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SARS-CoV-2 and Covid-19 Immunopathogenesis

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Abstract

The coronavirus disease 2019 (COVID-19) is now a global pandemic caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Unlike other known coronaviruses, such as the Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 reveals new clinical, immunological, and pathologic features. The lymphocyte depletion, macrophage and neutrophil hyperactivation, cytokine dysregulation, thrombophilia, delayed antiviral response, and immune exhaustion are key immunological findings linked to the clinical progression of this disease. Understanding and identifying the underlying immunological basis of COVID-19 is crucial to designing effective therapies. Here, we provide an overview of immunopathogenesis driven by SARS-CoV-2 after its interactions with the immune system.

1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causal agent of coronavirus disease 2019 (COVID-19). The virus was first isolated from the bronchoalveolar fluid collected from a 41-year-old man admitted to the Central Hospital of Wuhan (China) with SARS. Molecular analysis showed a new RNA virus strain of the Coronaviridae family, closely related to a SARS-like coronavirus previously described in bats (1). The infection was associated with a seafood and trading market of wild animals in Wuhan, China. The SARS-CoV-2 infection spreads by human-to-human transmission, primarily via respiratory droplets from sneezes and coughs. Indirect contact with contaminated surfaces is also a source of infection. Virus RNA is also found in human stools and semen (2; 3; 4; 5). Additionally, other infection routes, such as the fecal-oral transmission route, may

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be possible. Since December 2019, COVID-19 has rapidly become a worldwide emergency and has affected more than 100,000 patients globally, leading the World Health Organization (WHO) to recognize the outbreak as a global pandemic on March 11, 2020 (6).

Clinical diagnosis of COVID-19 is mainly based on epidemiological history, manifestations, and some auxiliary examinations (7). The clinical characteristics of COVID-19 include fever, coughing, sputum production, headaches, myalgia or fatigue, vomiting, and diarrhea (7; 8; 9). The majority of symptomatic patients that clinically present COVID-19 present a mild disease (80%), lymphocytopenia, and an elevation of C-reactive protein (60.7%) (9). In some cases, increased prothrombin time as well as increased levels of D-dimer (59.6%), lactate dehydrogenase (41%), and creatine kinase are present (13%). Among these symptomatic patients, 14% experience severe pneumonia, progressive hypoxemia, and dyspnea, along with a pulmonary infiltration of 50% or more. Approximately 30% of hospitalized patients require respiratory support in an intensive care unit (ICU) (7). The mortality rate of patients that require invasive ventilation is approximately 80% (10). Radiological findings have shown bilateral involvement with patchy ground-glass opacities and patchy consolidation. Peripheral distribution and lower zone dominance have also been observed (11; 12; 13). Long-term pulmonary fibrosis and respiratory functional impairment are also concerns for COVID-19 patients (14).

Cumulative evidence has indicated the importance of person-to-person transmission efficiency from asymptomatic patients, making epidemic control challenging (15; 16). Asymptomatic or minimally symptomatic individuals can contaminate their surroundings (17) and are diagnosed by reverse transcription-polymerase chain reaction (RT-PCR) tests only. The estimated rate of asymptomatic individuals is approximately 20.8%. Abnormal radiological findings confined to a single lung have been observed in 66.7% of patients with COVID-19 and in both lungs for 33.7% of patients with COVID-19 (18). Although asymptomatic individuals have a similar viral load to symptomatic patients, they show a distinct immunological profile (18).

Although the pathophysiological features of COVID-19 have not yet been fully understood, these clinical and laboratory characteristics are a result of the host-pathogen interactions with the immune system and body tissues (19). This severe illness is not only triggered by the viral load; an excessive inflammatory response to SARS-CoV-2 plays a major role in disease severity and likelihood of death (20; 21). A large number of pro-inflammatory cytokines and chemokines produced by immune cells have been documented for the inflammatory phase (22; 23). The development of new treatments for SARS-CoV-2 and a deep clinical understanding of COVID-19 could be achieved by gaining further knowledge of the immunopathogenesis. Therefore, this review focused on the critical immunological features and plausible inflammatory pathways of COVID-19.

2 Major autopsy and pathological findings

The pathological features of COVID-19 greatly resemble those observed in SARS and Middle East respiratory syndrome coronavirus (MERS-CoV) infection (24). SARS-CoV-2 has multiorgan viral tropism beyond the respiratory tract, including the kidneys, liver, heart, and brain (25). The initial examination of lung specimens from the early phases of COVID-19 reveals some important histological changes: (1) interstitial edema and proteinaceous and fibrin exudate; (2) reactive pneumocyte hyperplasia with patchy inflammatory cellular infiltration, corresponding to the ground-glass radiology findings; (3) thickening of alveolar walls and septa due to fibroblastic proliferation and type II pneumocyte hyperplasia, consistent with early diffuse alveolar damage patterns; and (4) abundant macrophage infiltration (26). Later stages have shown bilateral diffuse

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alveolar damage and fibromyxoid exudates (27). Liver examinations have shown moderate microvesicular steatosis and mild lobular and portal activity as well as mild myocarditis with interstitial mononuclear inflammatory infiltrates. Complete autopsies, including postmortem computed tomography and histopathologic analysis, have revealed a high incidence of thromboembolic events, including pulmonary embolism and deep vein thrombosis (28). Histologic and lung microvascular examinations have revealed endothelialitis, angiogenesis, and microthrombosis (29). Histologic hypoxic-ischemic changes of the brains of patients with COVID-19 have also been observed (30; 31)

3 General molecular features of SARS-CoV-2

Overall, 39 species in 27 subgenera, five genera, and two subfamilies have been identified as belonging to the family Coronaviridae (32). There are seven human coronaviruses, with SARS-CoV, MERS-CoV, and SARS-CoV-2 being the human pathogenic species. These three viruses cause SARS but, surprisingly, the CoV responsible for COVID-19 is the least pathogenic of the three (33). Like other CoVs, SARS-CoV-2 is a spherical positive-sense RNA virus that projects spikes, giving it the appearance of a solar corona. Its envelope (E protein) holds the helically symmetrical nucleocapsids and its 26 to 32 kb length genome, making it similar to the other CoVs, which are known for their large genome for RNA viruses (34). In spite of the 79% similarity of the SARS-CoV-2 genome sequence with SARS-CoV (35), questions regarding what makes the novel coronavirus different in terms of its spread needed to be investigated.

Since SARS-CoV-2 belongs to the same family, Coronaviridae of the order Nidovirales, as SARS-CoV and MERS-Cov, they have several similarities. The novel coronavirus has the capacity to cause massive tissue damage by an uncontrolled response of the innate immune system and an impaired adaptive response, affecting the body locally and systemically (36).

All the coronaviruses have common organization and expression of their genome, in which 16 nonstructural proteins (nsp1 through nsp16), encoded by open reading frame (ORF) 1a/b at the 50 ends, are followed by the structural proteins S, envelope (E), membrane (M), and nucleocapsid (N), which are encoded by other ORFs at the 30 ends (37). The non-structural section is responsible for virus replication, including RNA-dependent RNA polymerase, proteases, and helicase (38). The M protein is responsible for maintaining the shape of the virion (39). The E protein has a multifunctional role in the pathogenesis, assembly, and release of the virus and is related to the virulence (40). The N protein is bound to the nucleic acid material of the virus (41). The SARS-CoV N protein acts as an antagonist to the type I interferon (IFN) pathway by regulating its signaling and synthesis (42). Alongside multiple characteristics that make SARS-CoV-2 distinct one of the other seven coronaviruses, there are 12 extra nucleotides in one of the cleavage sites forming a new sequence that is similar to a canonical furin-like cleavage site, promoting a higher spreading capacity compared to the other beta coronaviruses (33).

Several studies have elucidated why the primary targets are the airway and alveolar epithelial cells and vascular endothelial cells. By expressing the angiotensin-converting enzyme 2 (ACE2), and the higher affinity to the receptor of SARS-CoV-2 compared to SARS-CoV, the infection continually presents in different ways. Between these coronaviruses, SARS-CoV and SARS-CoV-2 utilize the host cell ACE2 receptor, MERS-CoV binds to dipeptidyl-peptidase 4 to enter human cells.

The mechanism used by SARS-COV-2 to infect host cells has been determined. Its entry is mediated by the transmembrane spike (S) glycoprotein (43), which comprises two functional subunits; S1 and S2, respectively responsible for binding to the host cell receptor (ACE2) as it is a receptor-binding domain (RBD), and for the fusion of viral and cellular membranes by being a fusion peptide (FP) (43;

44). Normally, CoVs use particular domains within the S1 to recognize a variety of attachment and entry receptors, depending on the viral species; however, SARS-CoV and several SARS-related coronaviruses (SARS-CoV) attach via their S domain B (Sb) (43; 45). Both subunits act together to stabilize and activate the protein for membrane fusion via extensive irreversible conformational changes (46).

Following receptor binding, the virus enters the host cell cytosol via the acid-dependent proteolytic cleavage of the S protein by a cathepsin, TMPRRS2, or another protease, followed by fusion of the viral and cellular membranes (47). Furthermore, the cathepsin TMPRSS2 is highly expressed in the lungs and kidneys, but only in low to moderate levels in the heart and blood vessels, which suggests there could be another mechanism of injury for the latter organ systems (48).

4 ACE2 receptor and SARS-CoV-2

The ACE2 was discovered in 2000 by two different groups of scientists (49). This enzyme is a zinc metalloproteinase and key regulator of the renin-angiotensin system (RAS). It has, in its catalytic domain, 42% identical residues compared to endothelial ACE (50; 51). ACE2 cleaves only one amino acid from angiotensin I (ANG) to form angiotensin (1-9), which is then converted to Angiotensin II by ACE (52). There is another important action of ACE2, which is metabolized ANG II to form peptide ANG (1–7), a vasodepressor responsible for decreasing the vasopressor peptide ANG II levels in the circulatory system and tissues. Additionally, the activity and action of ACE2 are not affected by ACE inhibitors, further distinguishing ACE2 from the classic ACE (53). Therefore, the pathophysiology of ACE2 needs to be explored when it comes to understanding cell infection and the influence of COVID-19 on the body.

