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REVIEW ARTICLE

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Mechanistic inferences from clinical reports of SARS-CoV-2

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ABSTRACT

SARS-CoV-2 was identified as the causative pathogen in an outbreak of viral pneumonia cases originating in Wuhan, China, with an ensuing rapid global spread that led it to be declared a pandemic by the WHO on March 11, 2020. Given the threat to public health posed by sequelae of SARS-CoV-2 infection, the literature surrounding patient presentation in severe and non-severe cases, transmission rates and routes, management strategies, and initial clinical trial results have become available at an unprecedented pace. In this review we collate current clinical and immunologic reports, comparing these to reports of previous coronaviruses to identify mechanisms driving progression to severe disease in some patients. In brief, we propose a model wherein dysregulated type I interferon signalling leads to aberrant recruitment and accumulation of innate immune lineages in the lung, impairing establishment of productive adaptive responses, and permitting a pathologic pro-inflammatory state. Finally, we extend these findings to suggest possible treatment options that may merit investigation in randomized clinical trials.

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Introduction

SARS-CoV-2 was determined to be the causative pathogen of the disease COVID-19 resulting in an outbreak of viral pneumonia cases originating in Wuhan, China in 2019. The rapid spread of the virus on a global scale led to WHO designation as a pandemic on 11 March 2020. At the time of this review, there are over 3.8 million confirmed global cases of COVID-19 and at least 270,000 attributable deaths, with over 1.2 million cases and 76,000 attributable deaths in the United States [1]. Relative to annual influenza infections, COVID-19 appears to have significantly higher rates of patient morbidity and mortality, though they remain lower than what was seen for SARS-CoV and MERS. Unfortunately, no specific therapy is available and the management of infected patients has been variable among institutions and nations. Given the rapid pace at which the global situation and our understanding of the virus is progressing, this review intends to collate currently available published case series (peer-reviewed and pre-print) and integrate these clinical findings with immunologic insights from COVID-19, SARS-CoV, and MERS studies.

Clinical characteristics among patients with COVID-19 infection

Emerging data from Wuhan, China suggests that while some patients are hospitalized with more severe COVID-19 infections (17%), most cases are not life-threatening [2]. Preliminary estimates suggest the time from infection to symptom onset to be around 5 days, and a mean duration of 11–12.5 days from the time of infection to hospitalization [3]. Despite initial controversy, viral shedding and transmission from person-to-person does occur during an asymptomatic period [4], likely contributing to the rapid global spread. Indeed, most of the viral shedding occurs before symptom onset [5]. Of note, viral shedding has been found to align with cough symptoms, in nasopharyngeal samples and sputum. Shedding can also occur through stool although infectious viral transmission has not been reported through this route [6]. A notable limitation to most studies are the relatively small sample sizes and the relative exclusion of infected patients with only mild symptoms who do not seek medical attention. The severity of patient illness has repeatedly been found to correlate with symptomology, clinical parameters, and outcome; therefore, most published reports stratify patient parameters by 'non-severe' versus 'severe' disease (studies stratifying by 'acute respiratory distress syndrome (ARDS)' versus 'no ARDS', or 'intensive care unit (ICU)' versus 'no ICU' were conformed to this strategy for ease of reporting). Published case series indicate the most common presenting symptoms to include fever in the majority and cough with fatigue, sputum production, myalgias/arthralgias, and shortness of breath less commonly present but enriched in patients with severe disease [2,7–10]. Although distinct viral strains may predominate in different geographical regions, the clinical presentations from these Chinese studies were mirrored by laboratory-confirmed COVID-19 infected patients treated at an academic health system in New York, New York, USA [11,12], though respective frequencies of fever (61%) and cough (56%) were notably lower in an Italian case series [13] (Table 1).

Following hospital admission, clinical parameters tend to correlate with disease severity. The acute phase protein C-reactive protein (CRP) was significantly elevated in severe versus non-severe cases. Elevations in lactic acid dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were also more frequent and more significant in severe cases, suggesting a greater extent of systemic tissue damage and corroborating a more inflammatory environment in this cohort [2,7,9,14,15].

