pattern and/or damage-associated molecular pattern that bind to pattern recognition receptors such as Toll-like receptors on the surface of macrophages.⁹ The resident-activated alveolar macrophages, after several intracellular signaling cascades, generate TNF, IL-1 β , and IL-6 and trigger a systemic inflammatory response (Fig 1).³¹ This simultaneously prompts a well-coordinated local innate response composed of specific enzymes (defensins, mucins, lysozymes), nitric oxide, reactive oxygen species, platelet-activating factor, and other cytokines.³² Other key components of the innate immunity against viral infection are the type I IFNs. In contrast with findings on the influenza virus, patients with severe COVID-19 patients have minimal peripheral quantities of type I IFNs but increased IFNs and IFN genes in the bronchoalveolar environment, a discovery associated with CS development in a mouse model of SARS-CoV infection.³³ Furthermore, *in vitro*, SARS-CoV-2 failed to produce IFN expression in infected cells,³⁴ indicating a dampened early innate immune response.³⁵

Monocytes and macrophages play a central role and a disruption in the mononuclear phagocyte compartment is considered to increase the COVID-19-related hyperinflammation.^{36,37} Also, an increase in the CD14⁺CD16⁺ monocytes producing IL-6 has been noted in the peripheral blood of critically ill patients with COVID-19.^{38,39} Bruton's tyrosine kinase (BTK), an intracellular kinase, also appears to have a role in monocytes and macrophage activation and specifically in infection clearance by macrophages.⁴⁰

IL-1 β is produced after the inflammasome (especially nucleotide-binding and oligomerization domain-, leucine-rich repeats-, and pyrin domain-containing protein 3), activated by

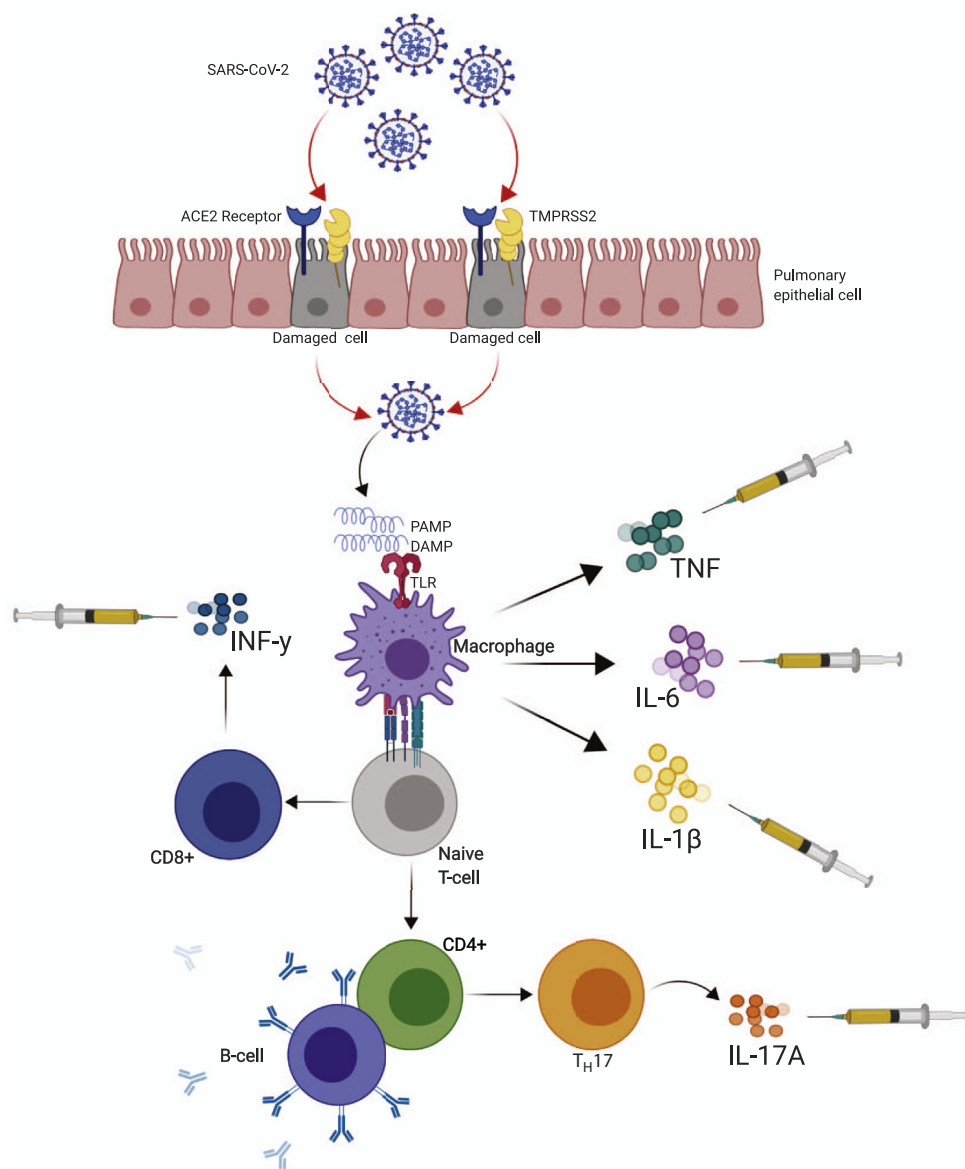


FIG 1. COVID-19 immunologic mechanisms for CS and the possible role of biologics. When SARS-CoV-2 PAMPs and/or DAMPs bind to TLRs on the surface of resident alveolar macrophages, they become activated and secrete TNF, IL-1 β , and IL-6. In increased levels, these cytokines will be the hallmark of the CS responsible for the ARDS and CS in COVID-19. The different targets of biologics are illustrated in the figure. Specifically, the downstream effect of IL-6 can be blocked with tocilizumab, sarilumab, or siltuximab and the effects of IL-1 β with anakinra or canakinumab. CD8⁺ T cells produce IFN- γ , causing direct tissue damage, whereas activated CD4⁺ T cells, in the presence of transforming growth factor β and IL-6, will differentiate into the T_H17-cell subset, responsible for secreting IL-17A and IL-17F, which, among numerous roles, target macrophages, dendritic cells, endothelial cells, and fibroblasts to increase the production of IL-1, IL-6, and TNF. *DAMP*, Damage-associated molecular pattern; *PAMP*, pathogen-associated molecular pattern; *TLR*, Toll-like receptor; *TMPRSS2*, transmembrane protease, serine 2.

absent in melanoma 2—sensing foreign DNA, induces the formation of caspase-1, which cleaves pro-IL-1 β into IL-1 β (Fig 2). In a study using single-cell RNA sequencing in the PBMCs of 10 patients with COVID-19 compared with 5 healthy controls, the authors reported an increased quantity of CD14⁺⁺ monocytes with inflammatory gene expression and CD14⁺⁺ IL-1 β ⁺ monocytes in the early recovery stages of SARS-CoV-2.⁴¹ IL-1 β is also implicated in the activity of nuclear factor kappa-light-chain-enhancer of activated B cells, inducing the synthesis of various

inflammatory genes of mediators such as IL-6.⁹ Thus, a reduction in IL-1 β activity would reduce IL-6 production.⁴²

Following initial escape of the innate response, recognition of virus promotes the migration of pulmonary dendritic cells to the lymph nodes for presentation of antigen to passing T cells for the development of more robust antigen-specific T- and B-cell adaptive response. During this response, soluble mediators play a role in cellular function and signal transduction by binding to specific receptors on the surface of target cells. For example,