The immunopathogenesis of COVID-19 is related to the ACE2 receptor in the following ways: (1) it affects many tissues as it is widely distributed and expressed at higher levels in at-risk groups with comorbidities, (2) the use of the receptor can cause downregulation that influences RAS homeostasis, (3) the binding between the virus and ACE2 modulates tumor necrosis factor (TNF)-α-converting enzyme (TACE) activity and (4) ACE2 tissue distribution (54; 55; 56; 57; 49).

4.1 Tissue distribution and expression according to comorbidities

ACE2 is expressed on the apical surface of type II alveolar cells of the lung, esophagus epithelial cells, enterocytes from the ileum and colon, cholangiocytes, myocardial cells, endothelium of the coronary and intrarenal vessels, kidney proximal tubule cells, bladder urothelial cells, and tongue epithelium (55). ACE2 is also found in central nervous system cells, indicating that SARS-CoV-2 affects neuronal cells after brain invasion via the olfactory epithelium (58). This tissue distribution explains the wide multiorgan virus tropism of SARS-CoV-2 (59).

Older patients with comorbidities, such as systemic hypertension, diabetes mellitus (DM), obesity, and respiratory diseases, as well as smokers and those with chronic obstructive pulmonary disease (COPD) (56; 57; 49), have a greater risk of developing the severe form of the disease. There is also a correlation between ACE-2 and a poor prognosis in pregnant women.

The expression of ACE2 is substantially increased in patients with type 1 or type 2 diabetes who are treated with ACE inhibitors and angiotensin II type I receptor blockers (56). Most studies have shown that DM is associated with more severe cases of COVID-19 and other viral infections, acute respiratory distress syndrome (ARDS), and increased mortality. These patients are more likely to be older than those without type II DM. Diabetes has a notable immune effect on the body, especially on the innate

immune response, the first line of defense against viruses, and it has been associated with exaggerated pro-inflammatory cytokine responses (notably interleukin (IL)-1, IL-6, and TNF-α)(60).

Systemic hypertension is associated with a severe form of COVID-19. A study enrolled 1,099 patients, of whom 173 had a severe disease with comorbidities, such as hypertension (23.7%) and coronary heart disease (5.8%) (9). Another study showed that among 140 patients who were COVID-19-positive, 30% had hypertension (61). Another study enrolled 78 patients with mild to moderate heart failure and found that the myocardium of these patients that had dilated or those who had ischemic cardiomyopathy had significantly increased expression, at mRNA and protein levels, of ACE and ACE2 compared to the control group (62).

Obesity is another risk factor that can lead to severe complications in SARS-CoV-2 infections (57). The ACE2 expression is higher in adipose tissue than in lung tissue (63). Individuals with obesity have more adipose tissue, so have an increased number of ACE2-expressing cells and, consequently, a higher predisposition to more severe infections by SARS-CoV-2 (64).

Most of the severe cases of COVID-19 have been described in those over the age of 55 years with significant comorbidities, such as COPD. ACE-2 expression in the human small airway epithelium was significantly increased in those with COPD compared to non-smokers, but not in healthy smokers (49). According to the Center for Disease Control and Prevention (CDC), 63.1% of adults over the age of 60 have hypertension, 38% over 65 have chronic kidney disease, and 26.8% over 65 have diabetes. Therefore, older individuals with these comorbidities may have an elevated risk and a more severe course of infection of SARS-CoV-2 (65).

For pregnant women during the COVID-19 pandemic, there are not, at present, enough data to define the risks of infection to both the mother and baby's development. One argument that theorizes the danger of SARS-CoV-2 during pregnancy is the significant expression of ACE2 in the human placenta, kidney, and uterus (66; 67), which suggests the possibility of pregnancy complications during the viral infection. Recently, the placenta of a COVID-19-positive and symptomatic woman, complicated by severe preeclampsia and placental abruption, was sampled for molecular immunohistochemical assays and electron microscopy. This analysis revealed that SARS-CoV-2 was localized on the syncytiotrophoblast cells at the maternal-fetal interface of the placenta as well as a dense macrophage infiltrate, without the typical vasculopathy associated with preeclampsia (68).

There has not yet been a substantial number of studies regarding rheumatic diseases and their relationship with high-risk infection by SARS-CoV-2. Therefore, a better understanding of the implications of COVID-19 in patients with immune-mediated inflammatory disease and the effects of anti-cytokines and other immunosuppressive therapies is urgently needed to guide clinicians in the care of patients with psoriasis, rheumatoid arthritis, psoriatic arthritis, inflammatory bowel disease, and related conditions.

4.2 Downregulation of ACE2

ACE2 is probably functionally removed from the external site of the membrane after virus entry (69). This is why SARS-CoV infection reduces ACE2 expression in lung cells, because the loss of pulmonary ACE2 function is associated with acute virus-induced lung injury (70). ACE2 regulates the RAS system, thus, the reduction after viral infection may result in system dysfunction and influence blood pressure regulation and fluid balance (71).

4.3 The effect on TACE activity

ACE2 is constitutively shed by TACE to release enzymatically active soluble ACE2 (sACE2) (72). The spike protein in SARS-CoV that binds to ACE2 has the ability to modulate the TACE activity through its cytoplasmatic domain (54). Inflammatory cytokines, such as IL1beta and TNF, and SARS-CoV can increase ACE2 shedding. Additionally, SARS-CoV S protein-induced ACE2 shedding has been found to be closely coupled with TNF-α production in cell culture conditions (73). Curiously, other CoVs do not share these same properties (74). Previous studies suggest that sACE2 may be directly involved in the inflammatory responses of SARS-CoV, and possibly SARS-CoV-2.

5 Viral infection, recognition, and escape mechanisms

Infection by SARS-CoV-2 begins with the binding of the ACE2 receptor to protein S, which comprises two subunits; S1 and S2. The S1 subunit consists of an amino terminal domain and a RBD (75; 76; 77). The S2 subunit acts as a membrane fusion subunit (78). Endocytosis is triggered by binding the RBD domain to the host cell's ACE2 receptor, exposing the SARS-CoV-2 virion to endosomal proteases (79). In addition, to properly process the SARS-CoV-2 spike protein and facilitate host cell entry, the cellular serine protease TMPRSS2 must be expressed in the host cell (80). After fusion, TMPRSS2 cleans ACE2 and activates protein S, leading to conformational changes, allowing the virus to enter the cells (41). On the other hand, the presence of a furin-like cleavage site in protein S, similar to MERS-CoV and human CoV OC43, increases the infectivity of SARS-CoV-2 compared to SARS-CoV (81).

Within the endosome, the S1 and S2 subunits carry out processes that culminate in the fusion of the viral membrane and the release of its content into the host cytoplasm. The S1 subunit is cleaved, exposing the fusion peptide, which inserts itself into the host membrane. The S2 region folds over itself to bring together the HR1 and HR2 regions, which merge to participate in the viral fusion process (82; 83; 84; 85). The viral entry is coupled with TNF-α production (86). In contrast, using a variety of pattern recognition receptors (PRRs), alveolar epithelial cells and alveolar macrophages detect the pathogen-associated molecular patterns (PAMPs), such as viral RNA, and damage-associated molecular patterns (DAMPs), including ATP, DNA, and ASC oligomers, initiating the innate immune response (Figure 1).

The PRRs that integrate the recognition systems of PAMPs and DAMPs across the cell surface are toll-like receptors (TLR) 1, 4, and 6, which activate the signaling pathway, leading to the activation of NF-kB and IRF3/IRF7, and the subsequent expression of pro-inflammatory cytokines and type I IFNs, acting on the depletion of viral infections (87; 88; 89; 90; 91). Curiously, previous studies have shown strong binding of protein S of SARS-CoV-2 with TLR4, and a lower binding intensity with TLRs 1 and 6 (92).

In the intracellular environment, viral recognition occurs through PRRs present in the endosome, cytosolic plasma, and inflammasome. The PAMPs are recognized by the endosomal RNA receptors TLR7 and TLR8 and the cytosolic RNA sensor, RIG-I/MDA5, the signaling cascade of the recognition results in the activation of NF-kB and IRF3 transcriptional activity, leading to the expression of IFN and pro-inflammatory cytokines (93; 94; 95). The pathway for NF-κB activation is transforming growth factor-β-activated kinase 1 (TAK1). Upon activation, TAK1 activates the downstream kinase IKK, thereby mediating IκBα phosphorylation and NF-κB activation (89; 96). The pathways for the expression of type I IFNs and IFN-inducible genes involve the recruitment of TRAF proteins, particularly TRAF3, to TRIF, and subsequent activation of TANK-binding kinase 1 (TBK1) and IKKε through TRAF3 ubiquitination and the ubiquitin-dependent recruitment of TBK1 and IKKε. Upon activation of TBK1 and IKKε, transcription factor IRF3 is phosphorylated, followed by dimerization

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of IRF3, the transcriptional induction of type I INF occurs (89; 96). The type I IFN via IFNAR activates the JAK-STAT pathway, and the JAK1 and TYK2 kinases phosphorylate STAT1 and STAT2. STAT1/2 form a complex with IRF9 and initiate the transcription of IFN-stimulated genes (93).

