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Severe COVID-19: what have we learned with the immunopathogenesis?

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Abstract

The COVID-19 outbreak caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global major concern. In this review, we addressed a theoretical model on immunopathogenesis associated with severe COVID-19, based on the current literature of SARS-CoV-2 and other epidemic pathogenic coronaviruses, such as SARS and MERS. Several studies have suggested that immune dysregulation and hyperinflammatory response induced by SARS-CoV-2 are more involved in disease severity than the virus itself.

Immune dysregulation due to COVID-19 is characterized by delayed and impaired interferon response, lymphocyte exhaustion and cytokine storm that ultimately lead to diffuse lung tissue damage and posterior thrombotic phenomena.

Considering there is a lack of clinical evidence provided by randomized clinical trials, the knowledge about SARS-CoV-2 disease pathogenesis and immune response is a cornerstone to develop rationale-based clinical therapeutic strategies. In this narrative review, the authors aimed to describe the immunopathogenesis of severe forms of COVID-19.

Keywords: COVID-19, SARS-CoV-2, Immunology, Inflammation, Cytokine storm, Cytokine, Macrophage activation syndrome, Thrombosis

Background

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense single-stranded RNA-enveloped virus, is the causative agent of coronavirus disease 2019 (COVID-19), being first identified in Wuhan, China, in December 2019. Previously, other epidemic coronavirus such as severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002 and the middle-east respiratory syndrome coronavirus (MERS-CoV) in 2012, had serious impact on human health and warned the world about the possible reemergence of new pathogenic strains [1]. Despite being a new virus, several common morpho-functional characteristics have been reported between SARS-CoV and the SARS-CoV-2, including the interaction of the viral spike (S) glycoprotein with the human angiotensin converting enzyme 2 (ACE2). These similarities may help understanding some pathophysiological mechanisms and pointing out possible therapeutic targets.

The first step for SARS-CoV-2 entry into the host cell is the interaction between the S glycoprotein and ACE2 on cell surface. Since the latter acts as a viral receptor, the virus will only infect ACE2 expressing cells, notably type II pneumocytes. These cells represent 83% of the ACE2-expressing cells in humans, but cells from other tissues and organs, such as heart, kidney, intestine and endothelium, can also express this receptor [2]. A host type 2 transmembrane serine protease, TMPRSS2, facilitates virus entry by priming S glycoprotein. TMPRSS2 entails S protein in subunits S1/S2 and S2′, allowing viral and cellular membrane fusion driven by S2 subunit [3]. Once inside the cell viral positive sense single strand RNA is translated into polyproteins that will form the replicase-transcriptase complex. This complex function as a viral factory producing new viral RNA and viral proteins for viral function and assembly [4]. Considering

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these particularities, the infection first begins on upper respiratory tract mucosa and then reaches the lungs. The primary tissue damage is related to the direct viral cytopathic effects. At this stage, the virus has the potential to evade the immune system, where an inadequate innate immune response can occur, depending on the viral load and other unknown genetic factors. Subsequently, tissue damage is induced by additional mechanisms derived from a dysregulated adaptive immune response [5].

Although most of COVID-19 cases have a mild clinical course, up to 14% can evolve to a severe form, with respiratory rate ≥ 30/min, hypoxemia with pulse oxygen saturation ≤ 93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300 and/or pulmonary infiltrates involving more than 50% of lung parenchyma within 24 to 48 h. Up to 5% of the cases can be critical, evolving with respiratory failure, septic shock and/or multiple organ dysfunction, presumably driven by a cytokine storm [6]. Host characteristics, including aging (immunosenescence) and comorbidities (hypertension, diabetes mellitus, lung and heart diseases) may influence the course of the disease [7]. The false paradox between inflammation and immunodeficiency is highlighted by the severe form of COVID-19. Thus, severe pneumonia caused by SARS-CoV-2 is marked by immune system dysfunction and hyperinflammation leading to acute respiratory distress syndrome (ARDS), macrophage activation, hypercytokinemia and coagulopathy [8].

Herein, we aim to review the factors related to the dysregulated immune response against the SARS-CoV-2, along with its relation with severe forms of COVID-19, namely ARDS and cytokine storm (CS).

Virus and host interaction

Mechanism of invasion and cell damage of SARS-CoV-2

The virus penetrates the body through the inhalation of contaminating particles, mainly droplets and aerosols from infected hosts, first lodging in the upper respiratory tract and then reaching the lungs. SARS-CoV-2 uses ACE2 to infect epithelial cells form pharynx, larynx, alveolus (type II pneumocytes), alveolar macrophages and endothelial cells [2, 9].

Glycoprotein S is present in homotrimers on the viral surface. It is divided into two subunits, S1 that bind ACE2, and S2 that fuses with the cell membrane. The S1 and S2 subunits sites are cleaved by a transmembrane serine protease called TMPRSS2. SARS-CoV-2 can also use endosomal proteases cathepsin B and L for S protein priming in TMPRSS2 non expressing cells [3]. After glycoprotein S-ACE2 interaction and membrane fusion, the virus enters the cell using the endossomic compartment alongside with the ACE2 receptor. This early endosome becomes a late endosome and merges with a lysosome to form an endolysosome. At this moment, the virus leaves the endolysosome and reaches the cytoplasm, where its viral genome will be translated [3].

Hydroxychloroquine and chloroquine are widely used to treat patients with rheumatic diseases, especially systemic erythematosus lupus, with a known antiviral effect against SARS and SARS-CoV-2 in vitro. In addition to anti-inflammatory effects by disrupting endosomal toll like receptors signaling, the endolysosomal pH increase would also hamper the fusion of virus membrane with the endosomal membrane inhibiting the viral entry into the cell [10]. Unfortunately, the antiviral action of hydroxychloroquine did not occur in vivo. Clinical studies have shown that there is no benefit of these antimalarial drugs for treating hospitalized patients or in post-exposure prophylaxis [11, 12].