Immunologic perturbations are increasingly recognized as a defining feature of severe infection with COVID-19. Serum cytokine derangements include a nearly invariable elevation in interleukin 6 (IL-6). IL-8 and type I interferon are also frequently elevated in the blood of patients with severe COVID-19 pneumonia [16,17]. Although increased circulating tumour necrosis factor alpha (TNFα) has been reported in more severe cases, differences in concentration appear to be inconsistent (Table 1).

Derangements in predominantly pro-inflammatory cytokines, or 'cytokine storm' suggest propagation of a systemic inflammatory state in hospitalized patients that develop severe disease. Specifically, elevated ferritin and IL-6 were shown to correlate with mortality in Wuhan, China [9]. The neutrophil-to-lymphocyte ratio (NLR) is a surrogate of systemic inflammation [18] and can serve as a predictor of poor prognosis in critically-ill septic [19,20] and cancer patients [21]. Extending its utility to COVID-19 patients, NLR is significantly elevated in those with severe disease [8,15,22], serving as an independent risk factor and prognosticator of patients at high risk for progression to severe pneumonia [10]. Specifically, circulating neutrophil counts are significantly elevated while

Table 1. Compiled clinical data from multiple studies of severe and non-severe COVID-19 patients.

	Non-severe	Severe	Reference no.
Symptoms (%)			
Fever	91.94 (83–97.7)	94.29 (87–100)	[2,7–10,14,15]
Cough	66.27 (33.7–82)	71.36 (33.6–85)	[2,7–10,14,15]
Sputum	31.61 (33.6–85)	42.59 (26–64.7)	[2,7–10,14,15]
Fatigue	35.54 (21–52.3)	42.57 (22–70.6)	[2,7–10,14,15]
SOB	30.18 (9.1–62)	52.02 (17.6–87)	[7,8,10,14,15]
Myalgia/arthralgia	16.23 (14.4–19.3)	18.47 (15–23.1)	[7–9]
Diarrhea	18.95 (11.4–26.5)	16.6 (5.9–27.3)	[8,10]
Comorbidity (%)			
Male	59.10 (47.7–68)	71 (58.8–85)	[2,10–12,14,15]
Obesity (BMI 30–40)	32.7 (31.9–33.5)	37.85 (32.3–43.4)	[11,12]
Chronic conditions	70.70	73.40	[11]
Cardiovascular conditions	9.57 (0–42)	18.23 (5.9–47.1)	[2,8,10,11,14,15]
Diabetes	15.82 (4.5–22.9)	19.41 (8–27.7)	[2,8,10–12,14,15]
Asthma/COPD	5.5 (0–12.2)	10.56 (3–17.6)	[2,10–12,14]
Hypertension	22.62 (13.6–48.3)	35.2 (15–53.8)	[2,8,10,12,14,15]
CKD	2.40	2.10	[8]
Cytokines (pg/mL)			
IL-1β	5.00	5.00	[8]
IL-6	9.22 (6.3–13.41)	18.55 (7.39–37.7)	[8,9,14–16]
IL-8	13.70	18.40	[8]
IL-10	3.73 (2.4–5)	5.6 (4.59–6.6)	[8,16]
TNFα	6.24 (4.1–8.4)	5.82 (2.9–8.7)	[8,16]
Labs			
CRP (mg/L)	22.6 (10.3–34.1)	77.7 (23.5–126.6)	[10,14,15]
LDH (U/L)	263.83 (253.5–281)	439 (396–521)	[2,9,15]
AST (U/L)	32 (30–34)	41 (38–44)	[2,15]
ALT (U/L)	27 (27–27)	41.33 (35–49)	[2,9,15]
D-dimer (μg/mL)	0.56 (0.52–0.6)	3.18 (1.16–5.2)	[9,15]
NLR	2.74 (2.2–3.2)	6.53 (3.6–10.5)	[8,10,15]
Leukocytes ($\times 10^9$/L)	4.8 (4.3–5.2)	6 (3.7–9.8)	[7,9,10]
Lymphocytes ($\times 10^9$/L)	1.05 (1–1.1)	0.68 (0.4–0.9)	[2,7,9,10]
Platelets ($\times 10^9$/L)	177.13 (149–220)	163 (137.5–196)	[2,7,9,10]
Neutrophils ($\times 10^9$/L)	2.89 (2.4–3.2)	4.71 (2.8–7.04)	[8,10,15]
Monocytes ($\times 10^9$/L)	0.37 (0.34–0.4)	0.35 (0.29–0.4)	[8,15]
B cells ($\times 10^6$/L)	181.05 (166–196.1)	147.15 (125.3–169)	[8,16]
Total T cells ($\times 10^6$/L)	648.4 (633–663.8)	454.05 (446.5–461.6)	[8,15]
CD4 T cells ($\times 10^6$/L)	414.27 (371–451.3)	260.77 (234–285.1)	[8,15,16]
Naive CD4 T cells ($\times$ 10^6/L)	35.00	44.50	[8]
Memory CD4 T cells ($\times 10^6$/L)	65.00	55.50	[8]
CD8 T cells ($\times 10^6$/L)	243.83 (201.9–288.6)	163.73 (154.7–179)	[8,15,16]
Treg ($\times 10^6$/L)	4.50	3.70	[8]