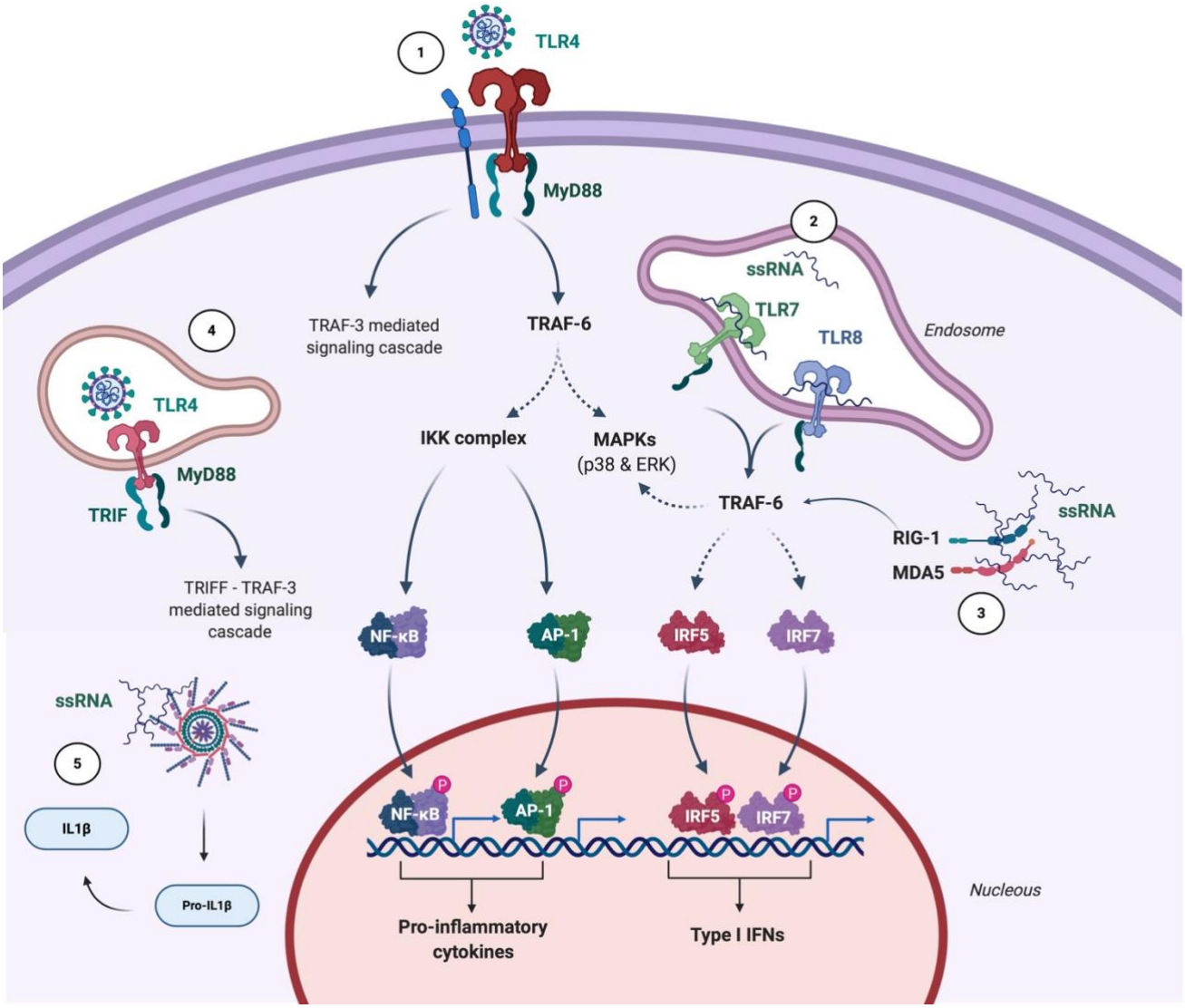

Figure 1. **SARS-CoV-2 Recognition by pattern recognition receptor and transcription factors pathway**. 1) molecular modeling shows that SARS-CoV-2 proteins have affinity to TLR-4 (membrane and endosomal), suggesting that virus can be recognized by this receptor. The SARS-CoV-2 ssRNA is mainly recognized by 2) endosomal TLR-7 and 8, 3) the cytoplasmatic receptors RIG-1 and MDA5. Downstream signaling pathways of these receptors activates NF-KB, AP1 and IRFs transcription factors. 4) Single strain RNA is also recognized by NLPR3-Inflammasome leading to IL1 and IL18 production.

Inflammasomes are multiprotein complexes that oligomerize through stimuli with ASC (the speckled protein associated with apoptosis of the adapter molecule that contains a CARD) and recruit pro-caspase-1 to cleave cytokine precursors pro-IL-1β and pro-IL-18 in mature IL-1β and IL-18. The Nod-like receptor family, pyrin domain-containing 3 (NLRP3) is the receptor related to viral RNA

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recognition, such as SARS-CoV and SARS-CoV-2 (97; 98). The activation of the NLRP3 inflammasome usually occurs in two stages; the first through the transcriptional expression of the NF-κB triggered by the aforementioned PRRs. The second through various PAMPs, DAMPs, and cell events, such as viral RNAs, ATP, K + efflux, Ca_2 + signaling, reactive oxygen species (ROS), oxidized mitochondrial DNA, lysosomal proteases, and viroporins (89; 96; 99; 97; 98).

As mentioned, activation of NF-κB triggers the transcription of additional pro-inflammatory cytokines, chemokines, and inflammatory mediators. The main cytokines induced and related to SARS-CoV-2 infection are IL-2, IL-6, IL-7, TNF, and precursor versions of IL-1β and IL-18 (100). IL-2 is produced by CD4+ and CD8+ T cells, dendritic cells, and natural killers (NK), promoting proliferation and differentiation of NK cells and T helper cells, development of regulatory T (Treg) cells as a B cell growth factor, and stimulating antibody (Ab) synthesis (101). IL-7 is a homeostatic cytokine involved in the maturation of T cells and NKs, the development of naïve and memory B and T cells, and is found mainly in T cells, B cell progenitors, and macrophages (101).

IL-1β is found in hematopoietic cells, such as monocytes, macrophages, such as microglia or Kupffer cells, and dendritic cells, and is activated through the inflammasome, initiating or improving the pro-inflammatory response (101; 102). Recent trials have associated epithelial and endothelial IL-1β with cardiovascular disease (103). IL-18, in collaboration with IL-12, promotes Th1 and Th2 responses and has a role in autoimmune diseases, such myocardial infarction and metabolic syndromes (101).

TNF is produced by T cells, NK cells, macrophages, and monocytes. The principal receptor is TNFR1 (55 kD) and TNFR2 (75 kD) and the binding of TNF-α and TNF-β with it triggers inflammatory reactions (101). TNF-α can provoke blood clotting, leading to disseminated intravascular coagulation (102).

IL-6 can promote the differentiation of CD4+ T cells via IL-21 production into Th17 effector cells. The increase of Il-6 levels in septic patients is correlated with gravity and organ failure (102). Additionally, IL-6 also suppresses Major Histocompatibility Complex (MHC) class II expression in dendritic cells via STAT3 activation (104).

According to previous studies, SARS-CoV-2 has a high inflammatory characteristic associated with macrophages and neutrophils, mainly due to the release of pro-inflammatory cytokines (105). The maintenance of this inflammatory state, especially in the presence of pro-inflammatory cytokines, is related to tissue damage in multi-organs, septic shock, and circulatory failure (106; 44).

5.1 Antigen-presenting cells (APCs) and the virus

SARS-CoV and SARS-CoV-2 target pneumocytes (both types I and II) and alveolar macrophages (107). After the infection of the host cell, the virus has its antigen presented to the APCs through the human leukocyte antigen (HLA) or by inducing the death and injury of infected cells and tissues as part of the virus replicative cycle (105).

The details of the antigen presentation in SARS-CoV-2 remain uncertain. However, in SARS-CoV, macrophage and dendritic cells represent SARS epitope by MHC II to recruit CD4+ helper cells (Th1) and the virus-infected epithelial cells present the SARS epitope by MHC I to recruit CD8+ cytotoxic T cells (108). The antigen presentation of coronaviruses via MHC I (HLA-A2) leads to the release of IL-12, driving Th1 cell differentiation (Figure 2) (109).

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APCs have PRRs, recognize PAMPs, and induce a signaling cascade to produce immune system cell effectors. Each PRR induces a different response to subsequent protein activation. The TLR-4 might recognize the protein spike, which will trigger the activation of NF-kB transcription factor and the pathogen-activated protein kinase (MAPK) pathway to induce proinflammatory proteins. The activation of TLR-3 and TLR could identify the RNA or dsRNA genome of CoV, conducting the recruitment of TRIF adapter protein. The TRIF activates the IRF3 and NF-kB transcription factors to induce IFN-a and TNF-b (41). In SARS-CoV, the expression of M protein suppresses TNFα-induced NF-κB activation (110).

