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A Review

COVID-19 and Hyperinflammatory Syndrome

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COVID-19, the multifactorial disease caused by the novel coronavirus SARS-CoV-2 is mediated by specific antiviral and inflammatory responses. It is now recognized that in most severe cases of COVID-19 an excessive and uncontrolled inflammatory response exacerbates lung damage caused by viral infection, and contributes to acute respiratory distress syndrome and respiratory failure. This hyperinflammatory syndrome is characterized by multiple cellular and molecular events, including aberrant neutrophil and lymphocyte function, amplification of the inflammatory response by release of damage associated molecular patterns, cytokine storm, lung damage and edema and a pro-fibrotic condition, ultimately leading to respiratory failure. This review discusses these molecular events in correlation with stages of viral infection and disease progression, underscoring the key points that characterize the clinical manifestations of the hyperinflammatory syndrome in COVID-19. Furthermore, it discusses the available or potential therapeutics that target various important mediators of this hyperinflammatory response that are being considered for treatment of COVID-19.

Keywords: Chemokine, Cytokine storm, Lung injury, Lymphohistiocytosis, Neutrophil

Introduction

In the eight months since the first cases of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection were reported from China in December 2019, the virus has caused a worldwide pandemic infecting more than 23 million people and causing more than 800, 000 deaths (https://coronavirus.jhu.edu/map.html). The epidemiology of COVID-19, the disease caused by SARS-CoV-2, differs from the earlier coronavirus-induced diseases caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) due to its greater transmission, resulting in a much larger number of people getting infected. Although the proportion of patients progressing to severe disease is less for SARS-CoV-2 than SARS-CoV or MERS-CoV, the vastly higher number of infections has caused greater number of patients with severe acute respiratory distress requiring intensive care and ultimately in much higher number of deaths. Understanding the characteristics of COVID-19, both in terms of the viral infection as well as the host response to the virus is crucial for developing effective therapeutic strategies to combat the multistage progression of the disease[1].

SARS-CoV-2 infects pneumocytes lining the alveoli of lungs through interaction of its spike (S) protein with angiotensinogen converting enzyme 2 (ACE2) expressed on cell surfaces and penetrates host cells on activation of the cellular transmembrane protease serine 2 (TMPS2)[2,3]. The destruction of the alveolar cells leads to atypical pneumonia progressing to acute lung injury and acute respiratory distress syndrome (ARDS) in many patients[4,5]. While most individuals with SARS-CoV-2 pneumonia clears the lung infection *via* host immune responses, resulting in mild or no symptoms, in most severe cases an aggressive and uncontrolled inflammatory response exacerbates the lung damage and can lead to respiratory failure. Therefore it is increasingly becoming apparent that disease severity in COVID-19 is related not only to alveolar cell damage caused by SARS-CoV-2 but also to a hyperinflammatory syndrome that leads to lung and multiorgan injury and mortality[6]. Understanding

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Abbreviations: ACE2, Angiotensinogen converting enzyme-2; ARDS, Acute respiratory distress syndrome; CRP, C-reactive protein; CRS, Cytokine release syndrome; DAMPs, Damage-associated molecular patterns; GO, Gene Ontology; IFN, Interferon; IL, Interleukin; LDH, Lactate dehdrogenase; MAS, Macrophage activation syndrome; MERS-CoV, Middle east respiratory syndrome coronavirus; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PAMPs, Pathogen-associated molecular patterns; S-protein, Spike protein; SARS-CoV, Severe acute respiratory syndrome coronavirus; sHLH, Secondary hemophagocytic lymphohistiocytosis; TMPS-2, Transmembrane protease serine-2

the molecular and cellular basis of this hyperinflammatory response is therefore of critical importance for deciding the course of treatment for COVID-19 patients with severe disease.