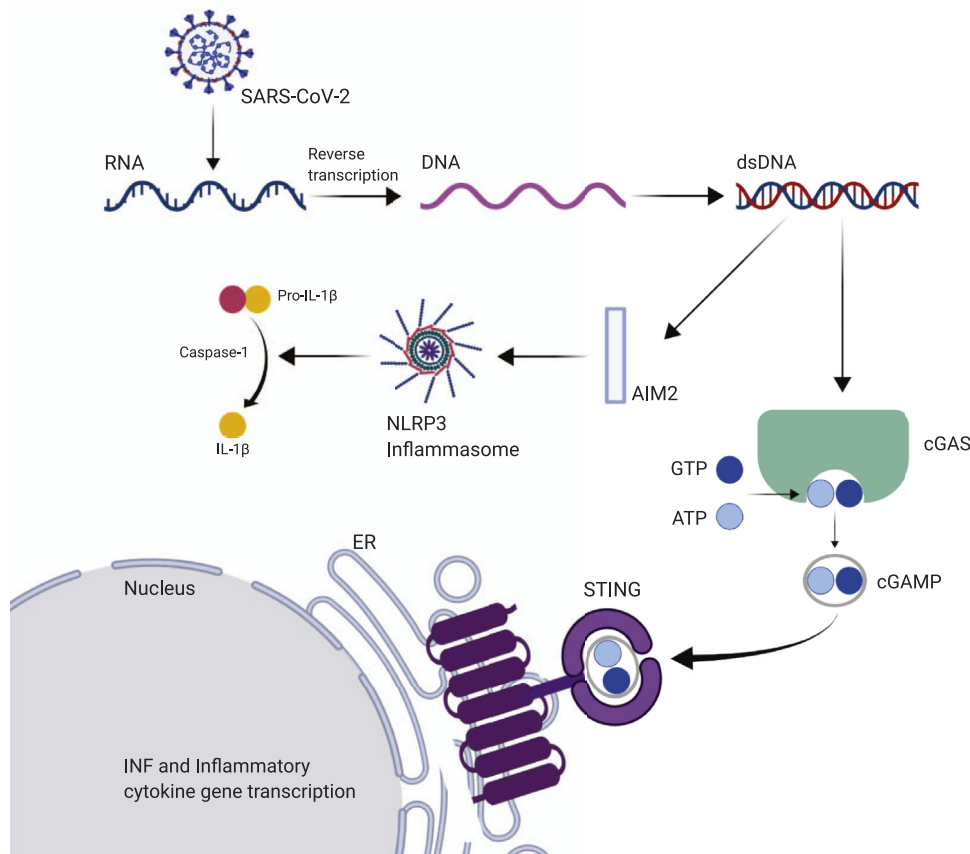


FIG 2. The implications of the STING pathway in coronavirus. The cGAS-STING pathway is activated by sensing foreign cytosolic DNA (obtained after reverse transcription from SARS-CoV-2). cGAS catalyzes the generation of cyclic GMP-AMP (cGAMP), which binds and activates STING in the ER, leading to the expression of IFNs and other cytokines. IL-1 β is produced after the NLRP3 inflammasome, activated by AIM2-sensing foreign DNA, induces the formation of caspase-1, which will cleave pro-IL-1 β into IL-1 β . AIM2, Absent in melanoma 2; AMP, adenosine monophosphate; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthetase; dsDNA, double-stranded DNA; GMP, guanosine monophosphate; GTP, guanosine triphosphate; ER, endoplasmic reticulum; NLRP3, nucleotide-binding and oligomerization domain-, leucine-rich repeats-, and pyrin domain-containing protein 3.

CD8⁺ T cells produce excessive amounts of TNF- α and IFN- γ , causing direct tissue damage, whereas activated CD4⁺ T cells, in the presence of transforming growth factor β and IL-6, will differentiate into T_H17-cell subset, important for extracellular pathogen elimination and autoimmunity (Fig 1).⁴³ The defining cytokines secreted by T_H17 cells are IL-17A and IL-17F, which primarily target macrophages, dendritic cells, endothelial cells, and fibroblasts to increase the production of IL-1, IL-6, and TNF- α .⁴³ In this particular setting, IL-6 will also inhibit the transforming growth factor β -dependent development of CD4⁺ regulatory T cells, a critical mediator of immune tolerance with a major role in regulating the effector T-cell response.⁴⁴

In the setting of CS syndromes, overactivation of effector CD4⁺ and CD8⁺ T cells and production of cytokines and chemokines generate an uncontrolled hyperinflammatory injury at the tissue level, resulting in local and distant injury.¹³ Increased inflammation is associated with peripheral blood lymphopenia, a significant drop in the lymphocyte to neutrophil ratio, and CD4⁺ T-cell dysfunction in observational studies but the mechanisms for these changes are unclear.^{38,45,46} In one of the first studies describing the postmortem pathological findings in 1 patient with COVID-19, peripheral flow cytometry indicated a

reduced CD4⁺ and CD8⁺ cell count but an increased proportion of activation markers such as HLA-DR and CD38 as well as an increased concentration of T_H17 cells.⁴⁷

Summary statement

Inflammatory cytokines and chemokines, including IL-6, IL-1 β , and TNF- α , are significantly elevated in patients with severe SARS-CoV-2 infection, suggesting that CS may play a role in the SARS-CoV-2 severity, morbidity, and mortality.

THE PLEIOTROPIC ROLE OF IL-6 IN INFLAMMATORY AND VIRAL RESPONSES

IL-6 is secreted by a plethora of immune and stromal cells including monocytes, macrophages, endothelial cells, B and T cells, hepatocytes, keratinocytes, adipocytes, dendritic cells, and fibroblasts. IL-6 exerts effects on a similarly broad array of cellular targets expressing the functional IL-6 receptor (IL-6R) such as T cells, B cells, vascular endothelial cells, monocytes, and hepatocytes.^{44,48} As may be expected, such diversity of targets translates into functional pleiotropy including the synthesis of acute-phase proteins in the liver, such as C-reactive protein

