COVID-19 Immunobiology: Lessons Learned, New Questions Arise

Aimilios Kaklamanos\textsuperscript{1,2}, Konstantinos Belogiannis\textsuperscript{3}, Panagiotis Skendros\textsuperscript{4}, Vassilis G. Gorgoulis\textsuperscript{3,5,6,7}, Panayiotis G. Vlachoyiannopoulos\textsuperscript{1,2†}, and Athanasios G. Tzioufas\textsuperscript{1,2*†}

\textsuperscript{1} Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, \textsuperscript{2} Institute for Autoimmune Systemic and Neurological Diseases, Athens, Greece, \textsuperscript{3} Molecular Carcinogenesis Group, Department of Histology and Embryology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, \textsuperscript{4} First Department of Internal Medicine and Laboratory of Molecular Hematology, University Hospital of Alexandroupolis, Democritus University of Thrace, Alexandroupolis, Greece, \textsuperscript{5} Faculty Institute for Cancer Sciences, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, United Kingdom, \textsuperscript{6} Basic Research Center, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece, \textsuperscript{7} Center for New Biotechnologies and Precision Medicine, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

There is strong evidence that COVID-19 pathophysiology is mainly driven by a spatiotemporal immune deregulation. Both its phenotypic heterogeneity, spanning from asymptomatic to severe disease/death, and its associated mortality, are dictated by and linked to maladaptive innate and adaptive immune responses against SARS-CoV-2, the etiologic factor of the disease. Deregulated interferon and cytokine responses, with the contribution of immune and cellular stress-response mediators (like cellular senescence or uncontrolled inflammatory cell death), result in innate and adaptive immune system malfunction, endothelial activation and inflammation (endothelitis), as well as immunothrombosis (with enhanced platelet activation, NET production/release and complement hyper-activation). All these factors play key roles in the development of severe COVID-19. Interestingly, another consequence of this immune deregulation, is the production of autoantibodies and the subsequent development of autoimmune phenomena observed in some COVID-19 patients with severe disease. These new aspects of the disease that are now emerging (like autoimmunity and cellular senescence), could offer us new opportunities in the field of disease prevention and treatment. Simultaneously, lessons already learned from the immunobiology of COVID-19 could offer new insights, not only for this disease, but also for a variety of chronic inflammatory responses observed in autoimmune and (auto)inflammatory diseases.

Keywords: SARS-CoV-2, COVID-19, immune deregulation, immunothrombosis, senescence, endothelitis, autoimmunity

INTRODUCTION

Coronavirus Disease-2019 (COVID-19) has costed millions of lives worldwide. Since the beginning of the pandemic, the scientific community has joined forces to understand the pathophysiology of the disease, aiming to develop effective preventive and therapeutic measures. The development of many vaccines complementing social and personal protective measures (distancing, masks, hand
SARS-COV-2: FROM INHALATION TO COVID-19

Viral Effects on the Respiratory System
SARS-CoV-2 is responsible for the so-called COVID-19, an airborne disease affecting initially the respiratory tract and eventually leading to a systemic disorder (1) (Table 1) (2–12). Through inhalation of air droplets and/or direct virus inoculation on mucosal surfaces, this novel viral strain can infect specific cells, of both the upper and lower respiratory tract, particularly those expressing high numbers of the Angiotensin Converting Enzyme-2 (ACE-2) receptor (Figure 1) (14–18). The ciliated epithelial cells appear particularly sensitive to this virus. Moving along the airways towards the lower parts of the respiratory tract, ACE-2 expression levels drop steadily, resulting in lower infection rates of type II pneumocytes (19). Ciliated epithelial cells and type II pneumocytes along with the resident lung macrophages -both alveolar and interstitial types- and vascular endothelial cells, are the main cellular targets for SARS-CoV-2 in the respiratory system (20, 21). Beyond the respiratory system, ACE2 has a ubiquitous expression in other human tissues and organs including the heart, kidney, intestine, vessels, liver, central nervous system, eyes etc. (22), and therefore, SARS-CoV-2 can potentially invade many different cells/tissues. Following the endocytosis of SARS-CoV-2 coupled with its receptor ACE2, the number of available molecules of ACE2 on cell membrane is reduced (20). As a result, the circulating angiotensin II is not degraded to angiotensin1–7 and the consequences of this phenomenon are depicted in Figure 2 (20, 23–25, 27–30).

In parallel, viral infection of host cells activates both the innate and adaptive arms of the immune system, mounting an immune response against SARS-CoV-2. Robust aberrant cellular alterations, including cellular senescence (31), apoptosis, with emphasis in the inflammatory subtypes thereof [pyroptosis (32), necroptosis (33), PANoptosis (34)] and NETosis are implicated in the disease course and severity.