Hyperinflammatory response and COVID-19 pathology

Inflammation is the primary protective response of the body to any infection or injury. However, inflammation is a double-edged sword as hyperactivation of the inflammatory response can lead to aberrant activation of inflammatory cells and protracted release of inflammatory mediators such as cytokines and chemokines[7]. The acute and protracted release of cytokines, referred to as "cytokine storm" or cytokine release syndrome (CRS) and aberrant activation of inflammatory cells such as neutrophils and monocytes cause host tissue damage, leading to morbidity and mortality[8]. Several studies have shown that COVID-19 is marked by a virus-induced shift from a protective antiviral immunity to a hyperinflammatory response observed in critically ill COVID-19 patients, as has been observed previously in SARS-CoV and MERS-CoV infections[9–11]. (Fig. 1) However, the role of infiltrating and peripheral immune cells in COVID-19 lung injury,

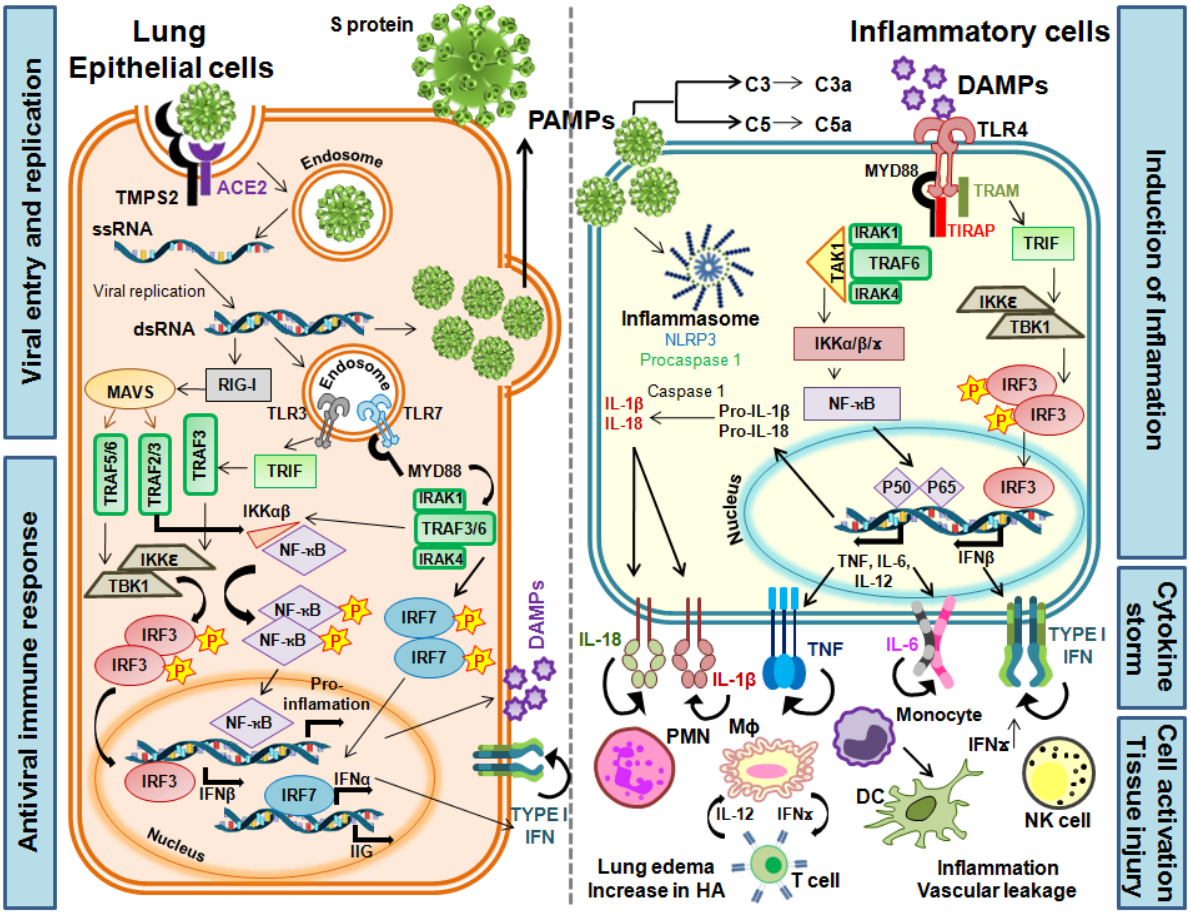

Fig. 1 — Course of viral infection and host immune response in COVID-19 hyperinflammatory syndrome. SARS-CoV-2 enters lung epithelial cells *via* interaction with cell surface proteins ACE2 and TMPS2. After endocytosis, the viral genomic (+ve strand) RNA enters the cytoplasm and is replicated to generate double stranded RNA intermediates and translated to generate viral proteins giving rise to new virus particles which can infect surrounding cells. As cells are lysed DAMPs are released. Intracellular innate immune receptors such as TLRs and RIG-1 recognize viral RNA and initiate signaling pathways activating transcription factors such as NFκB, resulting in the production of inflammatory cytokines such as Interferons. DAMPs released from dying cells, PAMPs from virus particles and the cytokines released by infected cells activate pro-inflammatory pathways in immune cells such as neutrophils and macrophages in the lung. Complement cascade is also activated by virus particles. Activation of the NLRP3 inflammasome complex leads to activation and release of IL-1β and IL-18 by the activated cells, which acts in autocrine and paracrine manner to cause high level of cytokine and chemokine secretion, leading to "cytokine storm". The cytokine storm initiates positive feedback loops leading to hyperactivation of immune cells such as PMN (polymorphonuclear leukocytes), Mϕ (macrophages), DC (dendritic cells), T lymphocytes and NK (natural killer) cells and a hyperinflammatory syndrome resulting in severe tissue injury

cytokine release syndrome, and sepsis are not well characterized, and efforts to understand the multi-stage immunological process are required[12].