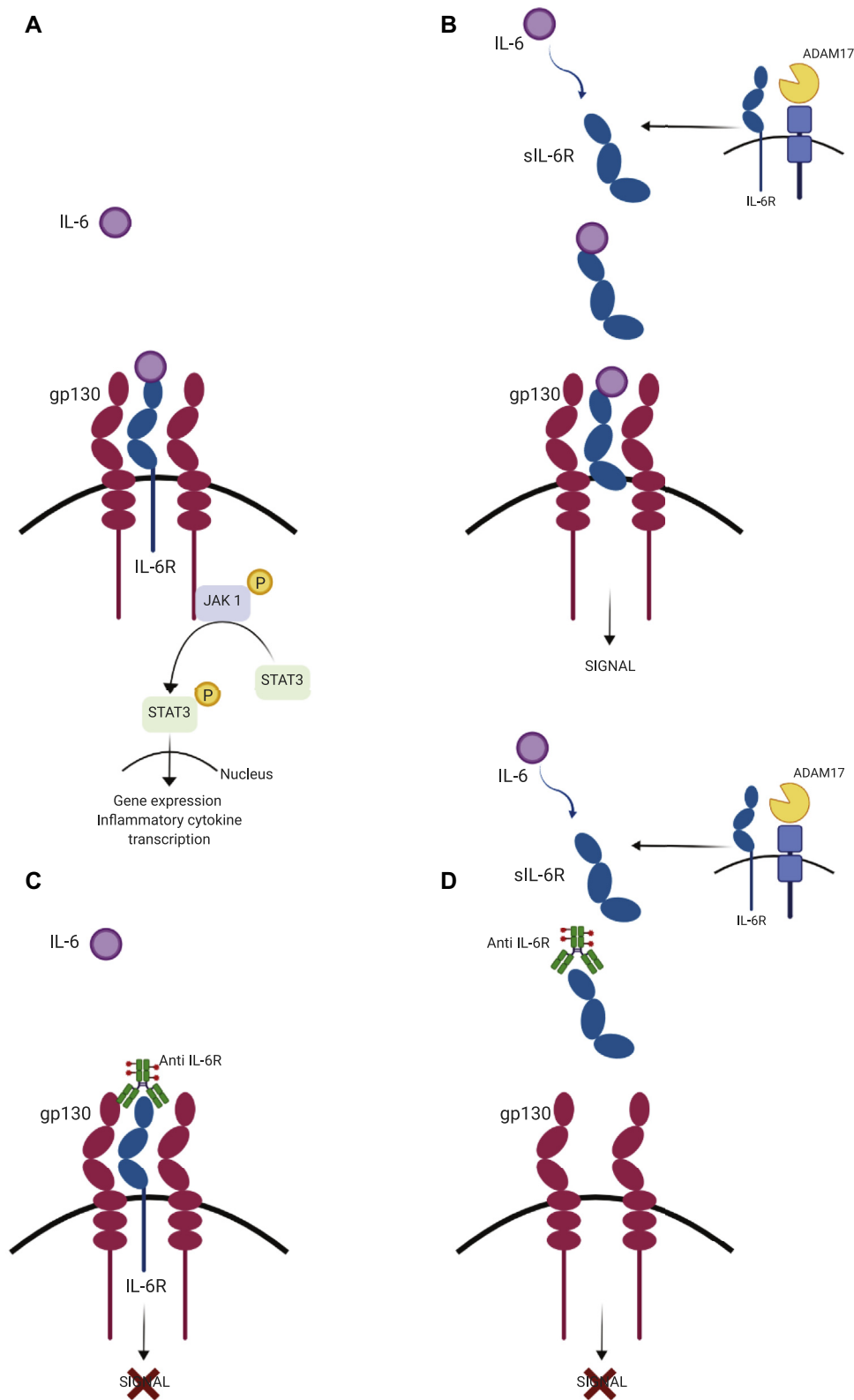


FIG 3. Classic and trans-signaling IL-6R. **A** and **B**, Different signaling pathways stimulated by IL-6. Binding of IL-6 to the membrane-bound or soluble IL-6 receptor (IL-6R) leads to gp130 dimerization and JAK 1–STAT 3 signaling and activation, leading to gene expression of inflammatory cytokines. This pathway is represented only in Fig 3, **A**, and replaced by the word “SIGNAL” in Fig 3, **B**. **A**, Classic signaling, which is restricted to several cell types, is initiated through binding of IL-6 to the membrane IL-6R and forms a complex with gp130. **B**, Trans-signaling is driven by IL-6 in all gp130-expressing cells. Proinflammatory functions have been found to be mediated through binding of soluble IL-6R shredded from cells undergoing ADAM17-mediated apoptosis. **C** and **D**, IL-6 blockade therapy using a humanized anti-IL-6R mAb. A humanized anti-IL-6R antibody blocks IL-6-mediated signaling pathway by inhibiting IL-6 binding to the membrane (Fig 3, **C**) and soluble (Fig 3, **D**) receptors. ADAM17, A disintegrin and metalloprotease family protein; gp130, glycoprotein 130; IL-6R, IL-6 receptor; sIL-6R, soluble IL-6R; STAT, signal transducer and activator of transcription.

TABLE I. Clinical and immunologic parameters associated with in-hospital mortality in COVID-19

Parameter	Studies				
	Zhou et al, ¹ 2020	Ruan et al, ⁷ 2020	Wu et al, ³ 2020	Wang et al, ⁶ 2020	Li et al, ⁵⁷ 2020
Country	China	China	China	China	China
Type of study	Retrospective cohort	Retrospective cohort	Retrospective cohort	Retrospective cohort	Retrospective cohort
Patients	191	150	201	33	548
Comorbidities*	Age > 69 y HTN CAD Diabetes	Age > 68 y HTN CAD	Age > 65 y HTN	NA	Age > 65 y HTN Male
Clinical*	↑ SOFA score† > 4.5	Dyspnea Respiratory failure ARDS AKI Other infection	Dyspnea	NA	NA
Laboratory*	Lymphopenia < 0.6 × 10 ⁹ /L Leucopenia < 4 × 10 ⁹ /L ↑ Procalcitonin < 0.1 ng/mL ↑ Creatinine > 133 μmol/L ↑ D-dimer > 1 μg/mL ↑ ALT > 40 U/L ↑ LDH > 245 U/L ↑ Troponin I > 28 pg/mL ↑ CK > 185 U/L ↑ Ferritin > 300 μg/L	Lymphopenia < 0.6 × 10 ⁹ /L Leucocytosis > 10.6 × 10 ⁹ /L ↑ CRP > 126.6 mg/L ↑ Creatinine > 91.2 μmol/L ↑ Urea > 8.6 μmol/L ↑ Troponin I > 30 pg/mL ↑ Myoglobin > 258.9 ng/mL	Lymphopenia < 0.6 × 10 ⁹ /L ↑ Urea > 7.4 μmol/L ↑ D-dimer > 3.95 μg/mL ↑ LDH > 484 U/L	Lymphopenia < 1.1 × 10 ⁹ /L Neutrophilia > 6 × 10 ⁹ /L ↑ Creatinine > 100 μmol/L ↑ Urea > 7.5 μmol/L ↑ D-dimer > 500 μg/L	Leucocytosis ↑ LDH > 445 U/L
IL-6	Nonsurvivors (N = 54) 11 (7.5-14.4) pg/mL Survivors (N = 137) 6.3 (5.0-7.9) pg/mL	Nonsurvivors‡ (N = 68) 11.4 ± 8.5 ng/mL Survivors‡ (N = 82) 6.8 ± 3.6 ng/mL (N = 82)	Nonsurvivors‡ (N = 44) 10.1 (7.4-14.8) pg/L Survivors‡ (N = 117) 6.3 (5.4-7.8) pg/L	NA	NA

AKI, Acute kidney injury; ALT, alanine transaminase; CAD, coronary artery disease; CK, creatine kinase; HTN, hypertension; LDH, lactate dehydrogenase; NA, not available; SOFA, Sequential Organ Failure Assessment.

Values are expressed as mean ± SD or mean (interquartile range).

*Only statistically significant variables are presented ($P < .05$).

†SOFA score: This score includes multiples parameters such as assessment of respiratory status (partial pressure of oxygen, fraction of inspired oxygen and oxygen saturation), coagulation parameters (platelets), liver function (bilirubin), hypotension, central nervous assessment with Glasgow coma score, and renal function (creatinine).⁵⁸

‡The IL-6 units reported in these studies do not compare with the units generally presented. Unfortunately, the method used for measuring IL-6 was not provided.

(CRP), which is a surrogate for IL-6; the decreased production of proteins such as albumin; the differentiation of B cells into plasma cells; and hematopoiesis and other metabolic and neurologic processes.^{44,48} CRP is an acute-phase reactant that binds the phospholipid component of microorganisms and damaged cells that is frequently used as a screening marker of infection and/or inflammation.³¹

IL-6 affects cellular immunity with both proinflammatory and anti-inflammatory functions. IL-6 genetic knockout mice present with varied impairments of inflammatory response including a well-documented increased susceptibility to microbial infection, whereas humans expressing defective IL-6 receptors experience a hyper-IgE syndrome-like disorder that clinically manifests as dermatitis and recurrent (staphylococcal and mycotic) infections, highlighting the important role that IL-6 likely plays in the diverse pathways of IgE-mediated allergy and microbial defense.⁴⁹

Contrasting inflammatory functions of IL-6 are mediated through its modality of receptor binding. Classical binding of IL-6 to the membrane-bound IL-6 receptor (IL-6R) leads to glycoprotein 130 dimerization, Janus-associated kinase (JAK) 1 signaling, and activation, among others, of the classical RAS/RAF/mitogen-activated protein kinase pathways, leading to anti-inflammatory responses (Fig 3).⁵⁰ Although all human cells display preformed, inactive glycoprotein 130 receptors on their cell surface, this receptor

remains inactive without the presence of IL-6R, which is expressed only on certain cell types.⁵¹ However, proinflammatory functions have been found to be mediated through binding of soluble IL-6R, termed trans-signaling, with important ramifications for potential therapeutic targeting.⁵² It has been shown that an important source of soluble IL-6R is shredded from cells undergoing ADAM17-mediated apoptosis, which controls mononuclear phagocyte recruitment, leading to amplified inflammatory response.⁵³ The proinflammatory responses of IL-6 are mediated by trans-signaling, whereas the anti-inflammatory functions are probably realized by classic signaling (Fig 3).⁵⁰ Selective blockage of this trans-signaling pathway is likely to have the beneficial effect of blocking inflammation without the undesirable off-target effects of broad immune suppression.