TABLE 1 | COVID-19 is a systemic disease affecting many different organs/systems.

| Systemic symptoms (2) | Mild: fever, malaise, fatigue, cough, dyspnea, chest pain, nasal congestion, and sore throat |
|-----------------------|------------------------------------------------------------------------------------------|
|                       | Severe: pneumonia, pulmonary edema, acute respiratory failure, ARDS, sepsis, multiorgan failure, shock, and death |
| cardiovascular system (3–5) | myocardial injury, myocarditis, acute coronary syndrome, heart failure, arrhythmia, sudden cardiac death, or thrombosis with subsequent thromboembolic events like ischemia, deep venous thrombosis and pulmonary embolism |
| gastrointestinal system (6, 7) | loss of appetite, diarrhea, nausea, vomiting and abdominal pain, elevated liver enzymes (bilirubin, AST, ALT), while the virus can be found in stool samples |
| kidneys (5) | acute kidney injury |
| nervous system (9) | headache, dizziness, anosmia, ageusia, encephalopathy, encephalitis, ischemic stroke, neuroinflammation or rarely immune-mediated complications such as ADEM, acute necrotizing hemorrhagic encephalopathy or Guillain-Barre syndrome |
| skin (10) | maculopapular, morbilliform, urticarial, vesicular, chilblain-like, petechiae/purpura and livedoid, or maculo-papular, or erythema multiforme-like, or diffuse erythodermic rash associated with a hyperinflammatory Kawasaki-like syndrome in children |
| muscular system (11) | myopathy |
| hematologic parameters (12) | elevated CRP - LDH - ferritin - CK levels, decreased albumin and elevated levels of various inflammatory cytokines like TNFα and IL-6 |

ADEM, acute disseminated encephalomyelitis; ARDS, acute respiratory distress syndrome; ALT, alanine aminotransferase; AST, aspartic aminotransferase; CK, creatine kinase; CRP, C-reactive protein; LDH, lactate dehydrogenase.

MM and severe refer to the severity of systemic COVID-19 symptoms. So it is “MM symptoms” and “severe symptoms”.

Innate Immune Response Against SARS-CoV-2
Innate Immunity, which is the first line of defence in response to infection, is initiated by Pattern Recognition Receptors (PRRs)-mediated identification of Damage-Associated Molecular Patterns (DAMPs) and Pathogen-Associated Molecular Patterns (PAMPs). The former are released by the infected cell and the latter are virus-associated molecules, such as ssRNA, acting as danger signals, which eventually activate the immune system. More specifically, as depicted in Figure 3 (35, 36), Toll-like receptors (TLRs) bind their ligands, and subsequently activate cytosolic RLRs and NLRs (37–39), leading to the production of interferons, cytokines and chemokines.

The earliest innate antiviral events, occurring within hours post infection, are Type I and Type III Interferon (IFN) responses, that act as barriers to viral replication and
dissemination. Type I interferons can directly inhibit the viral replication and enhance both innate and adaptive immunity, via various mechanisms, including: 1) MX1-GTPase polymerization, 2) IFN-inducible dsRNA-dependent protein kinase R (PKR), 3) 2'-5'-oligoadenylatesynthetase (OAS), 4) IFN-induced transmembrane protein (IFITMs), 5) APOBEC1 and the TRIM family of molecules (40). The main cellular source of type I IFNs in response to SARS-CoV-2, appears to be the plasmacytoid Dendritic Cells (pDCs), activated via the TLR7, as is the case for other coronaviruses (41).

These pathways have attracted the attention of current research since coronaviruses (CoVs) possess an intrinsic capacity to interfere with various IFN pathway-related molecules. Indeed, the implementation of specific structural and non-structural proteins has revealed that SARS-CoV-2 induces diminished or delayed Type I Interferon responses (42–44),
thus generating, early in the disease course, a replication permissive microenvironment. This phenomenon probably explains the high viral loads upon the onset of disease symptoms seen in COVID-19 patients (41), the unusually long viral incubation time and the high rates of asymptomatic or pauci-symptomatic patients (44). The mechanisms utilized by the coronaviruses to evade the initial immune recognition and response via IFNs can be divided into three categories: (1) avoidance of the recognition by the PRRs, (2) suppression of the transcription of interferons, and (3) the suppression of IFN signaling by the viral proteins through inhibition of IFNAR signaling or autoantibodies against IFN pathway elements that neutralize IFN actions. The precise mechanisms used by SARS-CoV-2 are yet unknown, but their majority is expected to share the same basis among different coronaviruses (45) as shown in Figure 4 (35, 41, 46). In addition to these mechanisms, SARS-CoV-2 uses its own capping machinery (nsp10, nsp13, nsp16), to generate 2-o-methyltransferase caps. These RNA caps are indistinguishable from cellular mRNA caps, therefore, the RNA detection and degradation by the type I IFN-inducible proteins, MDA-5 and IFIT, are circumvented. Furthermore, SARS-CoV-2 uses replicase-transcriptase complexes that protect the virus during the maturation process. Moreover, corona viruses use glycans and/or other post-translational modifications to mask the immunogenic viral protein epitopes (35). SARS-CoV-2 can also infect antigen-presenting cells (APCs), inducing epigenetic changes that cause an HLA-II downregulation, as described in both monocytes/macrophages, and B cells (47), probably via an IL-6-dependent pathway (48). Furthermore, the blockade of interferon signaling limits the Interferon-Stimulated Genes (ISGs) expression in APCs, including the cytokine-induced MHC-II expression. Moreover, SARS-CoV-2 protein Nsp5 interacts with the epigenetic regulator Histone Deacetylase 2 (HDAC2), which controls the MHC-II expression and cytokine production (49, 50). Finally, ORF8 of SARS-CoV-2 can directly bind to MHC-I molecules in the ER, redirecting...
Cytokines of inflammation (TNF, IL-1, IL-6), Chemokines (CCL2, CXCL8), E-selectin, CD80, CD86