Regarding pathological findings, typical characteristics of ARDS such as epithelial desquamation, hyaline membrane and pulmonary edema are seen, but cytopathic induced damage is also noted. Despite the fact that no intracytoplasmic viral inclusion was seen, multinucleated syncytial cells with atypical enlarged pneumocytes were observed, characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli, which indicates direct viral damage. Another important feature seen was the pulmonary infiltration of mononuclear cells and neutrophils [13]. Intense replication of SARS-CoV-2 leading to inflammatory cell death is an essential component of COVID-19 pathogenesis mainly in the initial phases but can be present along all disease process [14].

The fast intracellular viral replication leads to apoptosis and pyroptosis of infected cells, causing capillary leakage and the release of several pro-inflammatory cytokines. Pyroptosis is a pro-inflammatory cell death process induced by the assembly of a multiprotein complex called inflammasome [15]. The inflammasome activation is a well-known mechanism of tissue damage related to viral infection. It can be triggered by endoplasmic reticulum stress response or by ion influx through viroporins [16, 17].

Viroporin are viral proteins that undergo oligomerization forming ions channel and have been related to many functions in multiple stages of viral life cycle and enhancement of pathogenic effects. Coronaviruses encodes two viroporins: E and 3a. SARS-CoV-2 viroporin E acts as a Ca^{2+} selective ion channel that also activates the nucleotide-binding domain, leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome [17–19]. Viroporin 3a forms a homotetramer complex that work as an ion channel to promote virus release. The viroporin 3a ion channel leads to K^+ efflux and mitochondrial reactive oxygen species production that activate NLRP3 inflammasome. Thus, both viroporins are responsible for inflammasome activation, with subsequent release of IL-1ß and pyroptosis [18].

Inflammasome promotes the proteolytic cleavage of pro-IL-1β into the active form IL-1β, a pro-inflammatory cytokine, as well as cleavage of Gasdermin-D into Gasdermin N, forming pores and inducing the inflammatory cell death [20]. The death of the infected cell releases cellular and viral fragments known as damage associated molecular patterns (DAMPs) and pathogens associates molecular patterns (PAMPs), respectively, that may be sensed by Toll-like-receptor (TLR) of myeloid cells, and leads to the production and release of more inflammatory cytokines [21]. In addition, IL-1β triggers other pro-inflammatory cytokines through a paracrine way [22]. The probable role of pyroptosis and high levels of IL1- β in the pathogenesis of severe COVID-19 provided the rational to development of clinical trials to address the efficacy and safety of anti-IL-1 targeted therapy [23]. In a prospective non-randomized trial compared with a historical control cohort, anakinra, a recombinant, nonglycosylated human interleukin-1 receptor inhibitor, was associated with lower need for invasive mechanical ventilation and mortality rate in patients with severe forms of COVID-19 [24].

Despite enthusiasm for cytokine targeted drugs, the use of more available and safer drugs has been pursued. In this context, colchicine is a low-cost, widely available drug to treat diseases with an auto-inflammatory phenotype such as familial Mediterranean fever, Behçet's disease and gouty arthritis. Anti-inflammatory properties of colchicine, mainly neutrophil chemotaxis inhibition and blockage of NLRP3 inflammasome are important targets in COVID-19 pathogenesis and are being evaluated in clinical trials [25].

ACE2 downregulation

Another important mechanism of tissue damage is mediated by downregulation of ACE2 and its protective functions. ACE2 is an enzyme that cleaves angiotensin II (Ang II) and angiotensin I (Ang I) into angiotensin 1–7 and angiotensin 1–9, respectively [26]. It can be found as a transmembrane protein, acting as a receptor to SARS-CoV-2 attachment, or as a soluble protein. Angiotensin II has several deleterious effects that can contribute to lung injury through the AT1a receptor, comprising vasoconstriction, cell proliferation, inflammation, increased vascular permeability and fibrosis. On the other hand, Ang 1–7 present beneficial effects through signaling by Mas receptor, that leads to vasodilatation, anti-inflammatory and anti-fibrosis activity [27]. ACE2 receptor-mediated virus endocytosis leads to its downregulation, which might contribute to vasoconstriction, inflammation, vascular leakage and lung injury [27] (Fig. 1). In a study with ARDS murine model, the administration of recombinant ACE2 mitigated the progression to severe acute lung injury [29]. Similarly, another study with ACE2 knockout mice demonstrated more severe lung inflammation after acid inhalation when compared to wild-type mice [30].