Summary statement

IL-6 has major effects on cellular immunity with both proinflammatory and anti-inflammatory functions.

The role of IL-6 and other mediators in the response to SARS-CoV-2 infection

A multitude of markers for COVID-19 severity have been proposed such as CD4⁺ and CD8⁺ T-cell lymphopenia^{3,5,54} as

TABLE II. Review of hospital admission IL-6 values in patients with COVID-19

Reference	Setting Country	N	Control* (pg/mL)	Cutoff (pg/mL)	Critically ill patients† (pg/mL)	Predictor of complications‡ (pg/mL)	Predictor of mortality‡ (pg/mL)	Method for IL-6 monitoring
4	Hospital Germany	40	19.6 (0-76.5), N = 27	80	NA	121.0 (19.2-430.0), N = 13	NA	NA
3§	Hospital China	201	6.3 (5.4-7.8) pg/L, N = 117	NA	6.1 (5.1-6.7) pg/L, N = 40	7.4 (5.6-10.9) pg/L, N = 84	10.1 (7.4-14.8) pg/L, N = 44	NA
7§	Hospital China	150	6.8 ± 3.6 ng/mL, N = 82	NA	NA	NA	11.4 ± 8.5 ng/mL, N = 68	NA
1	Hospital China	191	6.3 (5.0-7.9), N = 137	NA	NA	NA	11.0 (7.5-14.4), N = 54	NA
55	Hospital China	48	10.4 (3.8-31.0), N = 21	100	64 (25.6-111.9), N = 17	NA	NA	ECLIA (Roche Ltd)
56	Hospital China	43	10.6 (5.1-24.2), N = 28	24.3	NA	36.1 (23.0-59.2), N = 17	NA	ECLIA (Rochecobase601)
60	Hospital China	43	6.7 (4.4-12.4), N = 36	NA	NA	51.7 (34.3-161.7), N = 7	NA	NA
54	Hospital China	53	13.4 ± 1.8, N = 45	NA	37.8 ± 7.8, N = 18	NA	NA	FMBA (Qingdao Raisecare Biotechnology Co)

ECLIA, Electrochemiluminescence method; FMBA, flow cytometer microsphere-based assay; NA, not available.

Values are expressed as mean ± SD or mean (interquartile range). N is the number of patients included in each study.

*The "control" IL-6 value represents the patients diagnosed with mild symptoms of COVID-19 included in the studies (no healthy controls included).

†Some studies included IL-6 levels dosed after hospital admission and during disease progression.

‡This value was recorded upon hospital admission and predicted either sepsis or mortality.

§The IL-6 units reported in these studies do not match the units generally presented. Unfortunately, the method used for measuring IL-6 was not provided.

well as global lymphopenia. Homing of lymphocytes to the lungs is significantly increased in nonsurvivors compared with survivors.¹ A number of publications now highlight that an increase in IL-6 correlates with severe response to SARS-CoV-2, defined as the development of sepsis, ARDS, requirement for mechanical ventilation, and death.^{1,3,5,7,46,54-56} The clinical and immunologic parameters associated with in-hospital mortality in patients with COVID-19 are reported in Table I.^{57,58} The structural N protein of coronaviruses performs an essential role during host cell entry as well as virus particle assembly and release.¹¹ The N protein from SARS-CoV activates the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells and, subsequently, the expression of IL-6 in human airway epithelial cells, providing a biologically plausible explanation for the role of IL-6 in SARS-CoV-2 infection immune pathogenesis.¹¹

In a cohort of 60 patients hospitalized with SARS-CoV-2 infection, IL-6 concentrations were 163 ± 153 pg/mL for the group with mild symptoms and 517 ± 796 pg/mL for the patients with severe presentation (intensive care unit admitted).⁵⁹ A summary of the observations from studies of IL-6 in patients hospitalized with COVID-19 is presented in Table II.^{1,3,4,7,54-56,60} IL-6 may be both a biomarker and a potential therapeutic target for patients hospitalized with COVID-19, which is an attractive concept in the absence of alternative direct-acting antiviral strategies.

Importantly, proposed clinical cutoff values for IL-6 in this setting have started to emerge, with greater than 80 pg/mL determined to predict respiratory failure in a study with 40 patients with COVID-19 in which 13 required medical ventilation⁴ and greater than 100 pg/mL in patients with detectable serum SARS-CoV-2 nucleic acid was found to correlate with mortality (N = 17 of 48).⁵⁵ However, other authors have reported specificity at much lower concentrations, with a retrospective

study of 43 patients with COVID-19 from China indicating that severe cases could be predicted using an IL-6 value greater than 24.3 pg/mL (sensitivity of 73.3% and specificity of 89.3%).⁵⁶ Although studies with larger sample sizes are urgently required to determine the true IL-6 cutoff associated with severe disease, intensive care unit admission, and mortality, these initial results are promising. The presented data are limited by their retrospective nature and uncertainty whether IL-6 levels can be ascertained by clinical laboratories in an expedited fashion to guide therapy in real time.

Summary statement

Several recent publications have shown that an increase in the proinflammatory cytokine IL-6 correlates with disease severity, defined as sepsis, ARDS, or mechanical ventilation, and mortality in SARS-CoV-2.

Lessons from IL-6 and other proinflammatory states including sepsis

IL-6 levels are considered to be undetectable, or below 10 pg/mL (with some intertest variability), in healthy controls.^{12,61,62} Conversely, mean IL-6 levels at presentation appear highest in severe sepsis (51.4 pg/mL) compared with patients who do not develop severe sepsis (36.5 pg/mL; *P* < .03) in a study of community-acquired pneumonia.⁶³ This response is highly specific for severe disease, and some studies indicate a role in disease progression, demonstrating up to a 4-fold decrease in IL-6 3 days after initial diagnosis. Moreover, IL-6 values appear to drop abruptly in survivors while remaining higher in nonsurvivor groups.⁶²⁻⁶⁴ Nonetheless, IL-6 currently represents one of the best characterized markers of disease severity and an early rise in IL-6 is associated with sepsis, organ failure, and death.⁶⁵⁻⁶⁷

TABLE III. Literature review of hospital admission IL-6 values in critically ill patients

Reference	Population/IL-6 dosage technique	Setting Country	Study design	N	Control* (pg/mL)	Cutoff† (pg/mL)	Sepsis ‡ (pg/mL)	Predictor of sepsis§ (pg/mL)	Predictor of mortality (pg/mL)
12	Patients with SARS-CoV Detection level NA (CBA)	Hospital Taiwan	MCRC	88	7.5 ± 30.4	NA	245.7 ± 770.2	NA	387.2 ± 911.82
59	Patients with SARS-CoV Detection >10 pg/mL (ELISA)	Hospital China	SCPC	228	61.0 ± 10.1	NA	NA	163 ± 153 517 ± 796 (severe)	NA
68	Patients with SIRS, sepsis (S), and septic shock (C) Detection level NA (ELISA)	ED Korea	SCPC	142	23.6 (11.2-43.5)	52.60 (S) 348.9 (C)	NA	89.9 (45.2-272.6) (S) 1,378.6 (256.4-11,062.1) (C)	≥348.92 7,609.5 (4,526.0-12,208.4) (28 d)
70	Critically ill patients with organ dysfunction Detection level NA (RT)	ICU Japan	SCPC	100	104 (46-152)	152	NA	720 (183-7,656)	NA
62	Patients with severe sepsis Detection level NA (ELISA)	ED Taiwan	SCPC	76	32.9 (0-663.5)	NA	NA	223.4 (3.1-979.1) septic shock	196.3 (0.5-979.1)
73	Patients with sepsis Detection level NA (ELISA)	ICU Finland	MCPC	61	426 (234-1,000)	NA	NA	NA	1,000 (269-2,000)
74	Patients with SIRS Detection level > 9.7 pg/mL (ELISA)	ICU Malaysia	SCPC	239	183 (61-358)		238 (86-3,159)		1,127 (218-8,643) (30 d)
64	Patients with SIRS Detection level NA (ECLIA)	ED Korea	SCPC	177	55.3 ± 100.9	75 (sepsis) 145 (shock)	NA	900.1 ± 1,643.4	1,018.8
69	Patients with infection suspicion Detection level NA (ECLIA)	ED Finland	SCPC	539	15.3 (1.5-653)	NA	NA	93.5 (1.5-43 790)	NA
71	Patients with infection and SIRS Detection level NA (ECLIA)	ICU Switzerland	SCPC	78	44.2	200	NA	NA	1,000
75	Patients with major trauma (female vs male) Detection level NA (ELISA)	ED Germany	SCPC	343	163.7 ± 25.98	NA	NA	363.9 ± 72.58	NA
61	Patients with major trauma Detection level > 7.8 pg/mL (ELISA)	ICU France	SCPC	100	55.7 (45.9-83.8)	NA	NA	95.1 (71.3-210.3)	NA
65	Patients with major trauma Detection level NA (ELISA)	Trauma unit Switzerland	SCRC	1,032	282.1 ± 39.8	NA	NA	551.6 ± 124.1	NA
63	Patients with CAP Detection level > 5.9 pg/mL (ECLIA)	ED The United States	MCPC	1,426	38.7	NA	98.7	51.4	109.4 (90 d)