Data is compiled based on information provided in respective studies. Data is presented as mean (range) when applicable, only mean is presented if data is from a single study. If data conforming to the table format could not be extracted from a report then that data was excluded from the table. Abbreviations: shortness of breath (SOB), body mass index (BMI), chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), interleukin (IL), tumour necrosis factor (TNF), C-reactive protein (CRP), lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), neutrophil-to-lymphocyte ratio (NLR), regulatory T cell (Treg).

nearly all adaptive populations are negatively impacted by severe disease (Table 1).

Lessons from previous coronaviruses

With our understanding still in its infancy, mechanisms contributing to severe COVID-19 pneumonia are yet to be determined; however, lessons can be gleaned from the previous coronavirus epidemics (SARS-CoV and MERS-CoV). Sequencing of the SARS-CoV-2 viral genome revealed a concordance rate of approximately 80% with SARS-CoV, its closest relative aside from bat CoVs (the likely source of COVID-19) [23,24]. Both coronaviruses also bind Angiotensin Converting Enzyme 2 (ACE2), a surface enzyme expressed predominantly on type 2 epithelial cells of the lower respiratory tract, to gain cellular entry, though SARS-CoV-2 binds with 10–20 × greater affinity [25]. Clinically, patient presentation during the SARS-CoV epidemic in 2002–2003 bore striking similarities to that being reported during the current COVID-19 pandemic. Common symptoms of SARS-CoV included fever, chills, myalgia, and dry cough [26]. LDH and CK were frequently elevated in the context of neutrophilia and lymphopenia, again reflecting a systemic inflammatory state [26–29]. Here, a dysregulated immune response frequently resulted in ARDS, a pathologic outcome similar to COVID-19 [26], and similar findings on autopsy [30,31]. Given the substantial biochemical and

clinical similarities, it is logical to assume similar mechanisms dictate the immune response to these pathogens. Therefore, lessons learned in the study of SARS-CoV can be cautiously applied to our evolving understanding of COVID-19.

Putative mechanism

SARS-CoV and SARS-CoV-2 enter cells in the respiratory tract *via* ACE2 and release damage associated molecular patterns (DAMPs; e.g. ATP, HMGB1, nucleic acids, etc.) as well as viral particle-derived pathogen associated molecular patterns (PAMPs) into the extracellular environment. Binding of these molecules to cognate pattern recognition receptors (PRRs) stimulates an innate immune response. In this manner, lung dendritic cells recognize an infection, mature, and traffic to the draining lymph node wherein antigen is presented to T cells [32]. Stimulation of adaptive immunity then leads to viral clearance through cellular and humoral mechanisms–the likely scenario in asymptomatic patients, or with only mild disease. Progression to severe disease, however, is likely driven by dysregulation of this process.