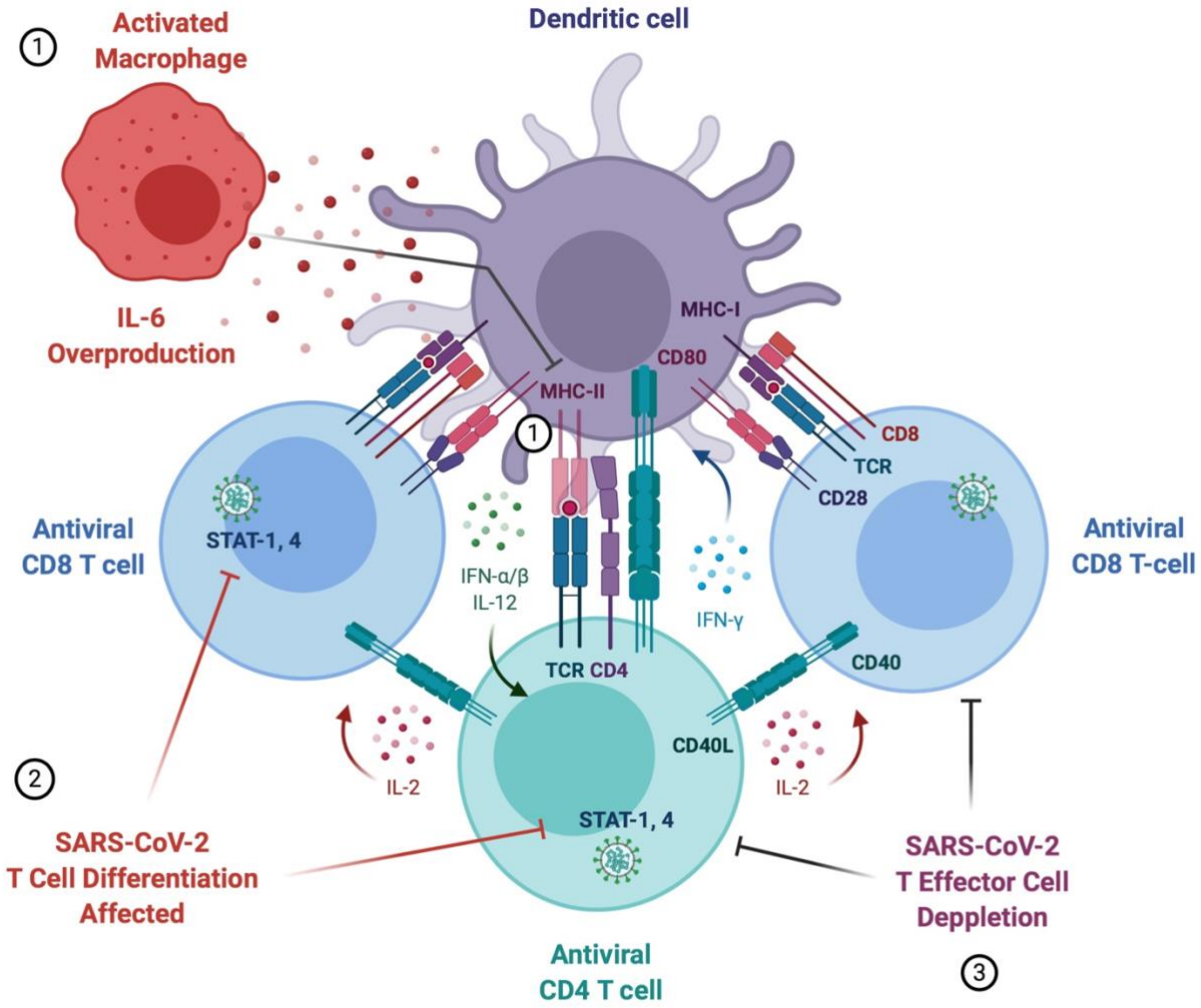

Figure 2. **Antigen-presentation cells (APC) and CD4+, CD8+ T cell interaction during SARS-CoV-2 infection**. Hypothetical antigen presentation to T CD4+ lymphocytes via Class II MHC and cross-presentation to CD8+ T lymphocytes via Class I MHC. 1) Excessive IL-6 secretion downregulates HLA-DR expression. 2) SARS-CoV-2 promotes both CD4 and CD8 cells depletion and differentiation.

In a previous case study with three RT-PCR assays positive for SARS-CoV-2, two patients had normal chest radiographs and one presented bilateral, patchy, ill-defined lung infiltrates. The posmRNA of

genes in the MHC class II, such as HLA-DRB1, HLA-DMA, and HLA-DMB, and T cell activation pathways, such as IL23A and CD74, were reduced in terms of abundance in the patient with altered chest radiographs (111).

High levels of IL-6 were identified in patients with more severe COVID-19 (112). IL-6 inhibited HLA D-related (HLA-DR) expression (104) and the low expression of HLA-DR on CD14 monocytes is characteristic of sepsis-induced immunoparalysis (113). In patients with pneumonia caused by SARS-CoV-2 when the number of molecules of HLA-DR on CD14 monocytes decrease, severe respiratory failure proceeds, which leads to the idea that high levels of IL-6 mediate the low expression of HLA-DR on CD14 monocytes of patients with severe COVID-19, affecting the antigen presentation to T CD4+ naive cells (114). Interestingly, the IL-6 inhibition with tocilizumab restores the expression of HLA-DR expression and antigen presentation, partially rescuing SARS-CoV-2- associated immune dysregulation (115).

5.2 Viral escape mechanisms

SARS-CoV-2 has different methods of exhausting the immune system, some of which are similar to the methods of SARS-CoV and MERS-CoV, which can provide important information on the evasion pathway of SARS-CoV-2 (116). Both viruses can form a double vesicle outside of the cell that prevents cytosolic PRR recognition to dsRNA (41). Protein M (ORF 4a, ORF 4b, and ORF 5) of MERS-CoV acts as a IFN antagonist (117). The M protein (structural protein of membrane) is present in SARS-CoV-2 so that the virus can inhibit the IFN pathway (105). MERS-CoV can also escape from the immune system by changing the antigen presentation using epigenetic modulation (118), and in the antigen presentation via MHC I/II, it was downregulated when MERS-CoV infected the macrophages or dendritic cells (119). In addition, SARS-CoV contains eight proteins that decrease IFN production; nps 1, nsp 7, nsp 14, nps 15, nps 16, papain-like protease (PLP), ORF 3b, and ORF 6. The nps 14 and nps 16 work together to build an RNA cap similar to that of the host and modify this to evade the immune system. The nps 1 (nonstructural proteins) have three functions; inactivation of the translational machinery, degradation of mRNAs of the cell, and inhibition of phosphorylation of STAT1. PLP blocks the phosphorylation of IRL3, nps 7, and 15 mechanisms, which are still unclear but recognized as possible inhibitors. ORF 3b inhibits IFN-β production mediated by RIG-I and MAVS, but not that which is mediated by TNF-α, and ORF 6 blocks nuclear translocation of the transcription factor STAT1 that interrupts the IFN pathway (120; 121). SARS-CoV-2 presents the capacity to produce nps 1-16 and the same ORF 6, but not ORF 3b (122), thus, this mechanism of exhaustion and the similarity of SARS-CoV-2, SARS-CoV, and MERS can assist in the development of treatment against SARS-CoV-2. Collectively, SARS-CoV-2 induces delayed INF and Th1 responses. The delayed IFN response may also account for the shift of Th1 to Th17 lymphocytes, leading to tissue accumulation of neutrophils and an increase in the neutrophil:lymphocyte ratio in blood (123).

6 Lymphocyte and cell depletion during COVID-19

Lymphocytes constitute protection for an organism by being part of the adaptive immunity and forming a humoral immune response by B lymphocytes or cell mediation by T lymphocytes. Normally, from the identification of the viral epitope by the APC, the T lymphocytes are activated via the TCR, and proliferates and differentiates into Th1 cells, becoming effector T cells acting on cell-mediated immunity for intracellular pathogens. B lymphocytes, on the other hand, will activate through the APC and become Ab-producing cells, resulting in humoral immunity. Both immunities appear around the

third day of infection and act to eliminate the antigen. SARS-CoV-2 appears to dump cellular and humoral responses depleting lymphocytes.

The new infection caused by SARS-CoV-2 reaches this lymphocyte barrier, generating lymphocytopenia through several defense mechanisms. Lymphocytopenia is a common marker presented by patients with COVID-19. A previous study indicated the presence of positive regulation in the mechanisms of autophagy, apoptosis, and p53 in peripheral blood mononuclear cells (PBMCs), mostly lymphocytes and a smaller number of monocytes, resulting in lymphocyte depletion (124). In terms of comparison, the MERS produced by MERS-CoV is capable of generating apoptosis of primary T lymphocytes through the extrinsic and intrinsic pathways, but it cannot replicate within the lymphocytes. SARS-CoV-2 is believed to have a similar mechanism that results in lymphocytopenia.

SARS-CoV-2 infection of lymphocytes is more competent compared to SARS-CoV. Protein S can considerably increase infection even in cells with low expression of hACE2 (125). However, lymphocytic infection is not limited to a single mechanism, as it was discovered that the new virus can infect T cells through the endocytosis pathway mediated by receptors, such as HR1, or through the fusion of membranes through the spike protein and its subunits. The S1 subunit assists in binding to the receptor and the S2 subunit facilitates membrane fusion.

Studies involving flow cytometry to evaluate cell phenotypes in patients with COVID-19 have resulted in markedly reduced total T cell number, B cell, and NK cell cellularity, more intensely in severe cases. Increases of naive T helper cells and decreases in memory T helper cells, both in patients with severe forms of the disease, and reduction of CD4+ T helper cells and CD8+ cytotoxic T cells in all infected individuals, were also demonstrated. In addition, there was an increase of naive T helper cells and a decrease in memory T helper cells. Concerning Tregs, an increase was identified in patients with a mild form of the disease; however, another study attested to a decrease of these cells in all patients with COVID-19, more intensely in severe patients (126; 127; 19). Moreover, the percentage of naive T helper cells ($CD3^{+}CD4^{+}CD45RA^{+}$) increased and memory T helper cells ($CD3^{+}CD4^{+}CD45RO^{+}$) decreased in severe cases, compared to less severe cases (128).

Postmortem examinations of human spleens and lymph nodes collected from six patients with COVID-19 showed distinct features of lymphocytopenia in SARS-CoV-2 infection (129). The histopathological examination of hilar lymph nodes from different patients showed architectural destruction caused by the viral infection to lymph follicles and paracortical areas, and a number of necrotic and apoptotic cells, leading to a significant reduction in total lymphocytes (both T and B cells). SARS-CoV-2 does not seem to infect B and T cells directly, but induces apoptosis since findings in the in situ TUNEL staining showed high apoptotic activity in lymphocytes from infected spleen and lymph nodes. The exact mechanisms that lead to lymphocyte apoptosis remain unknown, but it is believed to be due to the persistence of viral antigens in the lymphocytic tissue, which activates induced cell death through the Fas/FasL signaling pathway. Lastly, lymphocyte apoptosis can also be stimulated by proinflammatory cytokines, such as the macrophage-released IL-6 (129).

Intriguingly, it has been reported that seven patients with confirmed COVID-19, of which five had common variable immune deficiencies (CVIDs), resulting in a lack of B lymphocytes, and two had agammaglobulinemia, resulting in dysfunctional B lymphocytes (130). In patients with agammaglobulinemia, only mild symptoms were observed. The patients with CVID presented a severe form of the disease, requiring multiple pharmacological treatments and mechanical ventilation. This led to speculation on the role of B cells in determining lung inflammatory disorders with need for further confirmation with the acquisition of new data and future studies (130). Moreover, A study with

204 patients with confirmed COVID-19 showed that the number of B cells (CD19+) was significantly lower in the severe group compared to the non-severe group, and significantly higher levels of IgG and complement C3 and lower levels of IgM were reported in severe patients. However, there was no notable decrease in B cells between patients who improved and those who died in the group with severe cases of COVID-19 (131).

Transcriptomic analysis of both bronchoalveolar lavage fluid and PBMCs from patients with COVID-19 revealed that p53-related genes are overexpressed (132). Some viruses, such as the Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus (HIV) use the p53 pathway to arrest cycle cells to favor viral replication (133). The mechanism of p53-induced apoptosis is largely unknown. However, for cells infected with RNA viruses, the cellular stress responses mediated by p53 can trigger apoptosis and senescence (134).

The overall effect of cellular depletion in COVID-19 is the dumping of innate and adaptive immunity favoring viral replication, tissue dissemination, and persistence. An interesting drug approach would be T cell rescue and control of the cytokine release syndrome using IL-6, IL-10, and their receptors as targets of the drug (135).