CBA, Human T_H1/T_H2 cytokine or chemokine bead array kit; CAP, community-acquired pneumonia; ED, emergency department; ECLIA, electrochemiluminescence method; ICU, intensive care unit; NA, not available; RT, routine testing; SIRS, systemic inflammatory response syndrome.

Types of study design: SCRC, single-center retrospective cohort; SCPC, single-center prospective cohort; MCRC, multicenter retrospective cohort; MCPC, multicenter prospective cohort.

Values are expressed as mean ± SD or mean (interquartile range). N is the number of patients included in each study.

*The control group does not include any healthy controls.

†The authors calculated a cutoff value that could predict sepsis.

‡Some studies included IL-6 levels dosed after sepsis diagnostic.

§This initial value was recorded on admission to the hospital (ED) or ICU depending on the study and predicted either sepsis or mortality.

TABLE IV. Cytokine release storm—Grades and treatment⁷⁶

Cytokine release storm: A multiorgan clinical diagnostic involving constitutional, cardiovascular, hematological, gastrointestinal, cutaneous, and neurological manifestations		
Grade	Clinical manifestations	Recommended treatment
1: Mild	Patients require symptomatic treatment only Fever ± other constitutional symptoms (no organ dysfunction)	Supportive care (fluids, antipyretics, analgesics as needed)
2: Moderate	Symptoms respond to moderate intervention Hypoxia (oxygen requirement < 40% FiO ₂) or hypotension (responsive to IV fluids or low-dose vasopressors) Grade 2 organ toxicity*	Supportive care Cardiac and other organ function monitoring If comorbidities or older age, consider treatment as per grade 3
3: Severe	Symptoms respond to aggressive intervention Hypoxia (oxygen requirement ≥ 40% FiO ₂) or hypotension requiring high-dose or multiple vasopressors Grade 3 organ toxicity† or grade 4 transaminitis‡	Tocilizumab§ Adults: 4 mg/kg Children: 8 mg/kg Repeat the dose if clinical improvement does not occur within 24-48 h ± low-dose corticosteroids
4: Life-threatening	Requirement for mechanical ventilation or grade 4 organ toxicity‡ (excluding transaminitis)	Tocilizumab ± low-dose corticosteroids

CTCAE, Common Terminology Criteria for Adverse Events; FiO₂, fraction of inspired oxygen; IV, intravenous.

*CTCAE grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.

†CTCAE grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care.

‡CTCAE grade 4: Life-threatening consequences; urgent intervention indicated.

§Dose of tocilizumab approved for adults and children with rheumatoid arthritis.

||Data concerning the use of steroids in COVID-19 are limited. Please refer to the National Institutes of Health treatment guidelines.⁷⁷

Assigning discrete cutoff values for IL-6 to enable its use as a clinical diagnostic tool has remained ill-defined because of variations in the literature. Song et al⁶⁸ demonstrated in 142 patients that an IL-6 cutoff value of 52.60 pg/mL and 348.9 pg/mL was associated with a diagnostic and prognostic value, respectively, in patients with systemic inflammatory response syndrome.⁶⁸ In contrast, in another systemic inflammatory response syndrome cohort (N = 177), a cutoff of 75 pg/mL for sepsis and 145 pg/mL for septic shock was defined.⁶⁴ Thus, even if further clarification is required, the literature demonstrates that elevated IL-6 values are associated with sepsis or septic shock development.^{62,69-71} This is also supported by a 2016 meta-analysis of 2680 critically ill patients from 22 studies—the use of IL-6 was of moderate diagnostic capacity and relatively high specificity in defining sepsis from other systemic inflammatory response syndrome,⁷² and, thus, IL-6 may be of utility to confirm infectious causation in patients with complex presentation while considering the limitation in terms of availability of IL-6 levels. The specific IL-6 values from critically ill patients are represented in Table III.^{12,59,61-65,68-71,73-75}

Summary statement

As with the development of any novel diagnosis and because increased levels of IL-6 are associated with sepsis and septic shock, clinical cutoffs must be defined.

Lessons from IL-6 response in chimeric antigen receptor T-cell-associated cytokine release syndrome

There are similarities between the immunopathology of sepsis-associated CS and the cytokine release syndrome (CRS), a well-described complication of chimeric antigen receptor T-cell (CAR T-cell) therapy or hematopoietic cell transplantation. Although these terms should not be used interchangeably, CRS

was described as part of the CS syndromes.¹⁴ The CRS is a cytokine-mediated systemic inflammatory disease that groups signs and symptoms of multiple organ damage ranging from mild constitutional symptoms (grade 1) to end-organ damage (grade 4).^{23,24} Multiple grading systems for CRS have been provided in the literature, and a commonly used one is presented in Table IV. In the case of CRS, the cytokines are released directly by the infused CAR T cells or by other immune cells such as macrophages in response to the cytokines produced by the CAR T cells.²⁵ In some series of CRS, serum IL-6 levels correlated with the activation of potent T lymphocytes and CAR T-cell expansion, predicting subsequent therapeutic response and tumor control.^{78,79} Humanized IL-6R inhibitors such as tocilizumab have been integrated into CAR T-cell treatment protocols to preemptively manage CRS.

ROLE OF BIOLOGICAL IMMUNOTHERAPIES IN SARS-CoV-2 Targeting IL-6

The use of biomarkers such as IL-6 and downstream CRP to recognize early the hyperinflammatory state of SARS-CoV-2 infection has been proposed as a trigger point for using immunologic therapies. Importantly, many such immunotherapies are already available for different treatment indications including those that target the IL-6 and IL-6R. Tocilizumab is a humanized anti-IL-6R antibody engineered by grafting the complementarily determining regions of a mouse antihuman IL-6R antibody into a human IgG1κ to create a human antibody with a human IL-6R binding site. Critically for the opposing proinflammatory and anti-inflammatory functions previously discussed, tocilizumab binds to both membrane-bound and soluble IL-6R for total inhibition of IL-6 signal transduction (Fig 3). The main side effects of completely blocking IL-6 signaling are neutropenia, thrombocytopenia,