Adaptive dysregulation

Levels of CD4 and CD8 T cells negatively correlate with disease severity in COVID-19 patients and are similarly decreased in SARS-CoV patients [27,29]. Demonstrating their central role in viral clearance, adoptive transfer of virus-specific CD4 or CD8 T cells significantly improved mortality and expedited viral clearance in a lethal challenge model of SARS-CoV. Moreover, vaccination with peptide-coated DCs one week prior to infection was able to elicit a protective CD8 T cell response [33]. In a different approach, Chen et al. depleted T cell subsets before infection and found CD4, but not CD8, T cells to be critical for efficient mouse clearance of SARS-CoV infection. In this same study, the administration of neutralising antibodies following CD4 T cell depletion promoted viral clearance, suggesting a requirement for effective B cell help and production of neutralising antibodies for viral control [34]. In line with these findings, antibodies to type A blood antigens appear to be cross-reactive and somewhat protective, as patients with type B and O blood are less frequently infected with SARS-CoV and SARS-CoV-2 [35,36]. However, declining numbers of circulating lymphocytes in severe disease seemingly suggests impairment of these responses.

In COVID-19 mediated lymphopenia, B cells, activated CD4 T cells, memory CD4 T cells, and CD8 T cells are reduced. One proposed explanation is that SARS-CoV-2 might directly infect T cells and initiate cell death by viral lysis [31]. This outcome seems unlikely, as Banerjee et al. found viral-like particles in CD4 T cells but demonstrated an absence of viral replication in healthy donor PBMCs of any lineage [37]. Furthermore, single-cell RNA-sequencing of PBMCs from hospitalized COVID-19 patients failed to find SARS-CoV-2 viral reads in any samples [17]. Although lymphopenia in the circulation could be driven by massive recruitment of these cells into the lungs, autopsy of patients having succumbed to severe COVID-19 pneumonia showed a paucity of infiltrating lymphocytes [31], rendering this an unlikely scenario as well. The systemic inflammatory state imposed by severe COVID-19 disease, much like sepsis, may then be the impetus behind observed lymphopenia and elevated NLR [38]. In sepsis, circulating lymphocytes display signs of early apoptosis, Annexin V surface expression and lymphocyte shrinkage [39], implicating loss of these populations through programmed cell death [40]. Thus, it is possible that the systemic inflammatory state during severe COVID-19 pneumonia and/or viral sepsis induces lymphocyte apoptosis and dysregulated adaptive responses.

A recent report from China has found a positive correlation between abundance of SARS-CoV-2 Nucleoprotein (NP) neutralising antibodies and disease severity, noting that earlier, stronger responders for NP specific anti-IgG and anti-IgM associate with increased diseased severity. Conversely, patients with fewer circulating neutralising antibodies were found to have a decreased viral load [6]. In agreement with this, Wu et al. reported about 30% of non-severe patients generated very low neutralising antibody titres against the spike (S) protein. It was also found that patients who were older with lower lymphocyte counts and increased CRP had increased neutralising antibody titre, however, none of these patients had severe disease [41]. Although these are small observational studies, results are consistent with reports from MERS infection, where patients having succumbed to disease had robust neutralising antibody responses during infection [42]. Similarly, in SARS-CoV infection, patients with severe illness had higher antibody titres at earlier stages during infection [43].

A macaque model of SARS-CoV proposed one explanation for an ostensible role of neutralising antibodies in disease progression. Macaques vaccinated with S protein

and challenged with SARS-CoV developed diffuse alveolar damage compared to only mild pathology in controls. Disease severity was a consequence of increased inflammatory monocyte-macrophages in the lungs and was mediated in part by Fcγ receptor signalling, as the combination of blocking Fcγ receptors and giving anti-S IgG decreased macrophage production of IL-8 and MCP-1 *in vitro* [44]. A second explanation is that viral S protein and N protein activate complement directly and/or in complex with neutralising antibodies, contributing to the widespread complement activation that culminates in characteristic tissue damage and diffuse microthrombi [45,46]. Activated complement protein C5a is a potent anaphylatoxin that enhances FcγRIII signalling upon receptor binding (C5aR) to promote alveolar macrophage activation [47], potentially serving as a link between these two hypotheses. Indeed, this scenario accounts for observed elevations in D-dimer, reduced platelets, and diffuse tissue thrombi found on autopsy [48].

Antibody-mediated enhancement of viral disease—an increase in viral uptake, replication, and/or pathogenicity due to the binding of virions to antibodies—cannot be excluded. This mechanism has been demonstrated in various other respiratory infections, including influenza virus, respiratory syncytial virus, and is controversial in SARS-CoV and MERS [49].