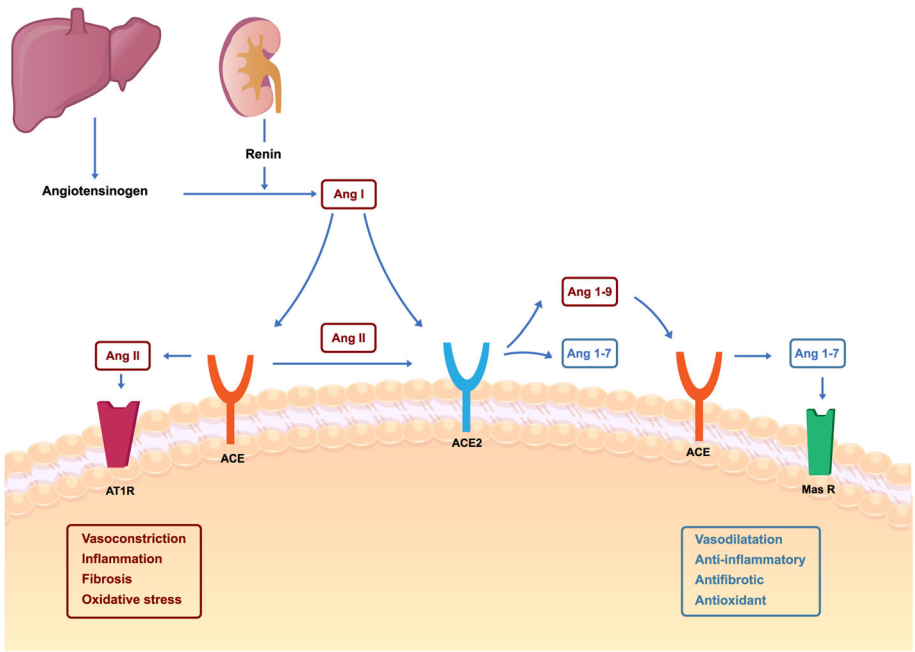

Fig. 1 Renin Aldosterone Angiotensin System in COVID-19. Angiotensinogen is produced by the liver and converted to angiotensin I by renin (produced by juxtaglomerular renal cells) in response to hypovolemia. Angiotensin I can be either converted by ACE into Angiotensin II or metabolized to Angiotensin 1–9 by ACE2. Moreover, angiotensin I can also be converted to Angiotensin 1–7 by ACE2. The Ang 1–7/MAS axis can have beneficial effects on lungs, while the Ang-II / AT1a axis have deleterious effects. Adapted from Yan, T, Xiao, R, Lin, G. 2020 [28]

A hypothesis that may partially explain the propensity of some individuals to develop more or less likely the severe forms of COVID-19 is concerning the ACE2 expression. Blood ACE2 activity is inversely proportional to body mass index and blood pressure [31], while estrogens act in its upregulation [32]. Also, children have higher levels of circulating ACE2. These findings may justify why women and children are less prone than adult or elderly men to develop the severe forms of the disease, especially those with obesity and hypertension [33]. However, other mechanisms explain its pathogenicity. The immune response plays an important role.

Immune and inflammatory response

Innate immunity cells such as macrophages, monocytes and dendritic cells are activated after recognizing DAMPs or PAMPs through their pattern recognition receptor (PRRs). This interaction trigger intracellular pathways that activates transcription factors and leads to cytokine, chemokine and adhesion molecule expression [21].

Viral PAMPs, specially viral RNA are sensed by immune system by endosomal TLRs, mainly TLR-7, and cytosolic nucleic acid sensors like retinoic-acid inducible gene 1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5), and that latter mechanism seems to be impaired in elderly [34]. SARS-CoV-2 S-glycoprotein can be sensed by TLR-4, activating the nuclear factor kappa-B (NF-kB) pathway [35]. The activation of TLRs and RIG-1 like receptors (RLR's) leads to the production and release of pro-inflammatory cytokines and interferons through the activation of the transcription factors NF-kB and Interferon Regulatory Factor (IRF) 3 and 7 [35].

In parallel, mainly dendritic cells present antigens to T helper cells and B lymphocytes in the lymphoid follicles through major histocompatibility complex (MHC) class II, triggering a more specific cellular and humoral response with effector cytotoxic lymphocytes differentiation and antibodies production, respectively [36, 37].

Alongside with mechanisms of activation of immune system, several mechanisms that suppress inflammatory process takes place in order to limit host damage by the immune system. Among them, NK cells may play an interesting role in immunomodulating the response against COVID-19. Activated myeloid cells upregulate the NKG2D ligands expression, triggering NK cells to kill them and hampering the resolution of inflammation by a contra-regulatory immune mechanism [38].

When there is an effective immune response, optimized viral clearance limits disease progression. In addition, viral antigens presentation by innate immunity cells to lymphocytes in the lymphoid organs enhances adaptive immunity and stimulates humoral immunity. B cells differentiate into plasma cells and produce antibodies that are able to neutralize and contain viral spreading. The coordinated and efficient immune response limits the infection.

Risk factors for development of severe forms

Age and sex

Advanced age hampers an efficient immune response. Several mechanisms are proposed to favor greater susceptibility to infections, poor vaccine response and degenerative inflammatory diseases. Immunosenescence evolves with reduced naïve T cells pool and memory T cells accumulation [39]. In the elderly, late differentiated effector memory T cells present a senescent phenotype with poor reproductive capacity, but higher capacity of pro-inflammatory cytokines production [40]. This concept is called inflammaging [41] and it is modulated by different factors, such as hormonal, microbiome, nutritional and comorbidities. Aged primates showed enhancement of NF-kB pathway activation, presenting a pro-inflammatory cytokine profile [42]. On the other hand, elderly is known to present an inefficient antiviral response due to an impaired cytokine release by DC. These cells are the most important source of type I interferons, which plays a major role in early antiviral response [39].

Interestingly, a higher male lethality is seen in COVID-19. Hormonal factors could be implicated, since chemotactic factors involved in neutrophils and monocytes recruitment such as CXCL1 and CCL20 are regulated by an androgen receptor. Noteworthy, estrogen receptor regulates immune response by enhancing interferon production and antiviral response. The selective estrogen receptor modulator (toremifene) have been proposed as a potential drug to treat coronavirus infection [43, 44].

Comorbidities

The majority of patients that develop severe forms of COVID-19 with CS, mononuclear lung cell infiltration and prothrombotic state have two or more comorbidities [45]. Despite being well described risk factors, the exact mechanism by which comorbidities (e.g. hypertension, diabetes and obesity) affect the clinical course is unclear.