TABLE V. Potential therapies for COVID-19 ARDS and CS

Name	Commercial name	Target	Role	FDA indications	Trials Country	Planned clinical trials					
Tocilizumab	Actemra RoActemra	Membrane or soluble IL-6R	Inhibits IL-6 signal transduction	CRS Rheumatoid arthritis Giant cell arteritis Juvenile idiopathic arthritis	COVACTA-the United States	NCT04320615 NCT04356937 NCT04331795					
					Italy	NCT04346355 NCT04332913 NCT04317092 NCT04315480					
					Spain	NCT04335305 NCT04332094					
					France	NCT04331808					
					Belgium	NCT04330638					
					Greece	NCT04339712					
					Switzerland	NCT04335071					
					Denmark	NCT04322773					
					Malaysia	NCT04345445					
					China	NCT04310228 NCT04306705					
					Sarilumab	Kevzara	Membrane or soluble IL-6R	Inhibits IL-6 signal transduction	Rheumatoid arthritis	International	NCT04327388
										The United States	NCT04315298
										Canada	NCT04321993
France	NCT04324073										
Spain	NCT04357808 NCT04357860										
Denmark	NCT04322773 NCT04345289										
Siltuximab	Sylvant	IL-6	Inhibits IL-6 signal transduction	Multicentric Castleman disease						Italy	NCT04322188
					Spain	NCT04329650					
					Belgium	NCT04330638					
Anakinra	Kineret	Type 1 IL-1 receptor	Inhibits IL-1 α and IL-1 β signal transduction	Rheumatoid arthritis NOMID	The United States	NCT04362111					
					Italy	NCT04324021					
					Greece	NCT04339712 NCT04357366					
					France	NCT04341584					
Canakinumab	Ilaris	IL-1 β	Blocking IL-1 β interaction with IL-1 receptors	Periodic fever syndromes Juvenile idiopathic arthritis	Italy	NCT04348448					
Ruxolitinib	Jakafi Jakavi	JAK1, JAK2 inhibitor	Inhibits cytokine-induced STAT phosphorylation	Myelofibrosis Polycythemia Vera Acute graft-versus-host disease	The United States	NCT04354714 NCT04348071					
					Canada	NCT04331665					
					Mexico	NCT04334044					
					Germany	NCT04359290					
					Spain	NCT04348695					
Tofacitinib	Xeljanz	JAK1, JAK2, JAK3, TYK2 inhibitor	Inhibits cytokine-induced STAT phosphorylation	Rheumatoid arthritis Psoriatic arthritis Ulcerative colitis	Italy	NCT04332042					
Baricitinib	Olumiant	JAK2 (JAK 1/3, TYK2), AAK1 inhibitor	Inhibits cytokine-induced STAT phosphorylation	Rheumatoid arthritis	The United States	NCT04340232					
					Canada	NCT04321993					
					Italy	NCT04320277					
Fedratinib	Inrebic	JAK2, FLT3, and BRD4 inhibitor	Inhibits cytokine-induced STAT phosphorylation	Myelofibrosis	None	None					
Acalabrutinib	Calquence	BTK	Inhibits BTK signaling/B-cell activation	Mantle cell lymphoma Chronic lymphocytic leukemia Small lymphocytic lymphoma	The United States	NCT04380688					
					Europe	NCT04346199					
Eculizumab	Soliris	Complement protein C5	Inhibits C5 cleavage to C5a and C5b (prevents formation of C5b-9)	Paroxysmal nocturnal hemoglobinuria (PNH)	The United States	NCT04288713					
					France	NCT04346797 NCT04355494					
					Ravulizumab	Ultomiris	Complement protein C5	Inhibits C5 cleavage to C5a and C5b (prevents formation of C5b-9)	Paroxysmal nocturnal hemoglobinuria (PNH)	The United States	NCT04369469 NCT04390464
Emapalumab	Gamifant	IFN- γ	Binds to and neutralizes IFN- γ	Primary HLH						Italy	NCT04324021

(Continued)

TABLE V. (Continued)

Name	Commercial name	Target	Role	FDA indications	Trials Country	Planned clinical trials
Adalimumab	Humira	TNF- α	Inhibits TNF- α signal transduction	Rheumatoid arthritis Psoriatic arthritis Ankylosing spondylitis Crohn disease Ulcerative colitis Plaque psoriasis Hidradenitis suppurativa	China	ChiCTR2000030089
Secukinumab	Cosentyx	IL-17A	Inhibits IL-17A signal transduction (via IL-17 receptor)	Psoriatic arthritis Ankylosing spondylitis Plaque psoriasis	None	None

AAK1, AP2-associated protein kinase 1; BRD4, bromodomain-containing protein 4; FLT3, fms-like tyrosine kinase 3; NOMID, neonatal-onset multisystem inflammatory; STAT, signal transducer and activator of transcription; TYK2, tyrosine kinase 2.

and liver enzyme abnormalities.⁸⁰ Serious infections have been reported in patients treated long-term with tocilizumab so caution should be used.⁸¹ Nonetheless, tocilizumab is Food and Drug Administration (FDA) approved for not only rheumatoid arthritis for which it was originally developed and provides beneficial relief from this largely T_H17-driven disease, but, more recently, for severe or life-threatening (grade 3 or 4) CRS associated with CAR T-cell therapy (Table IV) with a dramatic reversal of the clinical manifestations.²⁰ For CRS, initial studies dosed patients at 8 mg/kg and 12 mg/kg infused intravenously over 60 minutes, with up to 3 additional doses if needed (minimum 8 hours between consecutive doses).⁸² Responders were defined as patients with symptom resolution within 14 days.

Because of the proposed benefits of using tocilizumab in patients with CAR T-cell-induced CRS and the described similarities between CRS and CS following infection, randomized trials are recruiting in COVID-19. In certain centers, tocilizumab has been used in a compassionate access fashion in critically severe patients with COVID-19. A retrospective study from China (N = 21) that used tocilizumab 400 mg intravenous drip (single dose) with or without lopinavir/ritonavir and methylprednisolone demonstrated improvement in fever, hypoxemia, CRP levels, and pulmonary computed tomography imaging, without adverse events.⁸³ The mean CRP levels before the drug were 75.06 \pm 66.80 mg/L and decreased to 38.13 \pm 54.21 mg/L at day 1, 10.61 \pm 13.79 mg/L at day 3, and 2.72 \pm 3.60 mg/L at day 5.⁸³ The mean IL-6 level before the first dose of tocilizumab was 132.38 \pm 278.54 pg/mL.⁸³ Although follow-up IL-6 levels were not subsequently ascertained in this study, the pretreatment IL-6 concentration aligns with severe disease cutoffs in those studies mentioned earlier. Of immense importance for monitoring, increased serum IL-6 may be expected after initial treatment with tocilizumab.⁸⁴ Indeed, it is considered that the usual IL-6R-mediated consumption of IL-6 is altered by the bound between tocilizumab and IL-6R and that the IL-6 level during tocilizumab treatment probably reflects disease activity.⁸⁴ Furthermore, in this study, IL-6 was also significantly increased in 20 healthy volunteers 7 days after a single dose of tocilizumab (3.0 \pm 0.6 pg/mL at baseline and 9.3 \pm 1.0 pg/mL at day 7).⁸⁴ Therefore, it is proposed that posttocilizumab use, monitoring of CRP may be a more appropriate assay for monitoring inflammation.^{44,84} A French center has also shared its experience with tocilizumab 8 mg/kg (up to 2 doses) in 30 severe patients with SARS-CoV-2, defined as requiring more than 6 L/min oxygen

therapy with rapid changes in oxygen needs (increase of more than 3 L/min in 12 hours) and having a more than 5-day disease diagnosis.⁸⁵ The authors found that, when compared with a matched control group, the drug decreased the need for mechanical ventilation and intensive care unit admission (23 of 30).⁸⁵ Finally, in an observational study from the United States, 153 patients with severe COVID-19 (defined as patients requiring supplemental oxygen and critical care) were treated with an 8 mg/kg intravenous tocilizumab dose (maximum 800 mg). When compared with the nonsevere group, survival rates were similar (P = .11).⁸⁶

In light of these promising results, the FDA has approved a randomized, double-blind, placebo-controlled phase III clinical trial A Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia (COVACTA) with 55 locations in North America and Europe. This trial aims to test the efficacy and safety of intravenous tocilizumab in patients with severe SARS-CoV-2 infection (NCT04320615). Similarly, a multicenter, randomized controlled trial was started in China to test the efficacy and safety of tocilizumab in the treatment of patients with COVID-19 pneumonia and elevated IL-6 levels (ChiCTR2000029765). The Italian Regulatory Drug Agency (Agenzia italiana del farmaco; AIFA) has approved a multicenter, single-arm, open-label, phase 2 study (TOCOVID-19) where all the patients will be treated with tocilizumab 8 mg/kg intravenously (up to a maximum of 800 mg per dose), the primary goal being to assess the mortality rate after the first month (EudraCT: 2020-001110-38). There are currently more than 20 registered COVID-19-associated tocilizumab trials (Table V). A study registered in Greece proposes to individualize immunomodulatory treatment including tocilizumab or anakinra in COVID-19 depending on their cytokine profile (NCT04339712).