The progression of COVID-19 is marked by an early asymptomatic or mildly symptomatic stage with fever, dry cough, bodyache and fatigue and less frequently headache, productive cough and diarrhoea. Clinical symptoms include a decrease in the number of circulating lymphocytes to typically less than 1.0×10^6/mL. Transition to the severe stage is marked by dyspnea and hypoxia and is associated with abnormal lung CT scans showing typical ground glass opacities, increasing neutrophil counts and coagulopathies marked by increased prothrombin time and D-dimers. The severe stage is finally manifested by ARDS, caused by severe lung inflammation and damage, and is associated with increased levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, ferritin, troponin, N-terminal pro-brain-type natriuretic peptide (NT-proBNP) and interleukin (IL)-6, all of which are markers for hyperinflammation and tissue damage (Table 1). At this stage patients suffer from respiratory failure, in spite of mechanical ventilation, with diffuse vasculopathy and multi-organ involvement which can ultimately lead to death[4,5,13–16].

The hyperinflammatory response in severely ill COVID-19 patients is marked by increased plasma levels of pro- and anti-inflammtory cytokines such as IL-6, IL-1b, IL-7, IL-8, IL-9, IL-10, IFN-γ and TNF-α, chemokines such as MCP1, MIP1A and MIP1B which cause the migration of neutrophils, monocytes, T lymphocytes and NK cells to the lung alveoli and growth factors such as G-CSF and GM-CSF[4,6]. Elevated levels of IL-6 have been reported to have significant association with death in severe COVID-19 cases, making Tocilizumab, the humanized monoclonal antibody against the IL-6 receptor, a possible treatment for severely ill COVID-19 patients[17]. These clinical parameters suggest that the immunologic profile of severe COVID-19 patients is similar to CRS and secondary hemophagocytic lymphohistiocytosis (sHLH), also referred to as the macrophage activation syndrome (MAS)[18–20].

A detailed longitudinal study of cytokine profiles in COVID-19 patients with moderate and severe disease has exhibited a "core COVID-19 signature" shared by both moderate and severe disease patients defined by IL-1α, IL-1β, IL-17A, IL-12 p70 and IFNα[21]. Patients with severe disease are characterized by an additional inflammatory cluster consisting of thrombopoietin (TPO), IL-33, IL-16, IL-21, IL-23, IFNλ, eotaxin and eotaxin 3. Most of the patients with severe disease showed increased levels of the cytokines related to CRS, namely IL-1α, IL-1β, IL-6, IL-10, IL-18 and TNF. Longitudinal studies have shown that in the first ten days of symptom onset, both severe and moderate patients showed similar cytokine profiles, including the core COVID-19 signature. However, after ten days of symptom onset, these markers declined in patients with moderate disease but continued to remain elevated in patients with severe disease, together with emergence of additional cytokine markers, with an ultimately poor prognosis.