It is important to consider, however, that these clinical findings are subject to a temporal sampling bias in that patients do not typically seek medical attention until symptoms are relatively progressed. Therefore, it is difficult to discern whether antibody production contributed to a dysregulated immune response and disease severity, or whether disease severity and an altered cytokine milieu drove the production of higher antibody titres. In the latter case, neutralising antibodies could still be protective if present early after infection and would help explain the potential efficacy of passive immunisation. Indeed, Hoffmann et al. found SARS-CoV neutralising antibodies directed against the viral S protein to be cross-protective, inhibiting both SARS-CoV and SARS-CoV-2 host cell entry *in vitro* [50].

Innate dysregulation

Frequent and significant elevations in circulating IL-6 and IL-8, which contribute to neutrophil activation and recruitment, suggest a potential pathologic role for neutrophils in patients developing severe COVID-19 pneumonia. Using a rat model of SARS-CoV, Haick et al. showed a critical contribution of neutrophils to the initial anti-viral immune response, elaborating IL-1, TNFα, CXCL1, CXCL3, CCL2, and IP10 to orchestrate the recruitment of a productive cellular response to the lungs. Expectedly, depletion of neutrophils prior to infection impaired viral clearance. However, subsequent repopulation and influx of neutrophils to the lungs in late-stage disease contributed to significant morbidity and mortality without improvement in viral clearance [51]. Bulk RNA-sequencing and pathway analysis of PBMCs from COVID-19 patients with mild versus severe symptoms revealed upregulation of neutrophil chemotaxis, neutrophil activation, and type I IFN signalling pathways, consistent with these mouse studies. Virus-host interactome analysis then indicated that non-structural proteins 9 and 10 impaired the function of NKRF (a competitive inhibitor of NFκB) to induce IL-6 and IL-8 expression in lung epithelia, which the authors postulate could incite tissue destruction and eventual ARDS [52].

A role for type I IFN signalling

What is driving this perturbed immune response that causes significantly increased rates of mortality in the elderly and those with chronic disease? Accumulating evidence suggests type I interferon signalling, or dysregulation thereof, dictates establishment of a productive immune response to then pre-empt progressive viral replication, development of severe pneumonia, and ARDS. Channappanavar et al. showed that delayed type I interferon signalling promotes lethal respiratory infection with SARS-CoV in mice, while supplementation with IFNβ prior to peak viral replication abrogated mortality with no significant difference in pulmonary viral load. Knockout of the interferon alpha receptor led to reduced expression of CCL2 (among other chemokines), which functions to recruit inflammatory monocyte-macrophages *via* CCR2. Antibody-mediated depletion of this population led to reduced levels of TNFα, IL-1β, IL-6, and iNOS and ameliorated disease severity, directly linking pulmonary macrophages to morbidity and mortality in SARS-CoV [53]. Extending these findings, the late administration of type I interferon led to recruitment of pathogenic neutrophils and macrophages to the lung and development of fatal pneumonia in a mouse model of MERS-CoV [54]. Single-cell RNA-sequencing of PBMCs from four COVID-19 patients at discrete stages of disease severity (pre-ICU, ICU, and post-ICU samples) also identified a relationship between dysregulated type I interferon signalling in PBMCs and clinical status. This

correlated with drastically increased circulating inflammatory monocytes and fewer T and B cells while patients were in the intensive care unit [17]. In addition, autopsy of two patients having succumbed to COVID-19 infection showed significant aggregation and activation of infiltrating macrophages–homogeneously expressing IL-6, IL-10, and TNFα–amidst extensive tissue destruction, corroborating a direct role for these cells in pulmonary pathology [31].

Failure to clear viral infection early might then lead to prolonged type I signalling that can instead impair adaptive immune functions [55]. In support of this notion, flow cytometry on PBMCs from COVID-19 patients with mild versus severe disease found a significant decline in the frequency of multifunctional CD4 T cells in the severe cohort alongside the increased expression of activation and exhaustion surface proteins on CD8 T cells in the total T cell pool. The authors compare these phenotypic changes to an exhaustion-like state [56]. Indeed, prolonged type I interferon signalling is known to reinforce CD8 T cell exhaustion and impair viral control [57]. The extent to which the development of functional T cell exhaustion (that is, loss of cytokine production and cytotoxicity) contributes to disease severity on the timescale of COVID-19 infection and resolution remains to be determined.