An American study with 5700 patients revealed that the median age of the hospitalized patients was 63 years, 57% of them had hypertension, 42% were obese and 34% had diabetes [45]. Metabolic syndrome and obesity evolve with chronic inflammation due to higher NF-κB activity and pro-inflammatory cytokine production, such as IL-1, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF- α) [46]. Moreover, perturbation of hormonal and metabolic homeostasis seen in obesity could impair antiviral response, and bronchial epithelial cells from obese patients showed reduction of the interferon responses, increasing viral replication [47].

Patients with hypertension present with endothelial cells dysfunction and immunometabolic modifications that contribute to higher baseline inflammatory cytokine serum levels [48]. Diabetes is associated with higher risk of infection, and also present a pro-inflammatory cytokine profile, being considered as a risk factor for mortality in several viral pneumonias such as influenza (H1N1), SARS-CoV and MERS-CoV [49, 50]. Further studies are necessary to better understand the pathophysiological mechanisms through which comorbidities impact on severe COVID-19.

Laboratory abnormalities

Several laboratory risk factors for the development of severe forms have been identified. Most of them are related to hyperinflammation, immune dysregulation and hypercoagulability/ hyperfibrinolysis [51]. A Chinese retrospective, multicenter cohort study, with 191 patients showed higher mortality risk in patients with diabetes, coronary heart disease, advanced age, lymphopenia, leukocytosis, and elevated alanine aminotransferase, lactate dehydrogenase, high-sensitivity cardiac troponin I, creatine kinase, d-dimer, serum ferritin, IL-6 [52].

High ferritin serum levels are a well-known inflammation biomarker. Hyperferritinemic syndromes encompasses heterogeneous life-threatening conditions characterized by hyperinflammation and high ferritin levels [53]. Ferritin is not only a biomarker of acute phase response, but also seems to play a pathogenic role in inflammation. In patients with COVID-19, it is associated to poor prognosis, severe disease and mortality [52, 54].

IL-6 is an important inflammatory biomarker and is correlated with acute phase reactants. Several studies showed that high IL-6 plasmatic levels were related to COVID-19 severity [55], suggesting that IL-6 inhibitors, like Tocilizumab, could be a potential therapy for severe COVID-19 patients [23]. The pathogenic aspects regarding the IL-6 in COVID-19 will be addressed below.

D-dimer is a fibrin degradation product and its rising levels has been related to activation of coagulation and subsequent fibrinolysis. In addition, in a retrospective cohort, D-dimer plasmatic levels above 2.0 μg/mL, on admission, were considered as a relevant mortality predictor. More recently, a systematic review on biomarkers related to COVID-19 severity showed D-dimer has strong association with mortality [56]. Therefore, anticoagulation therapeutic strategies have been justified by higher thrombotic phenomena incidence [57].

Lymphopenia, which is seen in up to 72–85% of severe cases is a hallmark of COVID-19. Severe cases tend to have lower lymphocytes counts, higher leukocytes count and high neutrophil-lymphocyte-ratio (NLR). All lymphocytes subsets, including CD4+ T cells, CD8+ T cells, B cells and natural killer cells decreased in COVID-19, especially in patients who develop severe forms. CD8+ T cells and CD4+/CD8+ ratio showed a significant association with the inflammatory status in COVID-19 and were independent predictors for poor outcomes [58]. Patients with COVID-19 have lower level of regulatory T cells, mostly in severe cases [59].

Lymphopenia mechanism is not completely understood. One of the proposed mechanism is lung infiltration with lymphocyte sequestration [60]. Indeed, it is well-documented that genes related do lymphocytes apoptosis such as annexin V and exhaustion-related genes are upregulated [58, 61, 62]. Lymphocyte exhaustion will be discussed below.

Immunopathogenesis of severe forms

Inadequate interferon response

It is well-established that interferons, mainly types I and III, are crucial in promoting antiviral state, providing an effective viral clearance. Multiple pathways are involved in this complex response, including upregulation of Interferon Stimulated Genes (ISGs) that enhances MHC-I presentation, as well as immune cells activation, apoptosis of infected cells and production of antiviral proteins that suppress viral replication [35].

Once type I interferon binds to its receptor (IFNAR1 and INFAR2), it activates the JAK/ STAT signaling pathway, through which non-receptors tyrosine kinase JAK1 and TYK2 will determine the phosphorylation of STAT1 and STAT2 and finally the expression of ISGs [63]. Type III interferon (also known as lambda interferon) shares similar effects and intracellular signaling pathways with type I interferon, although using a different membrane receptor present only in mucosal sites [64]. Thus, interferons I and III are essential in the early antiviral response. Type II interferon (i.e. gamma interferon), in turn, plays a fundamental role on intracellular microorganism defense, inducing macrophage activation and Th1 response [65]. Dead and apoptotic cells can be phagocyted by macrophages (M0) and dendritic cells (DC) leading to antigen presentation by MHC-II to TCD4+ cells enhancing the adaptive immune response [66]. Moreover, viral antigens can be expressed by MHC-I molecules of infected cells activating cytotoxic TCD8+ cells and cellular immune response [67].

A striking feature of coronaviruses is their ability to inhibit interferon action. Although the SARS-CoV-2 has 3.2-fold higher replication rate, it induced lesser production and release of types I, II and III interferons when compared with SARS-CoV infection. Therefore, SARS-CoV-2 can reach high viral loads before activating an innate immune response [68].