Table 1 — Threshold levels of some inflammatory markers with diagnostic and prognostic values in severe COVID-19 cases

Inflammatory marker	Threshold level in severe cases of COVID-19
Interleukin-6 (IL-6)	≥ 80 pg/mL[50]
Lactate dehydrogenase (LDH)	≥ 240 to 253.2 U/L[51]
C-reactive protein (CRP)	>26.9 mg/L[52]
Ferritin	>800 μg/L[53]
D-dimer	> 2.0 mg/L[54]
N-terminal pro-brain-type natriuretic peptide (NT-proBNP)	>900 pg/mL[55]

The threshold values are indicative only, as they are calculated from meta-analysis of cumulated data from different reports or from large single centre studies

Early stage of infection - Role of neutrophils and lymphocytes

The hyperinflammatory response in COVID-19 is most likely primed by the multiple steps of the viral life cycle: virus entry, replication and cell lysis. SARS-CoV-2 entry into alveolar cells is mediated by the interaction of the S protein with the ACE2 receptor protein which is expressed on many cell types including lung alveoli, nasal, oral, skin and kidney epithelial cells, smooth muscle cells and endothelial cells of vessels in the gastrointestinal tract as well as in arteries and veins[22]. The interaction of the S protein with ACE2 might perturb the renin-angiotensin system, reducing the level of ACE2 and increasing the level of angiotensin II, and thereby affecting vascular homeostasis[23]. The role of ACE2 in vascular homeostasis is observed in ACE2 knockout mice, which when challenged with inflammatory insults showed increased vascular permeability, lung edema and neutrophil infiltration of lungs[24]. Similarly, infiltrating neutrophils and pro-inflammatory macrophages have been found in bronchoalveolar

lavage of severely ill COVID-19 patients[25], and COVID-19 patients suffering from ARDS have been reported to develop neutrophilia (high number of neutrophils) and show the presence of toxic granules in neutrophils, suggesting the role of infiltrating neutrophils in lung inflammation and damage[4,14].

A gene network approach which constructed a protein-protein interaction network with upto 100 proteins that directly interact with the seven putative SARS-CoV-2 receptors including ACE2 together with the peptidases DPP4 and ANPEP and pathogen-binding proteins CD209, CLEC4G, CLEC4M and CEACAM1, followed by network inflation to allow mining of possible functional pathways showed "neutrophil degranulation" as the main Gene Ontology (GO) term in the expanded network[26]. There were 70 proteins in this category, including eight neutrophil-enriched genes, suggesting that SARS-CoV-2 binding proteins, and the wider network of interacting proteins, may be directly involved in neutrophil activation and eliciting an inflammatory response. Neutrophils are involved in early antiviral immunity, but hyperactivation, degranulation and lysis can make them cytotoxic during severe pneumonia as observed in the case of other viral infections[27]. It is now clear that an increased peripheral neutrophil:lymphocyte ratio is observed in severe COVID-19 cases and is likely associated with an unfavourable prognosis[28]. Therefore the role of neutrophils in COVID-19 pneumonia and their possible importance as markers of disease progression and severity and also in therapeutic strategies require intense study.

The role of lymphocytes in COVID-19 is less well understood. However, many studies have now shown that lymphonenia is significantly associated with disease severity in COVID-19 and can be an early and useful prognostic marker in determining the clinical course of the disease[28]. It has been suggested that the hyperinflammatory response is the key factor behind the lymphopenia, as serum levels of pro-inflammatory cytokines such as TNFa and IL-6 have been closely associated with decrease in circulating lymphocytes[28]. Massive lymphocyte death in lymphoid organs of deceased patients has been attributed to high levels of IL-6 and Fas-Fas ligand interactions. Exhaustion of T cells have been also seen in COVID-19, with a study finding both $CD4^+$ and $CD8^+$ T cells from COVID-19 patients showing increased cell surface expression of programmed cell death protein 1 (PD1) and T cell immunoglobulin and mucin domain 3 (Tim-3), two well-known markers of T cell exhaustion[28]. Moreover, SARS-CoV-2 infection might also interfere with T cell expansion as genes involved in T cell activation and function such as MAP2K7 and SOS1 have been found to be downregulated in T cells of severe COVID-19 patients[13]. Therefore, although lymphocytes, especially T cells, likely play a crucial role in the early immune response to SARS-CoV-2 infection, but subsequently undergo exhaustion and depletion most likely due to high cytokine levels, leading to lymphopenia and a high neutrophil:lymphocyte ratio in severe COVID-19 patients[29].