Collectively, these studies suggest a model wherein a delayed initial immune response to infection, including type I interferon signalling, permits high levels of viral replication and persistence. Neutrophil recruitment to the lungs and subsequent activation then elaborates inflammatory cytokines (IL-6 and IL-8) and chemokines (notably CCL2), driving substantial monocyte recruitment and activation. It is likely a combination of these innate populations and diffuse thrombotic complications that mediate eventual tissue destruction manifested by severe disease. The systemic inflammatory state also hinders adaptive immunity, inducing lymphocyte apoptosis, and reducing the production of canonical T cell cytokines as well as neutralising antibodies, impairing viral clearance. These factors culminate in the cytokine storm with local and systemic tissue destruction observed in patients with severe COVID-19 pneumonia. Delayed and/or blunted immune responses characteristic in the elderly [58] and an already dysregulated inflammatory state present in common comorbidities (e.g. insulin resistance, hypertension) [59,60] compromise prompt responses to infection, potentially driving the significantly increased morbidity and mortality observed in these patient populations (Figure 1).

Immunological memory to COVID-19

Whether or not protective memory responses are established following infection with COVID-19 remains uncertain. Convalescent plasma transfer from recovered to currently infected patients has shown promise in reducing clinical severity and expediting clearance of disease [61,62], implying that antibodies to at least some viral epitopes can confer protection. Because the kinetics of B cell contraction in COVID-19 are unknown, it could be that memory cells persist for some time following infection resolution, or that antibodies produced by antibody secreting cells prior to contraction are predominantly responsible for observed benefits. Importantly, if activation induced cell death is occurring in the T lymphocytes, which has been shown in CD44-positive memory or antigen experienced cells, this mechanism could also impede memory cell formation, either by depleting memory B or T cells directly or by interfering with T-B cell help [63].

It appears that memory B cell responses in SARS-CoV are relatively short lived. Patients having recovered from SARS-CoV maintained neutralising antibodies for 16 months after infection, tending to peak 4 months after infection and decreasing thereafter [64]. After 6 years, anti-SARS antibodies were undetectable in 91% of patients and no SARS specific memory B cells were found. Memory T cells were, however, detectable 6 years after infection, but their ability to confer protective immunity remains uncertain [65]. If SARS-CoV-2 proves to behave similarly, it will be important to determine the cause of this temporary persistence and approaches to address it.

Immune dysregulation and tissue pathology

Early reports from China repeatedly identified elevated d-dimer levels, alongside thrombocytopenia in severe COVID19 disease [2,9,15] consistent with formation of disseminated microthrombi and significant necrosis observed on autopsy [46]. Beyond the lungs, SARS-CoV-2 infection can cause thrombotic complications in other systems, including the heart and brain [66,67]. Large vessel clots causing ischaemic stroke in younger patients with otherwise mild symptoms can also be precipitated by infection [68]. A few potential mechanisms explaining observed hypercoagulability have been proposed. In a study of 183 patients, Tang et al. showed that 71% of COVID19 non-survivors had disseminated intravascular coagulation (DIC) [69]. This process can be initiated by