Type I Interferons (IFN α/β) and type III Interferons (IFNα)

Interferon-stimulated Genes (ISGs)
them to autolysosomes for degradation and resulting in the inefficient elimination of the virus by Cytotoxic Lymphocytes (CTLs) (46).

Normally, Type I and III IFN responses activate and augment the function of immune system tissue resident cells including dendritic cells, alveolar and interstitial tissue resident macrophages and Natural Killer cells (NK-cells). For example, monocytes/macrophages phagocytose the infected cells and induce type I IFN responses and pro-inflammatory molecules. NK cells recognize peptides expressed on the surface of infected cells and destroy them via direct cytotoxicity, through perforins and granzyme B. All immune cells release cytokines and chemokines, such as among others, TNF-α, IL1β, IL6 and IL8, that amplify and propagate the immune response by recruiting more cells in the following time order: Neutrophils are recruited first (within 12-24 hours post-infection, usually peaking at day 2-3), followed by pDCs (41). Monocytes, T- and B-cells appear later, from day 4-5 onwards, thus switching the immune response to its adaptive arm.

**FIGURE 4** | Corona viruses exploit different mechanisms to avoid immune detection (35, 41, 46). 1) The N protein-targeted ubiquitination drives RIG-I for proteasomal degradation, depleting the cell of a critical PRR. 2) Furthermore, the IFN pathway is inhibited by SARS-CoV-2’s proteins through various mechanisms: a) ORF3b, highly expressed in the acute stage of the infection, downregulates the expression of IFNAR, and eventually IFN I responses. b) Nsp13, Nsp14, Nsp15, and ORF6 suppress IRF3 nuclear translocation, c) Nsp1, blocks mRNA translation and also decreases STAT phosphorylation, d) M protein as well as nsp10, nsp13, nsp14, nsp15, can inhibit the production of type I and III IFNs through interaction with the RIG-I/MDA-5/MAVS signaling pathway, e) ORF8 can directly bind to MHC I molecules at the endoplasmic reticulum and redirect them to autolysosomes for degradation, which results in its inefficient elimination by cytotoxic lymphocytes. IFN, interferon; IFNAR, interferon a receptor; IRF3, interferon regulatory factor 3; MAVS, mitochondrial antiviral-signaling protein; MDA-5, melanoma differentiation-associated gene 5; MHC, major histocompatibility complex; PRR, Pattern Recognition Receptor; RIG-I, retinoid inducible gene I; STAT, Signal transducer and activator of transcription; STING, Stimulator of Interferon Genes; TAB, Mitogen-activated protein kinase kinase binding protein; TAK1, TGFβ-activated kinase 1; TBK-1, TANK-binding kinase 1; TIRAP, Toll-interleukin 1 receptor (TIR) domain containing adapter protein; TRAM, Translocating chain-associated membrane protein; TRAF, TNF receptor-associated factor; TRIF, TIR-domain-containing adapter-inducing interferon-β; Tyk, Tyrosine kinase.
Adequate adaptive responses require approximately 6-10 days of priming, reflecting the physiological lag period (post innate pathogen sensing) that is needed for the generation of pathogen-specific T- and B-cell clonal selection (51).

In parallel, glycans attached on viral proteins that are expressed on the infected cell surface can activate the lectin complement pathway after binding on the Mannose-Binding Protein (MBP). Complement activation leads to further inflammatory cell activation and the generation of Membrane Attack Complex (C5b-C9) on the surface of alveolar and epithelial cells, resulting in their destruction (52).