7 Cytokine storm and inflammation

SARS-CoV-2 exhausts the immune system inhibiting the IFN pathway and causing lymphopenia (41; 136). Most patients with severe COVID-19 exhibit substantially elevated serum levels of pro-inflammatory cytokines, including IL-6, IL-1β, TNF, IL-2, IL-17, G-CSF, GM-CSF, and the chemokines CXCL8, CXCL10, CCL2, CCL3, and CCL7 (137; 138). These chemokines are attractive for neutrophils and monocytes at inflamed tissues, amplifying tissue damage. CCL7 and CXCL10 overproduction is linked to disease severity and fatal outcomes (138). IL-6 is significantly increased in these patients and continues to increase over time, mostly derived from lung-accumulated macrophages and neutrophils, being relatively more elevated in non-survivors than survivors (71; 139; 140). This abnormal cytokine and chemokine production leads to a cytokine storm profile with uncontrolled inflammation (Figure 3 and Figure 4), accounting for ARDS, sepsis and shock, and multiple organ failure due to tissue damage (141; 36; 112; 105).

For SARS-CoV-2 infection, shortly after the destruction of the pneumocystis, a large amount of PAMPs and DAMPs are identified by macrophages through the PRRs (44), which act as early activators of the inflammatory responses. This activation generates, locally, a large release of IL-6, IFNγ, CCL2, and CXCL10 (141; 44) proinflammatory cytokines and chemokines found during acute viral infections (142). Therefore, this type of signaling is responsible for mediating the Th1 response, which culminates in the recruitment of monocytes and lymphocytes to the active site of inflammation (44) and is key to the activation of the specific immune response. However, patients suffering from COVID-19 are committed by lymphopenia, mainly from CD4+ and CD8+ T cells (136; 36; 116; 143), B cells, and NK cells (126). This can lead to a low anti-viral capacity by the immune system, helping the persistence of high viremia titers along with the continued extinction of macrophages and their cytokines. Additionally, the combination of high IL-6 and lower delayed INF production could shift Th1 into a Th17 response, thereby reducing the anti-viral activity efficiency and enhancing tissue damage by neutrophil and macrophage accumulation (144). In a recruitment and activation positive loop, macrophages and neutrophils contribute, in turn, to the cytokine storm and hyperinflammatory state (100).

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Patients with milder COVID-19 had a higher number of Treg cells, along with low serum concentrations of IL-6 (126), possibly organizing regulated and more effective immunological responses. On the other hand, patients with severe COVID-19 had high titers of proinflammatory cytokines, as well as high viral titers (126). This situation, together with the low levels of anti-inflammatory cytokines, decreased the levels of T and B cells (143), along with causing a late Th1 response (145) and dysregulated macrophage response (100) as the main causes for an exacerbated amount of cytokines, contributing to inflammatory deregulation, leading to morbidity and mortality of serious cases of SARS.

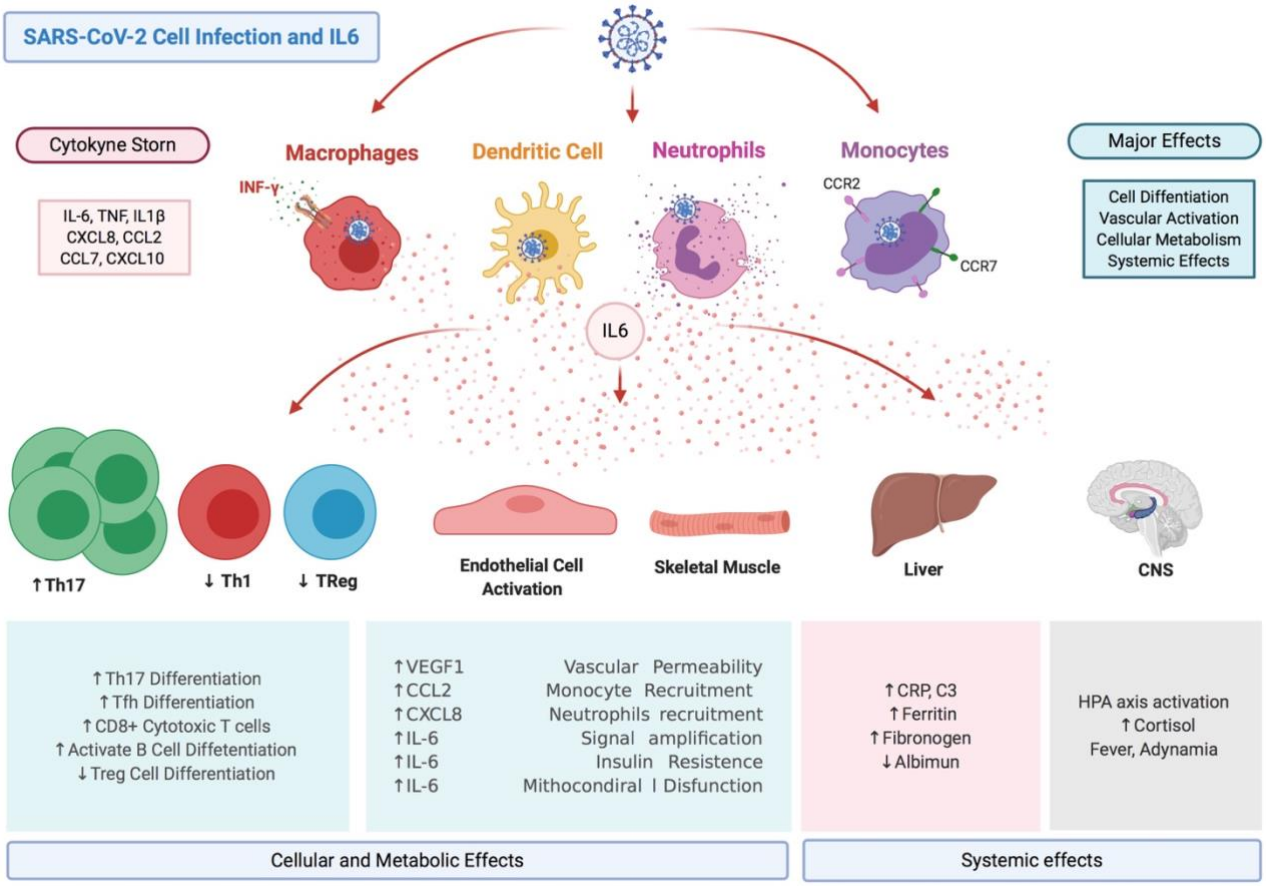

Figure 3. **Cytokine Storm during SARS-CoV-2 Infection**. Pro-inflammatory cytokines and chemokines produced by immune cells are mainly TNF, IL-1β, IL-6 with delayed interferon production. The major clinical and laboratory findings are related to IL6 overproduction.

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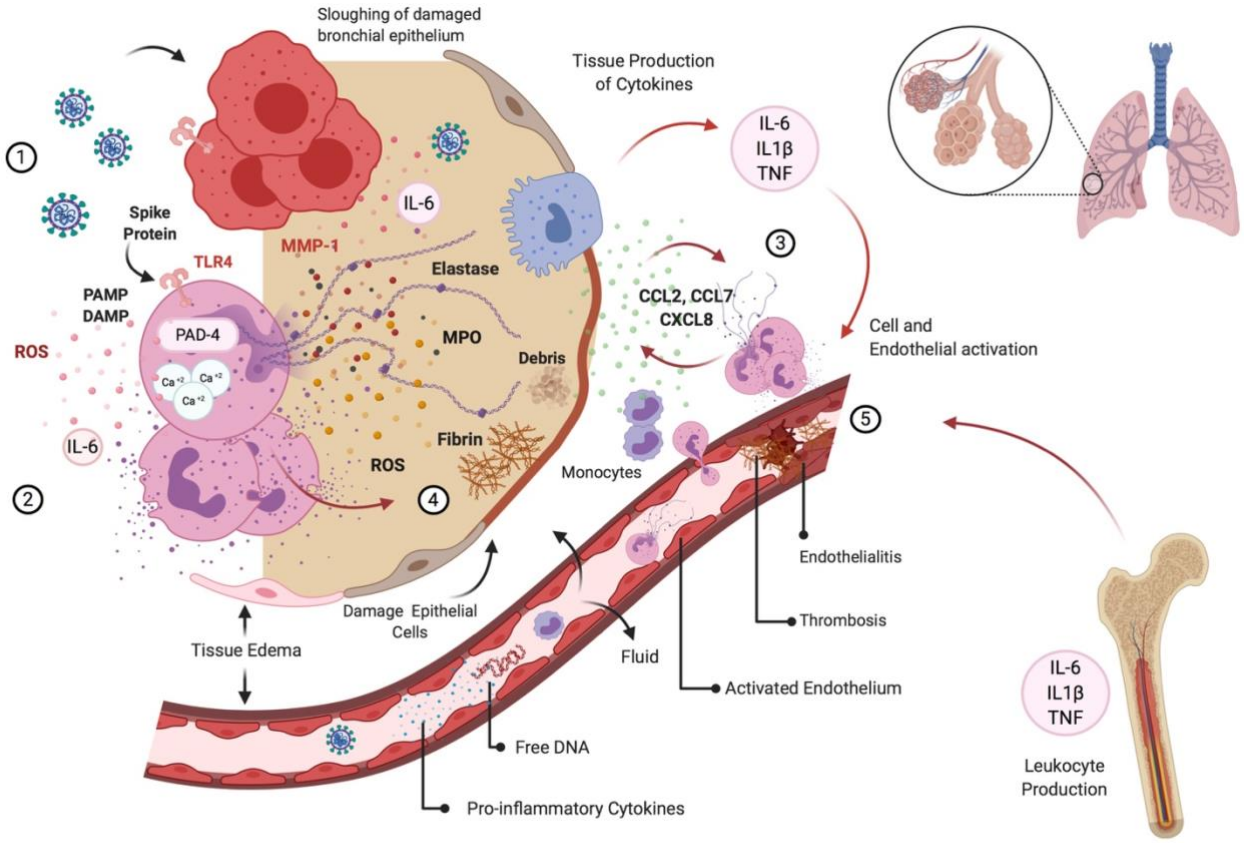

Figure 4. **Tissue Damage by macrophages and neutrophils during ARDS of SARS-CoV-Infection.** SARS-CoV-2 can activate 1) macrophages, 2) neutrophils with NETs formation and secondary secretion of reactive species of oxygen, proteases, pro-inflammatory cytokines, and chemokines. 3) The cytokine storm is a consequence of a state of continuous cell recruitment and activation. 4) Cell debris, protein-rich alveolar fluid, and hyaline membrane formation are tissue hallmarks of acute distress respiratory syndrome of Covid-19. 5) Endothelial activation, fluid leakage, and immune cell tissue homing are consequences of both inflammatory products and SARS-CoV-2. NETs formation is directly engaged in thrombophilia.