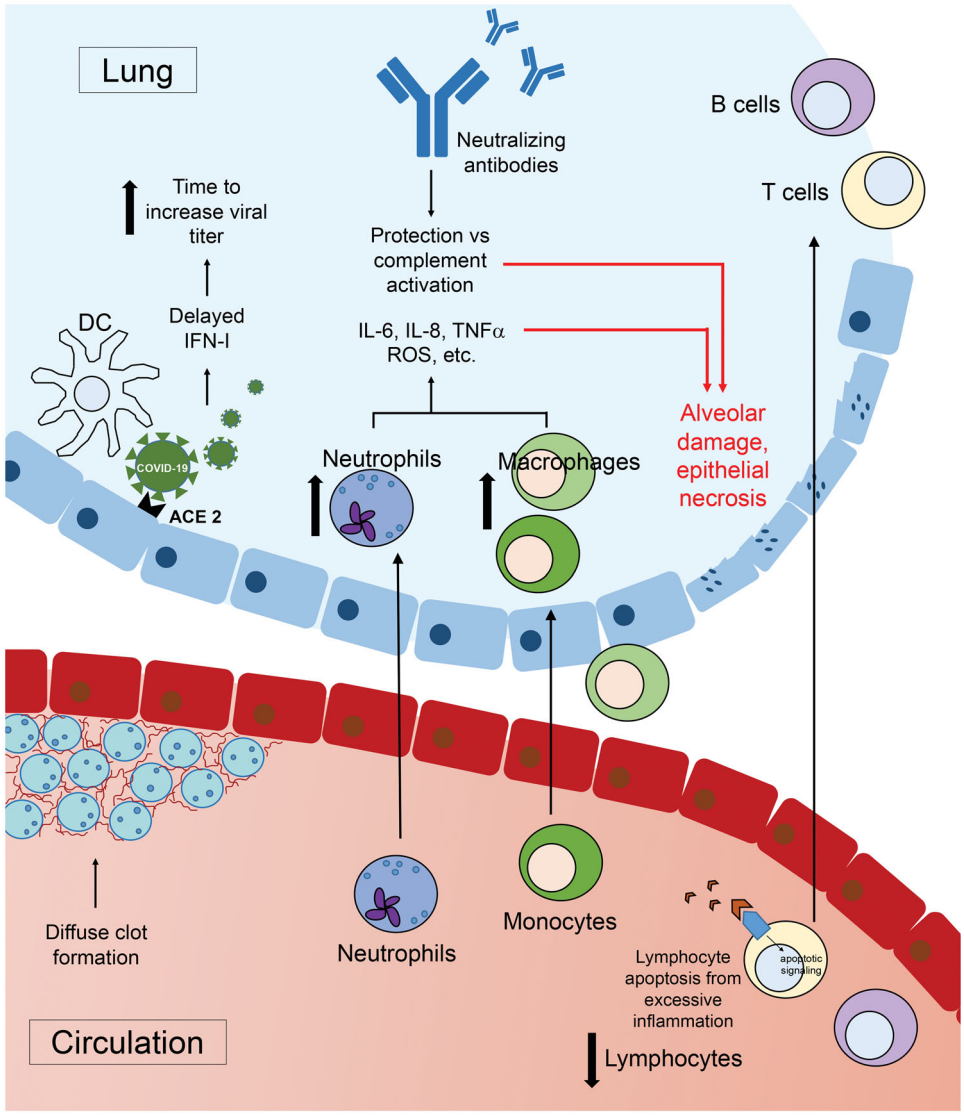

Figure 1. Proposed model of COVID-19 immune dysregulation leading to severe disease. Schematic of the proposed mechanism responsible for severe COVID-19 disease in some patients. Delayed type I IFN production by dendritic cells (DCs), or other IFN-producing populations, instigates a defective response that cascades into other responding immune lineages. This delay allows increased recruitment of neutrophils, secreting inflammatory cytokines and chemokines, then driving the accumulation of inflammatory monocyte-macrophages in the lungs, particularly the alveoli. Skewing of the innate response creates a potent inflammatory cytokine environment (including IL-6, IL-8, TNFα, reactive oxygen species (ROS), etc.) acting to propagate pulmonary pathology. Additionally, the systemic inflammatory state leads to lymphopenia and stymies establishment of a productive adaptive immune response.

pathogen-mediated activation of monocytes or epithelial cells with ensuing release of tissue factor (TF) and von Willebrand Factor (vWF) [70], Notably, proinflammatory cytokines such as TNFα, IL-6, and IL-1—frequently elevated in COVID-19—can also increase circulating vWF [71]. Observed increases in circulating vWF [72] would be expected to prolong the half-life and promote activity of factor VIII [73], potentially contributing to the diffuse microthrombi observed. Alternatively, engagement of the complement system may contribute to diffuse activation of the clotting cascade, and vice versa. N protein can directly activate complement [45] and co-localization of SARS-CoV-2 S protein with complement proteins C4d and C5b-9 (membrane attack complex) is observed in the lungs of patients having succumbed to COVID-19 [46], both suggesting a possible role for direct viral protein-mediated complement activation. The membrane attack complex components C5b-9 can then flip phosphatidylserine to the cell or platelet surface, providing support for coagulation. Moreover, C3a can induce platelet accumulation and C5a can produce pro-coagulant responses *via* cellular activation [74], highlighting the interplay between complement and coagulation pathways as well as potential contribution to diffuse coagulopathy. Mitigation of this induced hypercoagulable state with anticoagulants has

demonstrated improved survival in patients with 'sepsis-induced coagulopathy' [75] and those requiring mechanical ventilation [76].