Viral replication and host immune dysregulation

Post entry, replication of SARS-CoV-2 in host cells will lead to both single stranded and double stranded RNA intermediates, which will act as pathogen-associated molecular patterns (PAMPs) and be recognized by the host innate immune sensors TLR7 and TLR8 for ssRNA and TLR3, RIG-I and MDA5 for dsRNA[30]. The activation of these immune sensors usually lead to activation of host antiviral immune defences, starting with the type 1 interferon (IFN) pathway[31]. Studies have shown that SARS-CoV-2 can evade this innate immune response by actively blocking the induction of Type 1 interferon pathway using ORF6, ORF8 and nucleocapsid proteins[32]. Thus, evasion of the host RNA-specific innate immune response at the beginning of the infection will lead to enhanced virus replication and increase in the number of infected cells[33]. Lung epithelial cells with actively replicating virus will eventually die, releasing damage-associated molecular patterns (DAMPs) such as ferritin and high-mobility group box 1 (HMGB1) or high levels of IL-1β and IL-6 through NALRP3 inflammosome activation[34,35], which will be recognized by resident immune cells such as alveolar macrophages and infiltrating neutrophils. This will further amplify the inflammatory response and lead to cytokine release through autocrine loops of activation. Therefore modulation of the initial innate immune response activated by PAMPs and a secondary, amplified response mediated by DAMPs leads to a hyperinflammatory response to SARS-CoV-2 infection in lungs. This is further induced by the dysregulation of the complement system, as excessive activation of complement system components such as C3, C3a, C5, C5a and MASP2 has been associated with increased inflammation in SARS-CoV-2 infections[36,37]. This can activate alveolar macrophages

leading to release of IL-6 and other pro-inflammatory cytokines, further exacerbating the inflammation and lung damage.

Severe stage of disease - Hyperinflammation and acute respiratory distress syndrome

The progression to the severe stage of the disease could therefore be marked by this hyperinflammatory response induced by increased levels of IL-1β and IL-6 and DAMPS which will activate innate immune cells expressing IL-1R such as macrophages and NK cells. These will amplify the inflammation with release of pro-inflammatory cytokines IL-6, IL-18, TNFα and IL-1β by macrophages and IFN-γ by NK cells, which will act in a positive feedback manner to further enhance the inflammatory response, ultimately leading to a "cytokine storm". High levels of IL-6 and IFN-γ, found in the plasma of severely ill COVID-19 patients, are hallmarks of cytokine storm[20]. Increased level of IFN-γ is a hallmark of sHLH, as it activates monocytes and macrophages as also seen in severe COVID-19 cases[38]. IL-6 contributes to severe CRS by enhancing vascular permeability and further infiltration of inflammatory cells[17]. IL-1b and TNF has been shown to increase the levels of hyaluronan synthase 2 and consequently the level of hyaluronic acid (HA). HA, which can absorb high quantities of water, may contribute to the accumulation of fluids in the alveoli of COVID-19 patients, further affecting respiratory function[39,40]. These events will create a cytokine and chemokine-mediated hyperinflammatory environment in the lung epithelium which can recruit and hyperactive T cells, that can further exacerbate inflammatory damage of the tissue. The exhaustion of T cells and NK cells, observed in severe COVID-19 cases, might be subsequent to this hyperactivation[29]. Hypractivation and subsequent exhaustion of T cells in progressive COVID-19 disease may also be associated by the decrease in CD4+ T_{Reg} cells, which play a key role in controlling hyperinflammation and protection from tissue damage[41]. Together, these immunological and physiological events lead to extensive diffuse alveolar damage, including desquamation of alveolar cells, hyaline membrane formation and pulmonary oedema, most likely independent of and secondary to the viral infection, leading to ARDS in severe COVID-19 patients[6].