8 Immune T cell exhaustion during COVID-19 infection

More than two decades ago, dysfunctional but persistent CD8+ T cells were described during chronic lymphocytic choriomeningitis virus infection (146). In the following years, T cell exhaustion has been evident in humans with chronic infections, such as HIV, hepatitis C virus, and cancer (147). This process of T cell exhaustion is a result of the persistence of antigen stimulation and inflammation in these pathological conditions (148).

Exhausted T cells demonstrate a loss in the effector function, modified metabolism, high and sustained expression of inhibitory receptors, epigenetic and transcriptional profiles as well as the absence of a clear module of quiescence, a transition that memory T cells usually undergo in typical circumstances (149; 150; 151). Although exhausted T cells are not effective in eliminating tumors or pathogens, they have crucial functions, despite suboptimal conditions, in controlling tumor progression and pathogen replication (148). Exhaustion is not limited to CD8+ T cells, this response can also happen in a variety of immune cells, such as CD4+ T cell, natural killer cells and B cells (151).

Naive T cells are activated and differentiated into effector T cells in one or two weeks during acute infections or vaccinations (148). In the ensuing antigen clearance and resolution of the inflammation, most activated T cells die, but a small percentage (5-10%) endures and develop into memory T cells (152). Memory T cells downregulate the program of effector cells and develop into a type of stem cell with a characteristic antigen-independent self-renewal, essentially through the influence of IL-7 and IL-15, and can reactivate effector functions promptly in a secondary infection. It is important to understand that, for an effective memory T cell to occur, it is crucial that the memory T cell differentiation happens in the absence of ongoing antigen stimulation and high levels of persisting inflammation after the effector phase (148).

In the context of the COVID-19 pandemic, it is important to highlight that one of the signs of exhaustion is the overexpression of receptor NKG2A (NK group 2 member A) on NK cells and CD8+ T cells. This receptor is responsible for the regulation of cytotoxicity and inhibition of cytokine production by both cellular types (153). In a recent study analyzing peripheral blood samples of healthy controls and patients with mild and severe infections of SARS-CoV-2, the NKG2A receptor was upregulated in NK cells and cytotoxic lymphocytes (CTLs) in these patients, which is associated with reduced capacity to synthesize functional markers, such as CD107a, IFN-γ, IL-2, granzyme B, and TNF-α. However, the NKG2A+ CTL levels decreased as the infected patients revived. These findings suggest not only the correlation between the expression of NKG2A and CTL functional exhaustion but also establish this receptor as an indicator of efficient control of the disease (154).

Other functional exhaustion markers are the receptors programmed cell death protein 1 (PD-1) and T-cel Ig- and mucin-domain-containing molecule-3 (Tim-3). The former is involved in limiting immune-mediated damage during infection (155), and the latter can suppress IFN- γ production on the cells in which it is expressed (156). According to the findings of cellular functional exhaustion, another study measuring T cell exhaustion markers from the blood samples of 14 COVID-19 cases showed infected patients had higher percentages of CD8+ and CD4+ T cells positive for PD-1. In addition, it was detected in three patients of the study a progressive increase of levels of PD-1+ and Tim-3+ on CD8+ T cells following the disease course through worsening symptoms. In the same study, the severe group of patients had some particularities, including lower frequency of multifunctional (positive for at least two cytokines) CD4+ T cells, high levels of non-functional (IFN-γ-TNF-α-IL-2-) CD4+ T cells, and a decreased frequency of non-exhausted (PD-1-CTLA-4-TIGIT-) CD8+ T cells compared to the healthy and mild groups. These outcomes propose that this exhaustion is capable of damaging the cellular immune response in SARS-CoV-2, making individuals susceptible to the severe form of COVID-19 (157).

These exhausted T cells are a unique immune cell phenotype and are the targets of an assortment of immunotherapies, e.g., by targeting PD-1, and could eventually become pivotal in a myriad of clinical opportunities (151). Furthermore, many studies have established that T cell exhaustion can be reversible in cancer or chronic viral infections, for example, by blocking the PD-1 pathway with an important clinical response (158; 156). These findings suggest a variety of clinical opportunities in the use of immunotherapy to reverse T cell exhaustion, which could be an alternative for the treatment of COVID-19 (148; 154).

9 Antibody response to SARS-CoV-2 in patients with COVID-19

When it comes to Ab testing in patients with the new SARS-CoV-2 onset, one of the main concerns is how the levels of total Abs, IgG, and IgM behave through the course of the disease. The analysis of 173 patients' serum demonstrated that the rate of seroconversion is high, showing total anti-SARS-

CoV of 93.1%, IgM of 82.7%, and IgG of 64.7%. These changes were observed on day 11, 12, and 14, respectively. In the course of the first two weeks, Ab levels and seroconversion rates increased fast, as the cumulative seropositive rate hit 50% on day 11 and 100% by the day 39 of disease onset (159). However, another study found that 100% of patients were positive to virus-specific IgG around 17-19 days after symptom onset. Interestingly, for IgM, the peak took more days to occur and was a little lower, reaching 94.1% by days 20-22. No association between plateau IgG levels and the clinical characteristics of the patients was found. A small number of patients initially presented as seronegative; however, by the end of the third week all patients achieved seroconversion of IgG or IgM (160).

Regarding Ab levels at the late stage of the infection and their association with virus clearance, it was shown, in agreement with data presented previously, that IgG was first detected within an average of 15 days. The IgM begins to decline and reaches lower levels by week five, and almost disappears by week seven, whereas IgG persists beyond seven weeks (161). Most importantly, it has also been found that SARS-CoV-2 can coexist with specific Abs for 36-50 days, which leads to a new question: how can the virus circulate in the presence of these for such a long time? Additionally, it enhances the importance of innate and adaptative immunity in the resolution of COVID-19 (162).

Severe cases of COVID-19 are more frequently found in patients with high levels of IgG (51.8%) compared to those with lower levels (32.3%). With these findings, it is proposed that the Ab response might be linked to secondary organ damage, other than the antiviral activity (163). Additionally, the association between IgG levels and the severity of symptoms, a positive correlation with Ab titers for two weeks after onset, was found. It is suggested that high Ab levels alone might be a risk factor, separate to other known risk factors, such as the presence of comorbidities, being elderly, and being male (159). The combined detection of specific IgM and IgG against viral nucleotides (N-IgM, N-IgG) and spike proteins (S-IgM, S-IgG) can be used as an efficient method of early detection of SARS-CoV-2 infection since the seropositive rate of these four Abs combined reaches 75% after the first week. By the third week, the seropositive rates of N-IgG and S-IgG hits 100% (164). Thereafter, the most sensitive and earliest serological marker is total Abs, levels of which begin to increase from the second week of symptom onset (165).

The impact of these specific Abs in predicting a patient´s prognosis is another point debated in the same study. After analyzing blood samples from 38 patients (both ICU and non-ICU patients), differences were observed in the IgM to IgG class-switch between these two groups that might reflect distinct clinical outcomes. The ICU group presented an elevated production of N-IgM and N-IgG, but lower levels of S-IgG compared to non-ICU patients. High N-IgG levels are believed to indicate more severe illness than S-IgG levels, and, thus, a worse prognosis. On the other hand, non-ICU patients switched from IgM to IgG more quickly and showed a positive correlation between the increase of S-IgG and decrease of C-Reactive Protein (CRP) (a protein that marks systemic inflammation) (164).

The neutralizing Ab (NAb) response, especially spike binding Ab levels (targeting RBD, and subunits S1 and S2) has been evaluated through the plasma analysis of 175 recovered patients of SARS-CoV-2. Interestingly, around 30% of these patients generated a very low level of NAb titles, with one third of them being below the limit of detection and not developing NAbs afterward. Elderly patients, on the other hand, had higher levels of NAbs and these were negatively correlated with lymphocyte counts and positively correlated with blood CRP levels (166).

Although the RT-PCR test has been used as a standard method to diagnose SARS-CoV-2 infection globally, the reports of false-negative cases are afflicting and epidemiologically threatening. A Chinese study analyzed 610 patients diagnosed with COVID-19 by the recommended protocol. In the first test,

at least 63.0% (384) of the patients returned negative PCR results and 57 were dubiously positive (9.3%). In addition, 18 patients had a positive result after two consecutive negative results, showing an oscillating pattern (167). Therefore, the search for a better understanding of more specific methods of diagnosis has grown. Therefore, the measurement of virus-specific total Abs, as well as IgG and IgM, represents an effective supplementary method for COVID-19 diagnosis, since it also shows an intimate relationship to the clinical course and prognosis (163; 159; 168). A recent study used a colloidal gold-based immunochromatographic (ICG) strip targeting viral IgM or IgG Abs in confirmed patients, as well as those with negative RT-PCR. In accordance with the data presented earlier, it was found that positive rates of both IgM and IgG increase with the development of the disease. The ICG showed that the IgM positive rate went from 11.1% to 78.6% and 74.2% considered the early (1-7 days after onset), intermediate (8-14 days), and late (over 15 days) stages, respectively. The IgG positive results were 3.6% in early, 57.1% in intermediate, and 96.8% in late stages. Therefore, combining both IgM and IgG rates has been proven to enhance the sensitivity of the ICG assay (168). Although sensitive detection methods have been developed, some concerns regarding the validation of the majority of available testes have increased, especially considering the false-negative tests (169). The inaccuracy of non-validated and low-quality SARS-CoV-2 detection methods have two main consequences (1) the false-positive that labels persons erroneously as having COVID-19 with unnecessary quarantine and (2) the false-negative that is especially harmful due to misidentification of infected persons. Therefore, validation should be addressed for the available and developing tests as a way to improve public health measures of epidemic control.