Implications for treatment strategies

As of May 8th, the NIH shows over 1,300 clinical trials registered in association with COVID-19 (search term 'COVID19' in clinicaltrials.gov). In addition, new studies are becoming available on preprint servers daily, giving insight into new therapy options and preliminary results thereof. Of note, the SARS-CoV-2 viral and protease structure, which was found to be highly conserved throughout CoVs, has been identified, and specific protease inhibitors with antiviral effects *in vitro* are currently under investigation [77,78]. The development of specific anti-viral small molecule inhibitors along with a vaccine capable of generating protective immunity is undoubtedly critical to managing the COVID-19 pandemic.

Until such targeted therapies are available, potential points of clinical intervention for patients at high risk of progression can be surmised from the available literature. Administration of type I interferon quickly after infection may be sufficient to expedite a productive immune response. Unfortunately, this is not likely to be a clinically viable approach given the brief window of opportunity between infection and intervention, well before the onset of symptoms. Interrupting early stages of the pathologic response might curtail subsequent immune derangements and facilitate viral clearance. To this end, Li et al. proposed to inhibit neutrophil production of IL-6 and IL-8 using small molecule JAK inhibitors or IL-6 neutralising antibodies [52]. Because anti-IL-6 has proven efficacious in the alleviation of chimeric antigen receptor T cell therapy cytokine release syndrome [79], it is possibly a useful adjuvant to any treatment strategy for severe COVID-19 pneumonia. Alternatively, blocking CCL2-mediated recruitment of monocytes to the lung with neutralising antibodies may be efficacious over a longer period and more closely correspond with the development of symptoms (given the role of macrophages in lung pathology). Inhibitors of activated complement components may similarly impede recruitment of these populations and limit tissue destruction [80]. Of course, a patient's clinical picture should be evaluated when considering administration of broadly immunosuppressive agents. Giving these during the critical initial stages of response, which seem to determine whether severe disease will occur, might be detrimental to patient outcome. In these initial stages blocking monocyte recruitment, for example, may prove more advantageous.

For patients that do not come to clinical attention until pathologic inflammation has been established, or for whom clinical progression is imminent, management should focus on targeted immunosuppression and conferral of passive immunity. Early management of such patients has included broad-spectrum anti-virals, prophylactic antibiotics, and rather indiscriminate immunosuppression with corticosteroids [2]. More selective immune suppression could be achieved by specifically targeting neutrophils or macrophages. The depletion of these populations responsible for lung pathology might help to restore balance and promote an effective anti-viral immune response. Additionally, blocking type I interferons with neutralising antibodies in advanced disease may delimit lung pathology [81].

Once pulmonary pathology becomes sufficiently advanced, however, the ability to stimulate a productive immune response is compromised and passive immunisation with virus-neutralising antibodies might be of greatest benefit. This approach recently demonstrated favourable outcomes in two of three patients, while the failed case seems to have been an error in cross-matching [61]. A second study with 10 patients reported similarly auspicious outcomes [62]. According to the FDA's Recommendations for investigational COVID-19 convalescent plasma, plasma can be used in life-threatening COVID-19 cases in the United States, but trial results are still pending [82]. While seemingly promising, plasma transfer will likely be unable to reverse lung damage from immune dysregulation; therefore, the goal must remain improvement of screening measures and early identification of patients at high risk of severe pneumonia. Furthermore, these and other potentially beneficial therapies need to be studied in randomised control studies in sufficient numbers of patients to accurately assess their utility.

Concluding remarks

Our understanding of biologic parameters governing patient presentation following infection with SARS-CoV-2 is expanding at an extraordinary pace. Here we have integrated clinical data from mild and severe COVID-19 patients with mechanistic findings from similar coronaviruses to propose a model wherein an aberrant initial innate immune response cascades into dysregulation of adaptive immunity, preventing efficient viral clearance. Exploring this possible mechanism for severe COVID-19

pathogenesis identified several treatment options meriting continued investigation.

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