Several coronavirus non-structural proteins (NsP), like Nsp1, Nsp3, Nsp14, ORF3b, ORF6 and the structural M and N proteins could impair the interferon production,

signalization and antiviral activity through many different mechanisms [35]. Among some trickeries used by coronaviruses to evade the immune system is the fact that they replicate within double membrane vesicles, hiding the viral RNA from cytosolic RIG-like receptors, a characteristic of the *Nidovirus* order [69].

In a SARS-COV murine model, Channappanavar et al. demonstrated that the Interferon (IFN) late response is associated with severe forms of pneumonia and ARDS. It is characterized by accumulation of pathogenic inflammatory monocyte-macrophages (IMMs) that results in high cytokine/chemokine concentration in lung tissue, vascular leakage, and impaired virus-specific T cell responses [70].

The same authors reported the relevance of interferon kinetics in MERS murine infection. When administered on the first day after infection, IFN-beta had a protective effect due to increased viral clearance. However, if administered later, not only was the viral clearance not induced, but also an increased pulmonary inflammatory infiltration by macrophages and monocytes happened, resulting in a CS [71].

Thus, an inadequate antiviral response, either by viral immune evasive mechanisms or by an impaired host response to interferon, leads to an insufficient viral clearance, with higher risk of developing pneumonia, ARDS and CS [62]. This immune dysregulation phenotype found in COVID-19 infection is characterized by impaired interferon I response and downregulation of interferon stimulated genes.

An impaired antiviral interferon response may lead to a high viral load at the time of antibody formation. In SARS outbreak, seroconversion occurs on the eighth day of symptoms and it coincides with the worsening of the disease in 80% of cases. This dynamic process suggests that antibody-immune enhancement may plays an important role in the pathophysiology of COVID-19 severe forms [72] (Fig. 2).

Humoral response and antibody-dependent enhancement

Humoral response is a pivotal component against viral infections. SARS-CoV-2 cause a strong B cell activation, maturation and antibody production. Neutralizing antibodies (Nab) against the receptor biding domain of glycoprotein S is present in the vast majority of patients with COVID-19 following infection. Nab block the viral-receptor interaction and inhibit the viral entrance in the host cell [73].

Antibody-dependent enhancement (ADE) is a phenomenon in which subneutralizing antibodies enhance the entry of virus into monocytes/macrophages and granulocytic cells through interaction with Fc and/or complement receptors [74]. Clinical deterioration associated with ADE is a well described phenomenon in several viral infections such as the Dengue, Zika, Ebola, Influenza and veterinary coronavirus that causes feline infectious peritonitis [75–79]. A prime example is the development of the most severe form of dengue, the dengue hemorrhagic fever (DHF), that develop in patients with previous infection by a different serotype. In this situation, the risk of DHF is enhanced due to the presence of subneutralizing antibodies against the previous serotype [75].

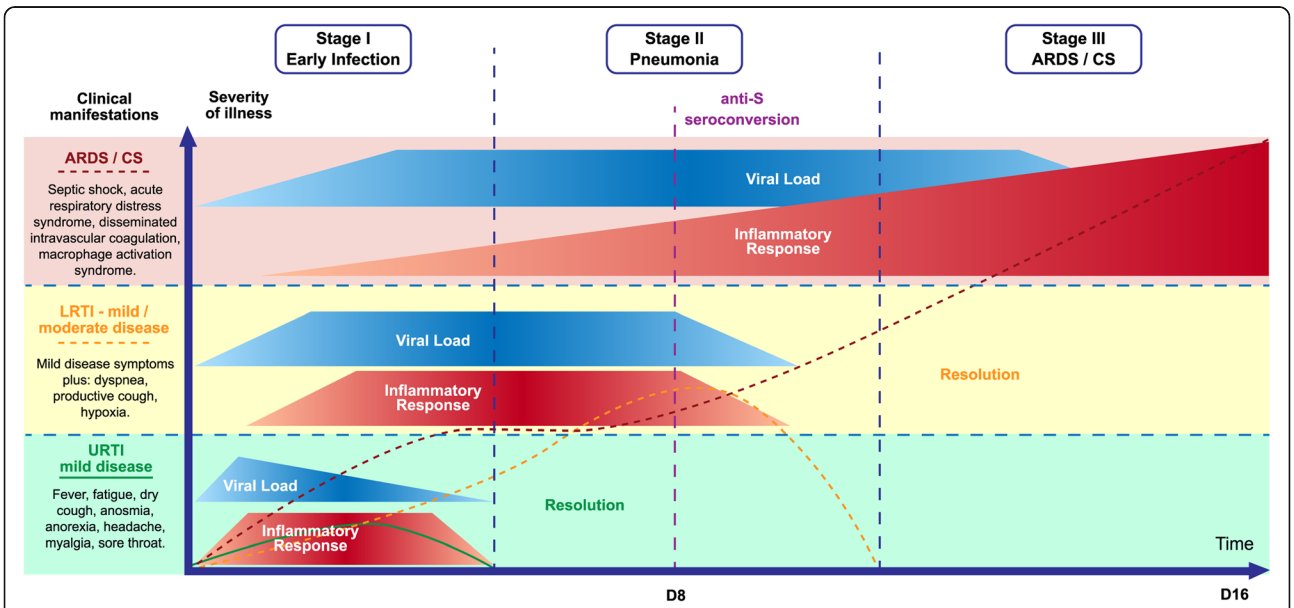

Fig. 2 Spectrum of severity and stages of COVID-19. UPRT – Upper Respiratory Tract Infection; LRTI – Lower Respiratory Tract Infection; ARDS – Acute Respiratory Distress Syndrome; CS – Cytokine Storm

The fact that seasonal human coronaviruses such as NL63, 229E, OC43, HKU-1 are responsible for 8–18.4% of all respiratory tract infections, usually common cold help to explain the rationale behind the hypothesis of ADE in SARS-CoV-2 infection [80].