Sarilumab, an mAb to IL-6 receptor, is also being investigated in COVID-19 trials. A French multicenter randomized controlled trial (Cohort Multiple Randomized Controlled Trials Open-label of Immune Modulatory Drugs and Other Treatments in COVID-19 Patients - Sarilumab Trial; CORIMUNO-SARI) aiming to assess the efficacy and safety of sarilumab versus standard of care is ongoing (NCT04324073). Two additional industry-driven clinical trials (NCT04315298 and NCT04327388) aiming to assess the efficacy and safety of sarilumab in patients hospitalized with COVID-19 are recruiting. Although the clinical outcome data for sarilumab are lacking, the comparative response with tocilizumab will be of interest given the longer half-life of sarilumab and greater affinity for the IL-6R.⁸⁰

Siltuximab, a chimeric mAb targeting IL-6 directly and preventing binding to both soluble and membrane-bound IL-6 receptors, is FDA approved for the multicentric Castleman disease.⁸⁷ There are currently 3 European trials recruiting patients diagnosed with COVID-19 (NCT04322188, NCT04329650, and NCT04330638).

Targeting IL-1 β

IL-1 β leads to an increase in body temperature, lung inflammation, and fibrosis.³⁶ Increased levels of IL-1 β were noted in patients diagnosed with SARS-CoV⁸⁸ and similar to IL-6, were associated with increased mortality in sepsis.⁹ Anakinra is a nonglycosylated human decoy IL-1 receptor antagonist (IL-1Ra) that binds to the type 1 IL-1 receptor and inhibits IL-1 α and IL-1 β signal transduction.⁸⁹ This drug is FDA approved for rheumatoid arthritis and neonatal-onset multisystem inflammatory disease⁹⁰ and suggested in the treatment algorithm for secondary HLH/MAS.⁹¹

A recent study found that the serum IL-1 β levels were undetectable in 100% (N = 17) of the patients with severe or moderate SARS-CoV-2 infection,⁵ an expected result considering the mechanism of action of this exocrine cytokine. Anakinra was used in a cohort from Italy to treat 29 adult patients diagnosed with COVID-19–related moderate to severe ARDS and hyperinflammation (defined as serum CRP \geq 100 mg/L, ferritin \geq 900 ng/mL, or both).⁹² Survival was 90% compared with 56% in a standard treatment group (N = 16) ($P = .009$).⁹² Other improvements included a reduction in CRP and a decrease in mechanical ventilation use. Posttreatment inflammatory relapse was not reported and the treatment was well tolerated.⁹²

Furthermore, the post hoc analysis of a phase III randomized controlled trial studying the use of anakinra in severe sepsis indicated a significant improvement in survival of patients with sepsis with features of MAS in the absence of any severe adverse reactions.²⁶ CORIMUNO-ANA is a trial that aims to determine the efficacy of anakinra in SARS-CoV-2–infected patients (NCT04341584). Anakinra will be administered twice daily as decreasing doses of intravenous infusions (400 mg on day 1, 2, and 3; 200 mg on day 4; and 100 mg on day 5). Canakinumab is a human anti-IL-1 β mAb that blocks IL-1 β interaction with the IL-1 receptor for which there is currently 1 registered observational study (NCT04348448).

Targeting TNF- α and IFN- γ

Similar to IL-1 β , TNF- α has a direct role in acute systemic inflammation and is increased in patients with severe SARS-CoV-2 infection.^{2,5,57} However, this finding is not consistent among the different studies.⁵⁴ Besides the observational reports that indicate an increase in the levels of this cytokine, a direct pathogenic mechanism of cellular viral entry involving the shedding of the coronavirus' functional receptor, the ACE2, was studied. This process of binding and shedding of the ACE2 is coupled with TNF- α production and the production of a TNF- α –converting enzyme.⁹³ Thus, it has been suggested that an anti-TNF drug could not only inhibit TNF- α directly but also downregulate the expression and shedding of ACE2. Also, some studies showed a decrease in sepsis-related mortality with anti-TNF treatment.⁹

There are multiple commercialized anti-TNF biologics. Adalimumab is a recombinant human IgG₁ mAb that specifically

binds to human TNF- α and blocks its interaction with the p55 and p75 cell-surface TNF receptors. This drug could be potentially useful in managing severe COVID-19 manifestations. To analyze the benefits of an anti-TNF- α treatment in COVID-19, a randomized controlled trial of adalimumab injection in severe patients with COVID-19 has been registered (ChiCTR2000030089).

Similar to TNF, the major proinflammatory cytokine IFN- γ is also increased in the CS associated with COVID-19. IFN- γ was particularly well described in patients with SARS-CoV¹² and may be targeted by emapalumab for which a comparative multicenter randomized clinical trial is also underway in combination with anakinra (NCT04324021).

Targeting IL-17

Another cytokine that could have a role in the CS caused by COVID-19 is IL-17. This was not a cytokine of interest in the recent SARS-CoV-2 studies, and the only study that characterized IL-17 in COVID-19 found normal levels using a flow cytometry method.⁵⁴

As described in this review, IL-17 stimulates the production of proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α . Secukinumab is a human IgG1 κ mAb that binds to IL-17A (inhibits the interaction with the IL-17 receptor) and is currently used for plaque psoriasis and several rheumatological conditions.⁹⁴ The further rationale for inhibiting IL-17 is that it is a proximal target to IL-1 and IL-6 and, hence, could reduce neutrophil recruitment to the lungs and prevent organ dysfunction in ARDS. To our knowledge, there are no ongoing trials involving this drug.

Targeting JAK

Targeting the T_H17 pathway, research on murine models showed promising results with the use of fedratinib, a JAK2 inhibitor. In this study, the drug decreased the expression of IL-17. Because IL-6 and IL-23 are signals for T_H17 cell initial differentiation and effector function through the JAK2-signal transducer and activator of transcription 3 pathway, the use of this inhibitor could decrease the proinflammatory function of T_H17.⁹⁵ This drug is currently FDA approved for myelofibrosis.⁹⁶ To our knowledge, there are no current registered trials involving this drug.

As mentioned, the cell-surface ACE2 receptor is needed for coronavirus endocytosis, and one of the regulators of this process is the AP2-associated protein kinase 1, part of the numb-associated kinase family.⁹⁷ AP2-associated protein kinase 1 inhibitors have been shown to prevent virus infections by disrupting viral cell invasion. Baricitinib is an oral JAK inhibitor (JAK1/JAK2, JAK1/JAK3, JAK1/tyrosine kinase 2, and JAK2/tyrosine kinase 2) but also an AP2-associated protein kinase 1 inhibitor, having direct antiviral activity, that is currently FDA approved for rheumatoid arthritis resistant to anti-TNF drugs.⁹⁸ Several trials are ongoing to confirm its safety and efficacy, and it is also being investigated in combination therapy with remdesivir (NCT04340232, NCT04321993, and NCT04320277). Remdesivir, an adenosine analogue with demonstrated antiviral activity against a broad range of RNA virus families, has been used in a randomized placebo-controlled trial showing a decrease in time to recovery (15 vs 11 days) and a trend toward decrease in

mortality.⁹⁹ This drug gained an FDA approval for use in children and adults with severe COVID-19.

Ruxolitinib is a JAK1 and JAK2 inhibitor that mediates the signaling of numerous cytokines such as IL-6, IFN- γ , and growth factors with essential roles in immune function and hematopoiesis. This drug is FDA approved for myelofibrosis, hydroxyurea-resistant polycythemia vera, and steroid-refractory acute graft-versus-host disease.¹⁰⁰ A multicenter, single-blinded, randomized trial (1:1) of 44 patients with COVID-19 showed a tendency (not statistically significant) toward improvement in clinical outcomes in the ruxolitinib group.¹⁰¹ Several larger clinical trials from North American and Europe are ongoing (Table V).