9.1 Does SARS-CoV-2 induce immunity?

One of the biggest questions surrounding SARS-CoV-2 is whether the immune system develops long-lasting immune responses after infection. As it has only been a few months since the spread of the virus, there is not currently sufficient evidence to answer this question, and further studies are needed. One of the main concerns is that other CoVs, such as SARS-CoV, showed a relatively low extent of immune response after infection (170). The levels of IgG and neutralizing Abs began to decrease after 16 months of disease onset, with IgG being undetectable for 25.8% and 16.1% of individuals at 36 months. Most patients (86%) persisted with Abs against MERS-CoV, including neutralizing Abs, after 34 months of infection (171). Mathematical modeling performed to understand the dynamics of SARS-CoV-2 through a post-pandemic period predicted a short duration of immunity (172). Therefore, the immune protection against these viruses may wane over time and immune protection after re-exposure is uncertain.

Remarkably, patients who recently recovered from the infection displayed serum neutralizing activities in a pseudotype entry assay, indicating mounted IgG and IgM responses to SARS-CoV-2 proteins, especially nucleocapsid protein and RBD of S protein, suggesting that the IgG amounts could be sustained for at least two weeks after discharge. Additionally, this suggests that most patients post-discharge have serum-neutralizing SARS-CoV-2 infections (173). It has also been shown that IgG specific to SARS-CoV-2 trimeric spike protein titers raised over the first three weeks from symptom onset, and fell during the second month after symptom onset but remained detectable (174).

RBD-specific monoclonal Abs (mAbs) derived from single B cells of eight SARS-CoV-2-infected patients showed potent neutralizing activity against pseudoviruses and lived SARS-CoV-2. It has also been demonstrated that none of the SARS-CoV-2 Abs nor the infected plasma cross-reacted with RBDs from either SARS-CoV or MERS-CoV, suggesting that the Ab response to RBDs is viral and species-specific (175). Over 1,100 isolated S-protein specific-memory B cells derived from seven COVID-19 convalescent donors showed that even though the frequency of S-protein specific-memory B cells is

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highly variable among donors, SARS-CoV-2-specific mAbs can be successfully isolated from most of them. Additionally, 17 of the mAbs were able to effectively neutralize SARS-CoV-2 with high potency when tested in vitro (176).

A recent study from China has demonstrated the lack of reinfection in rhesus monkeys after being submitted to a rechallenge infection of SARS-CoV-2 28 days post-initial challenge. The levels of Abs against SARS-CoV-2 were significantly higher 14 days post-rechallenge compared to 28 days after the initial infection. Additionally, there were no significant pathological findings that could be attached to possible reinfection. Although the results of this study were promising, there is a lack of evidence that the effects of a rechallenge would remain the same after an interval longer than six months (177). Moreover, emerging data comparing asymptomatic and symptomatic patients adjusting for individual characteristics are showing that IgG levels and neutralizing Abs start to decrease 2-3 months after the infection begins. These data indicate the risks of an "immune passport", relaxing public health measures, such as social distancing and widespread immune tests, and high-risk groups' isolation (18).

10 Thromboembolism, cell activation, and tissue damage

Beyond the immunological clues discussed in this paper for COVID-19, the underlying tissue damage effector mechanisms rely on (Figure 5): (1) SARS-CoV-2 target cell invasion, (2) depletion of ACE2 protein from the cell surface (71), (3) endothelial activation and thrombophilia leading to vascular occlusion and hypoxic-ischemic injuries, (4) immune cell-derived products, such as ROS, MPO, and elastase, from activated neutrophils and macrophages (178), (5) direct tissue damage by cytokine overproduction, and (6) complement activation microvascular injury (179).

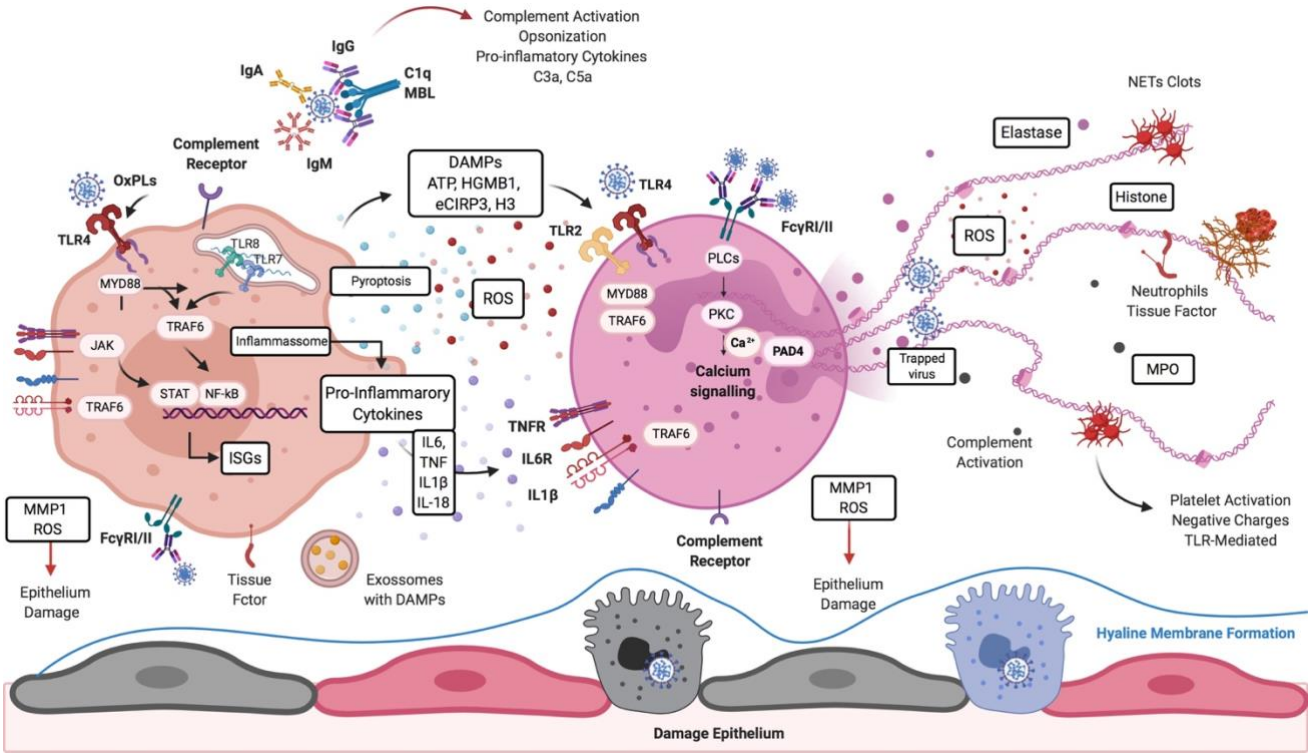

Figure 5. Macrophages and Neutrophils activation pathways in Covid-19. Multiple theoretical pathways of macrophages and neutrophils activation after SARS-CoV-2 recognition.

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10.1 Macrophage and systemic inflammation

Patients with severe COVID-19 pneumonia present features shared with other conditions of hyper-inflammation, such as macrophage activation syndrome (MAS), which is characterized by activation and expansion of T lymphocytes and hemophagocytic macrophages, and shows increased levels of numerous proinflammatory cytokines, high levels of C-reactive protein, increasing levels of serum D-dimers, cytopenias involving other cell lines, hyperferritinemia, liver dysfunction, coagulopathy, decreasing serum fibrinogen, and increasing triglyceride levels (180). These findings have also been found in severe cases of SARS-CoV-2, mainly cytokine storms with high levels of IL-2, IL-7, IL-10, GSCF, CXCL10, CCL2, CCL3, TNF-α, and IL-6 (23; 181), and T cell depletion in patients with SARS-CoV-2.

The role of SARS-CoV-2 in the lymphopenia and suppression of IFN production, with the high production of other pro-inflammatory cytokines, mainly IL6, and the presence of macrophages with high inflammatory and chemokine production capability (182), all create a constant state of systemic inflammation that activates more macrophages, causing hemophagocytosis, leading to multi-organ dysfunction and poor outcomes. Other features of MAS are not usually present in patients with COVID-19, such as hepatomegaly and splenomegaly, which may indicate a higher state of inflammation in the lungs.

An autopsy series correlating clinical and laboratory findings studied the reticuloendothelial organs (spleen, liver, and multiple pulmonary hilar/mediastinal lymph nodes) of four patients who died of COVID-19. Three cases had histological evidence of hemophagocytosis within pulmonary hilar/mediastinal lymph nodes, and one case showed hemophagocytosis in the spleen, but none showed hemophagocytosis in the liver or bone marrow. It was also found that lymphophagocytosis was the predominant form of hemophagocytosis. The clinical and laboratory data of one patient showed diagnostic features of hemophagocytic lymphohistiocytosis (HLH) with an H-score of 217, while a second patient was likely HLH with a partial H-score of 145 due to missing triglyceride levels (183).

10.2 Neutrophils and SARS-CoV-2-induced neutrophil-derived extracellular trap (NET) formation

The increased number of neutrophils is related to the severity of the respiratory syndrome and adverse outcomes in COVID-19 (9). NET formation is a key factor in tissue damage and organ failure during sepsis (184). NETs are networks of fibers composed of nuclear chromatin, nuclear histones, and granular anti-antimicrobial proteins. The NETs are triggered by activated PRR or chemokines, followed by ROS production and calcium mobilization (185). During NET formation, large amounts of ROS, myeloperoxidase, and elastase were released, with the aim to trap and kill pathogens (186). However, cumulative evidence has shown that NETs are largely involved in disease progression and pathogenesis (187; 188). Moreover, NETosis is linked to cytokine overproduction (21; 100), microthrombosis (186), acute lung injury (ALI), and ARDS (187; 189). Serum samples from COVID-19 patients reveal elevated levels of cell-free DNA, and NETs formation-specific markers, such as myeloperoxidase-DNA (MPO-DNA) and citrullinated histone H3 (Cit-H3) (190), demonstrated that the NETs were increased in plasma, tracheal aspirate, and lung tissue from patients with COVID-19 (178). SARS-CoV-2 induces the NET formation in a PAD-4-dependent manner and promotes injury to the epithelial lung cells in vitro. Additionally, the inhibitory agents of NET synthesis or fragmentation, such as CI-Amidine (PAD-4 Inhibitor), can halt the spontaneous NET formation by neutrophils from COVID-19 patients (178). It is interesting to note that NET formation and cell-free DNA are linked to anti-DNA Ab circulation and autoimmunity (191). Therefore, SARS-CoV-2-triggered autoimmunity in susceptible individuals should be investigated. Further cohort studies are

necessary to clarify the role of circulating NETs as a predictive biomarker and to what extend NETs should be explored as a therapeutic target (192; 186).