Viral entry via FC receptor can lead to productive infection, when virus can replicate inside myeloid cells or to unproductive infection when virus is destructed and no infective virus are released. Despite the fact that there is no evidence of SARS-CoV-2 replication inside myeloid cells, viral entry by Fc receptor, mainly Fc-gamma-RII (CD32) may lead to activation of endosomal TLR and release of proinflammatory cytokines. This phenomenon is known as antibody-immune-enhancement [81]. In the case of SARS-CoV-2, it is possible that a suboptimal humoral response leading to low titters of IgG anti-S or production of non-neutralizing antibodies can mediate antibody-immune enhancement [82].

Tseng et al. demonstrated that exposure to infectious SARS-CoV causes maturation and phenotypic alterations of dendritic cells enhancing T-cell-stimulatory capacity and cytokine release. In the same study, the authors showed that exposure to SARS-CoV led to diminished phagocytic capacity in macrophage and primes it leading to massive production and release of cytokines in response to low dose of LPS [81].

Noteworthy, the development of severe forms of disease occurs at the time of seroconversion in 80% of patients with SARS-CoV pneumonia [72]. In line with that, animal models actively immunized with anti-S IgG evidenced that these animals develop a more pronounced lung damage than non-immunized ones. This damage was mediated by lung infiltration by inflammatory monocytes and macrophages (IMM) [83].

Lymphocyte exhaustion

It is well known that persistent infections by several viruses can lead to immune exhaustion. Besides lymphopenia, lymphocytes in COVID-19 severe forms also exhibit an exhausted phenotype, characterized by impaired effector functions [84]. These exhausted T cells are more frequently found in those with severe forms [85].

The CD8+ T cells role in the immune response to coronavirus was highlighted in a bronchoalveolar lavage fluid analysis from patients with COVID-19. In mild symptomatic cases, a highly expanded clonal CD8+ T cell population was found, suggesting that a robust adaptive cellular immune response was related to a better disease control [5]. CD8+ T and NK cells from COVID-19 patients have increased expression of the inhibitory receptor NKG2A compared to healthy controls. This altered expression is normalized in convalescent patients [84].

Genome-wide transcriptional signature from exhausted CD8+ T cells showed altered expression of inhibitory and co-stimulatory receptors such as PD-1 and LAG-3 [86]. In this light, immune checkpoint inhibitors could restore effector functions and improve viral clearance [87, 88].

T cell exhaustion is a cornerstone of COVID-19 cytokine storm, since T cell activity is crucial for virus clearance and innate immune inflammation shutdown [84]. The inability to eliminate the virus due to lymphocyte exhaustion is both the cause and consequence of a high antigenic stimulus. This scenario favors continuous myeloid cell stimulation and hyperinflammation.

Inflammatory monocytes and macrophage lung infiltration

A minimally-invasive autopsy study demonstrated that inflammatory monocytes and macrophages accumulate in the lungs and are the likely source of pro-inflammatory cytokines and chemokines. Part of them were positive for SARS-CoV-2 by immunohistochemical staining [89].

The presence of anti-S antibody prior to the viral clearance appears to play a role in severe acute lung injury. In a previously cited study, SARS-CoV vaccinated primates had greater pulmonary infiltration of an inflammatory phenotype macrophages (MAC387 +, CD163 + and HAM56-), with higher IL-6 production than unvaccinated ones. Interestingly, despite alternatively activated macrophages (M2) were reduced in number, they showed a 10-fold increase in IL-6 production, 8-fold in MCP-1 production and 5-fold in IL-8 production. This would ultimately enhance inflammation and monocytes/neutrophils chemotaxis [83].

Therefore, lung accumulation of IMM with atypical macrophage activation and higher pro-inflammatory cytokine expression could provide a feedback loop that culminates in CS development.

Cytokine storm

CS is a serious life-threatening condition characterized by uncontrolled activation of macrophages and T cells, hypercytokinemia, hyperferritinemia that could finally cause multiple organ dysfunction. CS is the convergent pathway of several diseases that evolve with immune dysregulation and hyperinflammation [90]. The CS prototype is primary hemophagocytic lymphohistiocytosis (pHLH), caused by genetic defects that impair the cytotoxic capacity of $TCD8^{+}$ lymphocytes and NK cells [53].

NK cells are necessary for immune regulation. Through recognition of upregulated stress ligands, they are able to eliminate activated macrophages and other myeloid cells. Impaired cytotoxic capacity leads to loss of regulation, with persistent cytokine production by myeloid cell. As previously mentioned, patients infected

by SARS-CoV-2 had NK and $TCD8^+$ cells with an exhausted phenotype, characterized by expression of the NKG2A receptor, which impairs its immunoregulatory mechanism [84].

Besides pHLH, several diseases, such as, cancer and infections can lead to CS, often termed secondary hemophagocytic lymphohistiocytosis (sHLH). When the underlying disease is autoimmune or autoinflammatory, sHLH is called macrophage activation syndrome (MAS) [53]. Viruses are the most common infectious trigger for sHLH, but they may also trigger pHLH. The most implicated viruses are DNA viruses, like Epstein-Baar virus and Cytomegalovirus, and less frequently RNA viruses, such as Dengue, Influenza, HIV and highly pathogenic coronavirus [53, 91–95].

Regarding cytokine profile, COVID-19 severe cases have high IL-2R, IL-6, IL-10, TNF-α serum levels [51, 96–100], with conflicting data on IL-1β, IL-7, IL-8, IL-17, IFN-γ and G-CSF [101–105]. However, these findings must be cautiously interpreted since cytokine serum levels may not reflect tissue inflammatory process [106].