Another attractive drug is tofacitinib, which has been shown to inhibit the *in vitro* activity of JAK1/JAK2, JAK1/JAK3, and JAK2/JAK2 and thus decrease the related cytokines. According to the FDA, it can be used for rheumatoid arthritis, psoriatic arthritis, and ulcerative colitis.⁸¹ There is a planned Italian trial that aims to assess the advantage of early administration of tofacitinib in SARS-CoV-2-related interstitial pneumonia (NCT04332042).

Serious bacterial, mycobacterial, fungal, and viral infections have been reported with the use of JAK inhibitors. This potential off-target effect of these drugs combined with the decreased IFN innate response can lead to severe complications, and caution should be used in the SARS-CoV-2 context with theoretical benefit for the anti-JAK molecules that have more specific targets.

Other targeted immunomodulatory therapies and combination therapies

As described, the production of cytokines and chemokines by macrophages is regulated by the BTK. Thus, inhibition of this protein could be a promising strategy for reducing COVID-19-related complications, with therapeutic inhibition of BTK in patients with lymphoid malignancies resulting in decreased proinflammatory cytokines.

Company-sponsored trials with acalabrutinib, a small-molecule inhibitor of BTK enzymatic activity, that aim to study its efficacy and safety compared with best supportive care in hospitalized patients with COVID-19 are currently listed and will begin recruitment shortly in the United States and Europe (Table V).

By sensing self or pathogenic cytosolic double-stranded DNA, the cyclic guanosine monophosphate-adenosine monophosphate synthetase stimulator of IFN genes (STING) plays an important role in innate immunity and tumor development.¹⁰² STING is expressed in T cells, monocytes, natural killer cells, and dermal fibroblasts, and cyclic guanosine monophosphate-adenosine monophosphate synthetase-STING signaling promotes the production of IL-6 and the downstream activation of signal transducer and activator of transcription 3.¹⁰² The STING-IFN- β pathway is triggered by the binding of cyclic guanosine monophosphate-adenosine monophosphate synthetase to STING, which leads to IFN regulatory factor 3 phosphorylation and subsequent transcription of the gene encoding IFN- β .¹⁰³ The JAK receptors and their specific pathways are activated by the IFN- β binding to its receptor. The regulation of STING and other proinflammatory cytokine genes is also achieved with the synthesis and release of IFNs. Thus, this proinflammatory loop can be obstructed by JAK inhibition.¹⁰³

Combination therapy with lopinavir-ritonavir, ribavirin, and IFN- β 1b compared with lopinavir-ritonavir monotherapy was evaluated in an intention-to-treat multicenter, randomized phase 2 clinical trial from China. The primary end point was the time before a negative nasopharyngeal swab (RT-PCR) in patients with SARS-CoV-2, with the median time reported for the combination group (N = 86) being 7 days and the time in the control group (N = 41) being 12 days ($P = .0010$).¹⁰⁴ Anti-Coronavirus Therapies COVID-19 is a clinical trial that aims to evaluate the combination of chloroquine and azithromycin with subcutaneous injection of IFN- β 1b for SARS-CoV-2 prevention by assessing admission to intensive care, mechanical ventilation, and/or death (NCT04324463).

The complement system is also a potential therapeutic target in SARS-CoV-2 infection. Complement is key to the innate immune response to all viruses, and complement inhibition is a potential treatment for severe SARS-CoV-2 infection by reducing the severity and end-organ consequences of the innate immune response.^{105,106} A recent mouse model suggested that complement activation through C3 exacerbates SARS-CoV-associated ARDS and that C3-deficient mice infected with SARS-CoV showed less respiratory decline.¹⁰⁷ Lung biopsy samples from patients with SARS-CoV-2-associated ARDS showed evidence of complement activation with C3 fragment deposition and associated increased serum 5a levels.¹⁰⁵ However, there is little clinical data on the potential role of complement activation and its role in ARDS associated with SARS-CoV-2. There are now several proposed and ongoing studies examining the role of C5 inhibitors such as eculizumab and ravulizumab (Table V).

Convalescent plasma

Given the lack of evidence-based treatment and the novelty of this disease, CP has re-emerged as an emergency intervention passive immunization strategy aiming to decrease morbidity and mortality in critically ill patients with COVID-19.^{108,109} This treatment has been shown to be favorable during the SARS-CoV infection with a decrease in hospital stays and mortality compared with controls.^{110,111} Also, a recent systematic review, while acknowledging the limited data, indicated that CP is safe and clinically effective and can play a role in reducing mortality.¹¹² The described mechanisms of action are direct neutralization of the virus aimed at the spike viral protein^{113,114} as well as other immunomodulatory and anti-inflammatory functions such as neutralization of cytokines, complement, and autoantibodies.¹⁰⁸ A clinical report on the use of CP in critically ill patients with SARS-CoV-2 showed a hypothetical benefit with decrease in body temperature, increase in respiratory function, and ARDS resolution in 4 of the 5 patients included.¹¹⁴ In an open-label, multicenter, randomized clinical trial from China, adding convalescent plasma to the treatment plan did not result in increased clinical recovery.¹¹⁵ Several questions remain unanswered regarding CP, and there is a need for larger randomized controlled trials to answer these questions, but emerging successful reports related with its use in severe COVID-19 highlight the intense inflammatory response that accompanies this infection.

Summary statement

Clinical trials are urgently warranted to evaluate a therapeutic strategy targeting upstream and downstream pathways in SARS-CoV-2. The effective dose and the ideal administration

timing of the immunomodulatory drugs remain under investigation.

CONCLUSIONS

Severe SARS-CoV-2 infection is associated with CS producing a hyperinflammatory state and a clinical and laboratory picture similar to hemophagocytic lymphohistiocytosis that typically occurs 7 to 10 days after the onset of acute illness. In this setting, IL-6 levels correlate with respiratory failure, poor outcomes, and mortality. Blocking this and other appealing cytokines and signaling pathways at an early stage shows promise to target specific and undesirable immune responses in the setting of acute SARS-CoV-2 infection. Currently, studies examining the combination of direct antiviral agents with immunomodulatory therapy are ongoing and will be important in the quest to prevent acute respiratory deterioration, ventilation use, morbidity, and mortality from SARS-CoV-2 infection.

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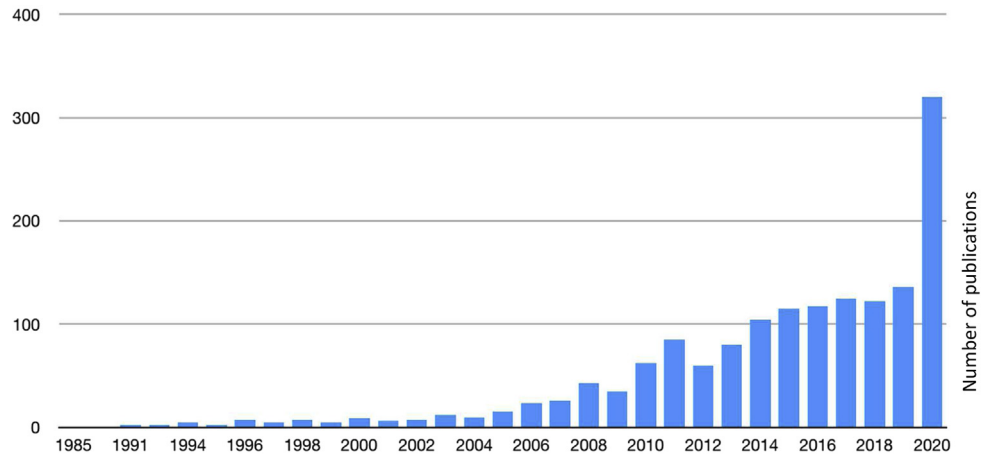


FIG E1. “Cytokine Storm” in PubMed Search (1985-May 2020). The figure represents the number of articles entered in PubMed from 1985 to May 2020. This marks several events that have led to CS including the 1985 original description in graft-versus-host disease, a small increase during the 2003-2005 SARS-CoV, a more significant number for the 2009-2010 H1N109, and the ongoing rise in publications associated with SARS-CoV-2.