The hyperinflammatory response in ARDS may also give rise to a pro-fibrotic condition in the lungs of severely ill COVID-19 patients, with major implications for treatment both during ARDS and in the long term for survivors of COVID-19[42]. Indeed, autopsy of 159 patients with ARDS has shown a high correlation of lung fibrosis with the duration of ARDS, suggesting that to be effective, potential antifibrotic therapy should be considered early in ARDS onset[43]. Dysregulation of multiple pathways during the hyperinflammatory response can lead to the pro-fibrotic condition, including the release of fibrotic mediators such as matrix metalloproteases which causes epithelial and endothelial injury and proliferation of fibroblasts. Inflammation leads to the activation of profibrotic pathways regulated by TGFβ and vascular dysfunction[44], mediated by VEGF and cytokines such as IL-1β and IL-6, can lead to fibrosis from ARDS[45,46], marked by the accumulation of fibroblasts and myofibroblasts and the excessive deposition of collagen and other components of the extracellular matrix. Recognition of this pro-fibrotic condition might help in deciding therapeutic strategies for treating severe COVID-19 and preventing the long-term fibrotic consequences in survivors[47].

Targeting hyperinflammation for therapy in COVID-19

The recognition of a hyperinflammatory syndrome as the cause of morbidity and mortality in a large number of severely ill COVID-19 patients has led to the consideration of a number of anti-inflammatory and immunosuppressive agents as potential therapeutics for COVID-19. Clinical trials are ongoing for multiple agents including complement inhibitors (eculizumab), recombinant human anti human IL-1β mAb (canakinumab), human IL-1R antagonist (anakinra), anti human IL-6 receptor mAb (tocilizumab and sarilumab), anti human IFNγ mAb (emapalumab), inhibitor of JAK1/2 pathway (ruxolitinib), human anti IL-17A mAb (secukinumab and ixekizumab) and others[1]. Treatment with the IL-6 inhibitor tocilizumab appears to show reduction in mortality in some retrospective studies, and has been approved for emergency usage by various countries, although results from randomized, controlled trials are still awaited. Anti-inflammatory glucocorticoids such as methylprednisolone may reduce the risk of death among severe COVID-19 patients with ARDS but are not recommended during early stage of viral infection as they may inhibit the antiviral immunity and affect virus clearance[19,48]. Hyaluronidase, the enzyme that catalyzes the degradation of hyaluronic acid and hymecromone, an inhibitor of hyaluronic acid synthetase are being considered as agents to decrease elevated hyaluronic acid and thereby relieve respiratory distress and lung damage

by decreasing fluid accumulation and reduce pulmonary edema[39]. Finally, anti-fibrotic agents with anti-inflammatory functions such as pirfenidone and the receptor tyrosine kinase inhibitor nintedanib are being considered to retard or reduce the pulmonary fibrosis associated with hyperinflammation[42]. Although these anti-inflammatory and immunoregulatory agents constitute important options in the therapeutic strategies against COVID-19, their usage translates into a tight-rope walk for clinicians treating COVID-19 patients as possible immunosuppression by these agents can negatively affect effective antiviral response, and may also fail to combat secondary bacterial infections with multidrug resistant bacterial strains which are a common feature, especially of intensive care setups[49]. A closer synergy between clinicians, intensivists and scientists, especially virologists and inflammation biologists, is therefore required for understanding the molecular and cellular basis of disease progression, and developing and implementing effective therapies against COVID-19.

Conclusion

Severe cases of COVID-19 are marked by an excessive and uncontrolled inflammatory response that exacerbates lung damage caused by viral infection, and contributes to acute respiratory distress syndrome and respiratory failure. This hyperinflammatory syndrome is characterized by multiple cellular and molecular events which results in lung damage ultimately leading to respiratory failure.The molecular and cellular mediators and markers for this hyperinflammatory syndrome are therefore important diagnostic, prognostic and therapeutic targets in COVID-19.

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Conflict of interest

All authors declare no conflict of interest.

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