In a study comparing host response to SARS-CoV-2 with other viruses (i.e. MERS, SARS, Respiratory Syncytial Virus and Influenza A), a distinct transcriptional profile was shown in ex vivo human bronchial epithelium model. SARS-CoV-2 induced a low or absent type I and III interferon response, while it promoted a strong pro-inflammatory cytokine (IL-1β, IL-6) and chemokines MCP-1 and CXCL-8 expression, attracting monocytes and neutrophils, respectively [107]. Besides type I and III interferon, IFN-γ expression also tended to be lower in CD4 + T cells from severe cases compared to moderate ones [51].

Data from SARS and MERS suggest that chemokines play a major role in inflammatory and immunity response to coronaviruses [93, 108]. Interferon-induced chemokines are crucial for an antiviral status. Among them, CXCL10 seems to be key for ARDS development as it has an important role on host viral defense [109, 110]. SARS-CoV infection of myeloid dendritic cells, even unproductive, leads to chemokines upregulation of CXCL10 (IP-10), CCL2 (MCP-1), CCL3 (MIP-1a) and CCL5 (RANTES) [111]. In a cluster of SARS-CoV-2 pneumonia, Huang et al. showed an upregulation of several chemokines in severe forms, such as CXCL10, CCL2 and CCL3 [96]. Thus, impaired interferon response seems to be related with a second wave of inflammation associated to lung monocyte infiltration, cytokine and chemokine production, and also tissue factor expression that leads to pro-thrombotic state [112] (Fig. 3).

Several studies are evaluating the effectiveness of treatments addressed to suppress CS, especially IL-6 inhibitors, such as tocilizumab and sarilumab. In some early case series, tocilizumab showed potential role to avoid mechanical ventilation and death [113, 114]. However, randomized clinical trials are important to demonstrate these beneficial effects. More recently, preliminary reports from randomized clinical trials failed to demonstrate a statistically significant benefit of IL-inhibitors. The COVACTA trial was interrupted due to side effects (opportunistic infections) [113, 115–117]. Probably this lack of benefit can be explained by the pleiotropic effect of pro-inflammatory cytokines.

The use of JAK inhibitors has been reported in clinical conditions with hypercytokininaemia, such as sHLH [118]. Because they act in the intracellular signaling pathway of several cytokines involved in CS, JAK inhibitors are potential treatments for severe forms of COVID-19. The main concern with its use is the inhibition of the antiviral response. Ongoing trials are evaluating the efficacy and safety of JAK inhibitors such as ruxolutinib and baricitinib in the treatment of COVID-19 [119, 120].

Up to date, dexamethasone is the only anti-inflammatory therapy that proves benefit in treatment of COVID-19 in a high quality randomized controlled trial. In preliminary report from RECOVERY trial, dexamethasone lower 28-day mortality among those who needs oxygen supplementation or respiratory support [121].

Neutrophil extracellular traps

Neutrophil extracellular traps (NET) are web-like structures released by neutrophils composed by chromatin, histones, and granule proteins (e.g. neutrophil elastase, myeloperoxidase) that aim to trap pathogens or infected cells [122].

Prior studies linked excessive NET formation to tissue damage and pulmonary diseases, especially ARDS [123, 124]. NET can induce macrophage secretion of IL1β, further enhancing NET formation and CS [125, 126]. Viruses are known triggers of NET [127]. Considering its close relation to ARDS, and also that neutrophilia, high levels of IL-1β, IL-6, and D-dimer are poor outcome predictors in COVID-19, some authors suggest that NET may play a major role in its pathogenesis. Zuo et al. reported that patients with COVID-19 have high NET biomarkers serum levels, including cell-free DNA, myeloperoxidase DNA and citrullinated histone [122]. Moreover, NET can trigger microvascular thrombosis, leading to damage in the lungs, heart, and kidneys [122]. The interaction between NET and coagulation is addressed in details bellow.

COVID-19 associated coagulopathy

The observation of high levels of fibrin degradation products in the serum of severe COVID-19 patients addressed the question on a specific coagulopathy in this