10.3 COVID-19 and hyperferritinemia

The severe form of COVID-19 is believed to be a part of the "hyperferritinemia syndrome" group since it presents features of high serum ferritin and systemic hyper-inflammation. The other four entities comprehended under this term include MAS, adult-onset Still's disease (AOSD), catastrophic anti-phospholipid syndrome (CAPS), and septic shock. Since these conditions share a common pathogenic background, COVID-19 patients could benefit from a similar therapeutic approach, including anti-inflammatory and immunomodulatory agents (193). In a study from China conducted with 21 severe COVID-19 patients, tocilizumab presented itself as an effective treatment, improving symptoms, and repressing clinical deterioration (194).

In order to understand the mechanisms of serum ferritin secretion and its relationship with inflammation, an experiment analyzing mouse serum ferritin secretion concluded that serum ferritin is not only a result of a cellular leak, but also actively secreted by macrophages through a nonclassical secretion process involving secretory lysosomes. After lysosomal processing, ferritin can be further degraded or secreted by cells that have a lysosomal secretory pathway, such as cells from the hematopoietic lineage (including macrophages) or renal tubular cells. Genetic findings with macrophages and the iron regulatory protein 2 (IRP2), and the fact that splenectomy caused decreased serum ferritin concentrations in mice, supported that macrophages contribute significantly to serum ferritin. Further evidence includes demonstrations that primary cultures of bone marrow-derived macrophages secrete ferritin into their culture medium. The results obtained seem to explain that serum ferritin is elevated in inflammation when increased hepcidin levels inhibit iron recycling from macrophages (195). In AOSD, high ferritin serum levels and findings of ferritin expression in the lymph node B area have been described, suggesting that macrophage activation might be related to hyperferritinemia (196).

A study aiming to evaluate clinical characteristics of infection markers in severe and very severe patients with COVID-19 showed that both groups exhibited increased serum ferritin levels, but the serum ferritin in the very severe COVID-19 group was significantly higher (1006.16 ng/ml) than that of the severe COVID-19 group (291.13 ng/ml). This increase in ferritin levels might be related to severe secondary bacterial infection in COVID-19 and a poor prognosis (197).

10.4 Role of HGMB-1 and TF in thrombotic events

High mobility group box 1 protein (HMGB-1) is an endogenous DAMP protein that can induce inflammation. HMGB-1 can be produced and released by damaged or dying cells and is involved in the innate immunity system. A study from 2015 sought to investigate platelet-derived HMGB1 with transgenic mice with platelet specific HMBG-1 ablation. It was reinforced that platelets store and express HMGB1 on their surfaces after activation and mediate platelet aggregation and thrombosis. It was also shown that the prothrombotic effect was mediated via platelet TLR-4 (198).

HMGB1-driven inflammation leads to intimal hyperplasia in arterial injury. It was identified that HMGB1 and TLR4 regulate cell migration, monocyte trafficking, and inflammatory mediator and growth factor production (199). High levels of HGMB1 in systemic circulation can lead to the development of DIC. Studies on the prothrombotic effect of this molecule show an intimate relationship with tissue factor (TF). The expression of TF on monocytes is increased by HGMB1 stimulation (200), as well as in vascular endothelial cells and macrophages. HMBG1 can induce TF

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expression through TLR4, TLR2, and RAGE. The activation of transcription factors was also described (201).

Considering the high number of reported cases on thrombosis related to COVID-19, a possible pathological mechanism is an association with HMGB1. A similar hypothesis was proposed for SARS-CoV in 2004. In this case, HMGB1 was related to lung injury and its release from endothelial alveolar damaged cells and innate immunity cells (such as macrophages and monocytes) (202). Due to its relationship to various SARS-CoV-2 symptoms and mortality, scientists point to HMGB1 as a therapeutic target for COVID-19 (203; 204).

10.5 The role of the ACE2 receptor at the endothelium and in thrombosis

ACE2 is widely expressed in the endothelium of blood vessels. This enzyme, along with Ang-(1-7), are able to inhibit early atherosclerotic lesion formation by protecting the physiological endothelium function and inhibiting the inflammatory response (205). Ang-(1-7) acts as an endogenous ligand for the G protein-coupled Mas receptor, which is highly expressed in the cardiovascular system. Moreover, the ACE2/Ang1-7/Mas pathway has anti-proliferative, anti-inflammatory, and anti-oxidative stress properties (206; 207; 208; 209; 210; 211).

On the other hand, the high expression of ACE2 by the endothelium contributes to infection by SARS-CoV-2. Therefore, it is possible that this infection leads to microvascular inflammation, microvascular dysfunction, and the release of pro-inflammatory cytokines, especially the cytokine storm already mentioned in this article. In this context, a series of cardiovascular complications, such as myocardial infarction, coagulation activation, and thromboembolic events, are possible, as already noted in the case reports of patients with COVID-19 (212; 213; 23; 7; 214; 215; 216).

Analyzing the current evidence, it can be hypothesized that the infection of SARS-CoV-2 might have an effect on the endothelium, leading to thrombosis by blocking the anti-inflammatory effects of ACE2. Virus-induced changes to the access to binding sites in the receptor as well as the inflammatory reaction may have a role in endothelium disfunction. However, further studies are necessary to clarify this topic.

10.6 Thrombosis and tissue oxygen delivery

The storm of proinflammatory cytokines from the SARS-CoV-2 infection contributes to plaque rupture, inducting procoagulant factors and hemodynamic changes, elevating the D-dimer levels and the prothrombotic state can lead to, as with other CoVs, vascular endothelial damage, causing disseminated intravascular coagulation (DIC), such as an increase of the risk of intracranial hemorrhage, leading to both arterial and venous thromboembolism and ischemia (217; 218; 219; 220). Being well-stablished, the association between SARS-CoV-2 infections and the risk of developing thrombosis is more specific to severe COVID-19 cases. The inflammation and injury of the myocardium predisposes thrombogenesis and also the risk of stroke (219).

The coagulation cascade is activated by an inflammatory response through polyphosphates, derived from microorganisms. These polyphosphates activate platelets, mast cells, and coagulation factor 12, intensifying the intrinsic coagulation response (221). In addition, other pathways contribute to the positive regulation of coagulation (222). Although extracellular NETs are present in thrombi, the individual NET components of cell-free DNA and histones activate the contact pathway and enhance other prothrombotic pathways, resulting in thrombin generation (223).

The International Society of Thrombosis and Haemostasis (ISTH) has developed and validated sepsis-induced coagulopathy (SIC), a type that is less severe and occurs earlier in patients than DIC (224). However, during SARS-CoV-2 infection, SIC can progress to DIC, and the reasons are still unclear. As discussed, the development of coagulation test abnormalities seen in SARS-CoV-2-infected patients is likely a result of the profound inflammatory response (217). In patients with sepsis-induced coagulopathy, the importance of evolution from adaptive hemostasis to pathologically-induced DIC with multiorgan failure continues to be evaluated.

10.7 Pathophysiology of thrombosis related to COVID-19

Studies have shown that SARS-CoV-2 induces a coagulopathy, namely DIC, with a high risk of venous thromboembolism (225). The mechanisms of DIC are based on inflammatory tissue factor (dependent coagulation cytokine, inefficient control of anticoagulant pathways, and plasminogen activator inhibitor 1)-mediated suppression of fibrinolysis that causes endothelial dysfunction and microvascular thrombosis (226). Patients in severe or fatal cases of COVID-19 present thrombocytopenia, elevated D-dimer levels, and prothrombin time prolongation, which suggests hyperfibrinolysis action (227). Plasmin has a fibrinolytic function and an important role in enhancing the virulence and pathogenicity of viruses containing furin in the envelope proteins. Plasmin activates the S protein of SARS-CoV and is also present in SARS-CoV-2, suggesting that the mechanism is similar (228; 229). Plasmin is elevated in patients with comorbidities, such as DM I or II, cardiovascular disease, hypertension, and cancer, which strengthens the link between elevated plasmin levels and worse outcomes (230).

During COVID-19 infection, changes in coagulability patterns may also be altered because patients affected by critical stages of the disease have manifestations compatible with sepsis (231). Sepsis and the systemic inflammatory response syndrome are major causes of DIC (232). This correlation exists because the severe inflammatory state secondary to infection leads to homeostatic disharmony (232). The DIC results from the activation of endothelial cells and monocytes by the cytokines released during tissue injury, cytokine storm, and together with the expression of tissue factor, Willebrand factor (233), factor VII, and fibrinogen (232). Another line of thought is that the origin of DIC in COVID-19 patients is based on a decrease in urokinase-type plasminogen activator (u-PA), plasmin rescue for SARS-CoV-2 protein S cleavage (inefficient control of anticoagulant pathway), and increases in plasminogen activator inhibitor 1 (PAI-1) and α2 antiplasmin (α2-AP) (234). Additionally, the cytokine storm and CID seem to maintain a feedback relationship; some cytokines present in the cytokine storm increase tissue factor expression storm (TNF, IL-1β, IL-6, CXCL8, IFN-γ, and the chemokine MCP-1) this favors hyperfibrinolysis, and in the same way, thrombin can further augment inflammation via proteinase-activated-receptors (235; 236; 237). Circulating microvesicles found in septic patients can also contribute to the state of hypercoagulability in patients affected with COVID-19 (232).

Therefore, disseminated intravascular coagulation causes venous thromboembolism, acute pulmonary embolism, deep-vein thrombosis, ischemic stroke, myocardial infarction, and systemic arterial embolism. Many studies seek the treatment of COVID-19 by focusing on the coagulation pathway; however, a general treatment has not been created yet, showing the complexity of this infection, and that more research must be done (238).

11 Conflict of Interest

The authors declare no conflict of interest

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