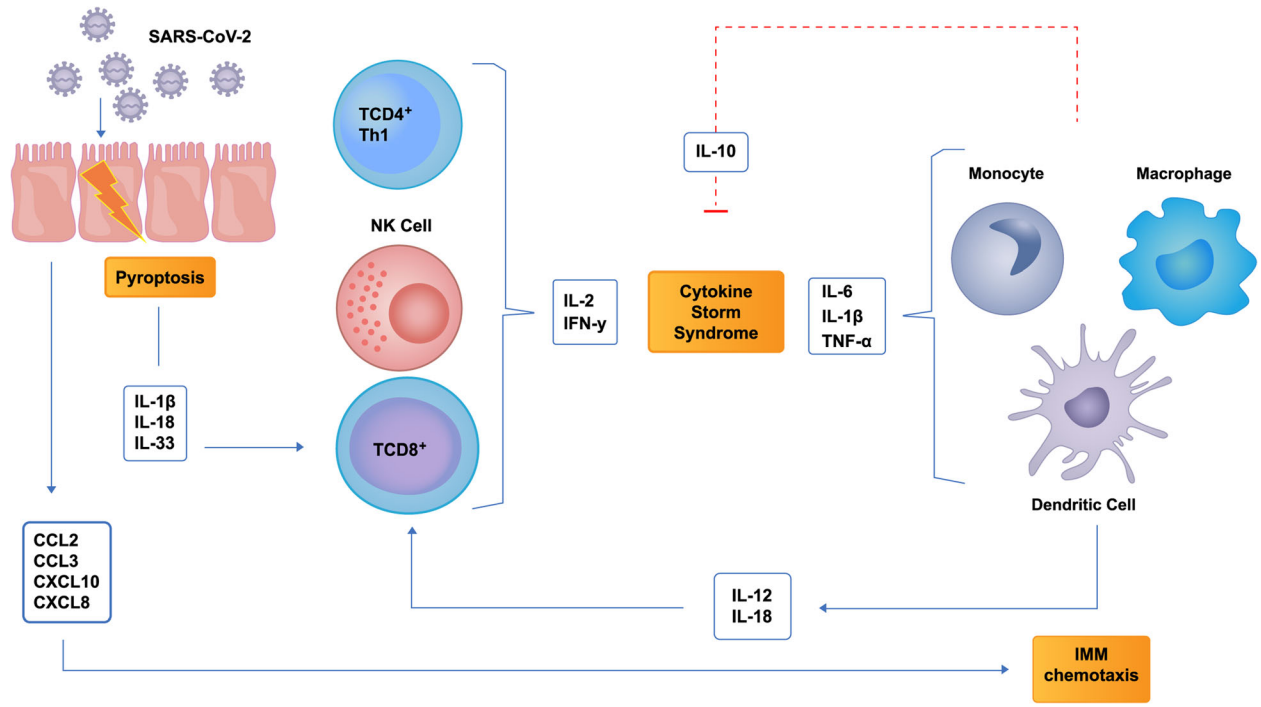

Fig. 3 Cytokine Storm from SARS-CoV-2. SARS-CoV-2 infection cause pyroptosis of alveolar epithelial cells (type II pneumocytes) leading to release of cytokines such as IL-1 ß, IL-18 and IL-33 (alarmins). In turn, IL-1ß stimulates a large amount of pro-inflammatory cytokines and IL-18 stimulates IFN-γ release by $TCD8^+$ effector cells, Th1 lymphocytes and NK Cells. Endothelial and epithelial damaged cells also release chemokines such as CCL2, CCL3 and CXCL10 that attract myeloid mononuclear cells that release more inflammatory cytokines, creating a positive feedback loop. IL-10 is released as an inefficient attempt to suppress inflammatory process

context [8]. Lung necropsy of SARS patients had already showed diffuse alveolar damage along with small vessel thrombosis [128]. Similarly, pulmonary pathological analysis in COVID-19 revealed modest vessel wall immune cell infiltration with hyaline thrombosis and infarction [129].

Another study evaluated 19 patients with ARDS due to COVID-19, and evidenced a severe endothelial injury, the presence of intracellular virus in endothelial cells, with disrupted cell membranes and widespread thrombosis with microangiopathy [130]. Indeed, infection of endothelial cells expressing ACE2 by SARS-CoV-2 cause an endothelitis and with a massive release of plasminogen activator [14].

In COVID-19, fibrinogen is usually high as part of the acute phase response, but platelet count remains normal and severe coagulopathy is only seen in very severe and late-stage forms. Besides, high serum levels of D-dimer, mild or unchanged prothrombin time are also seen [131]. This differs from sepsis intravascular coagulation/disseminated intravascular coagulation, since the latter usually evolves with thrombocytopenia and an increased prothrombin time [132].

Regardless of its clinical impact, coagulopathy seems to be triggered by hypercytokinemia [133]. Pro-inflammatory cytokines such as TNF-α and IL-6 induce tissue factor expression in monocytes and initiate coagulation cascade activation. In addition, these cytokines also suppress endogenous anticoagulant pathways [131]. MAS-like can cause local activation of endothelial cells from pulmonary vessels. The pro-inflammatory milieu leads to the upregulation of tissue factor and the reduction of fibrinolysis by Plasminogen activator inhibitor-1 [134].

In SARS-CoV associated coagulopathy, the high D-dimer levels seems to be result from upregulated urokinase-type plasminogen activator produced by alveolar macrophages [134].

Others proposed mechanisms for thrombotic phenomena in COVID-19 involves NET. First, it can activate the coagulation's contact pathway and pulmonary megakaryocytes [8], through electrostatic interactions between histones and platelet phospholipids. Also, NET can process natural anticoagulant molecules, such as antithrombin III and tissue factor pathway inhibitor [103]. Thus, NET could link several aspects related to infection, inflammation and thrombosis in COVID-19 pathogenesis [135].

Conclusions

Herein, we present the mechanisms of immune dysregulation, hyperinflammatory and immunothrombotic

state reported to date in severe forms of COVID-19. Data from other coronaviruses regarding antibody-dependent enhancement may be a concern for vaccine development. Knowledge about kinetics of immune response and viral course are essential for patient care and could provide insights for immunomodulatory therapeutic strategies. Further studies correlating clinical and laboratorial data with immune status and viral load may help choosing properly suitable candidates for targeted immune therapy.

Abbreviations

ACE2: Angiotensin-converting enzyme 2; ADE: Antibody-dependent enhancement; Ang-1: Angiotensin-1; Ang-2: Angiotensin-2; Ang-1-7: Angiotensin 1–7; Ang-1-9: Angiotensin 1–9; ARDS: Acute respiratory distress syndrome; DAMP: Damage associates molecular pattern; DHF: Dengue hemorrhagic fever; Nab: Neutralizing antibody; NET: Neutrophil extracellular traps; MAS: Macrophage Activation Syndrome; NLRP3: nucleotide-binding domain, leucine-rich repeat and pyrin domain-containing protein 3; PAMP: Pathogen associated molecular pattern; PRR: Pattern recognition receptors; pHLH: Primary hemophagocytic lymphohistiocytosis; sHLH: Secondary hemophagocytic lymphohistiocytosis

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Competing interests

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