Adaptive Immune Response Against SARS-CoV-2

A week after the onset of COVID-19 symptoms, if the above described mechanisms operate uninterrupted, adaptive immunity, - mediated by CD4⁺ T-cells, CD8⁺ cytotoxic T-cells and antibodies -, is activated (53). Glycans expressed on SARS-CoV-2 are recognized by DC-SIGN and other lectins, facilitating the viral uptake by DCs and pDCs, which in turn present viral antigens to CD4⁺ T-helper cells. This results in a Th1 response with enhanced IFN-γ, IL-2 and TNFα production/secretion (53, 54). CD8⁺ T-cell responses specific to SARS-CoV-2 also produce IFN-γ, IL-2 and TNFα, resulting in direct cytotoxicity against infected cells (55). Notably, memory T-cells have also been detected in individuals that do not produce antibodies against SARS-CoV-2, which suggests that cellular immunity operates together and/or independently of humoral immunity (55). Lymphocytes, and in particular CD4⁺ T-cells, are the main regulators of the adaptive immune response against the invader, and their chemotaxis to the lungs might be partly responsible for the lymphopenia observed in the peripheral blood of many COVID-19 patients, a hallmark of COVID-19 (56, 57).

Follicular T-helper cells, through the formation of germinal centers and the provision of B-cells with all the necessary costimulatory molecules (such as CD40L and cytokines like IL-21), drive the humoral immunity towards SARS-CoV-2 surface glycopolypeptides, mainly glycoprotein S and nucleocapsid protein (58, 59). Antibodies against the spike glycoprotein and especially its Receptor Binding Domain possess a neutralizing effect against SARS-CoV-2, preventing the binding of the virus to ACE2 (60). Usually the first antibodies produced are of the IgA class, during the first week of infection, followed by IgM (in 10-12 days) and after the first 3 weeks by IgG (61). Interestingly, approximately 10% of infected COVID-19 patients have no detectable antibodies even after the 4th week post symptom onset, leading to the hypothesis that antibody production is not required for SARS-CoV-2 resolution in these so-called “non-responder” patients. This paradox is apparently not the case, and a possible mechanistic explanation in a subset of these patients could be the masking of antibodies due to prolonged antigenemia/viremia (62), or some - until now not described in COVID-19 - antiidiotypic response. Although the clinical significance of the initial titers of the produced antibodies and the duration of humoral immune response are still under investigation (63, 64), it is widely accepted that irrespective of disease severity, both T- and B-cell function are critical for disease termination (51, 65).

COVID-19 IMMUNOBIOLOGY

From Normal to Aberrant Host Immune Response

In physiology, the immune response is well orchestrated in time and space (spatiotemporally), in a way that its ultimate actions - resolution of damage, repair, and healing - are accomplished. Prerequisite for the latter is the counterbalance of pro-inflammatory actions with equal and opposite anti-inflammatory actions. For reasons still under investigation, this is not the case for a significant number of COVID-19 patients who experience the severe or critical form of the disease. According to latest research findings, however, spatiotemporally dys-synchronised and deregulated immune responses -starting early at the level of Type I and III IFN responses- appear to support the immunopathogenesis of COVID-19, tipping the balance towards an excessive, uncontrolled viral replication and the subsequent robust release of cytokines with detrimental effects on the human tissues. A good example are patients with either inborn errors in type I interferon signaling, in the genes coding for TLR3, UNC93B1, TICAM1, TBK1, IRF3, IRF7, IFNAR1, IFNAR2 (66) or preexisting neutralizing autoantibodies against type I IFNs (67), who are at higher risk for life-threatening COVID-19 infection. Genetic predisposition is also regarded as a crucial element to explain the transition from normal to aberrant host immune responses in different COVID-19 patients. Numerous studies have identified such genetic susceptibility loci, like apolipoprotein E and the ABO blood group genes, but the importance of these findings has not been understood to its entirety hitherto (68).

Comparative studies between mild and severe disease are a prerequisite for understanding essential differences governing the phenotypic switch between different COVID-19 disease states and for deciphering its pathophysiology. Recent reports have shown, that the transition from mild to moderate and ultimately to severe disease phenotype is coupled with metabolic, as well as innate and adaptive immune alterations. Multicentric analysis of mild, moderate, and severe cases has revealed a pivotal change occurring between mild and moderate disease states, rendering the therapeutic interventions very critical at the moderate stage. This change is characterised by the emergence of unusual cell types, including preferentially exhausted CD4⁺ T-cells, cytotoxic CD4⁺ T-cells, proliferative exhausted CD8⁺ T-cells (loss of CD8⁺ T-cell poly-functional), S100Hig/HLA-DRlow monocytes and depletion of non-classical monocytes. This disturbance of cell populations is accompanied by metabolic depletion in lipids, amino acids and xenobiotics (69). Similarly, marked deregulation in lipid transport has also been shown by RNA-seq and high-resolution mass spectrometry, associated with abnormal complement activation, and neutrophil degranulation in severe cases (70). In line with that, two large
Deregulated Interferon Responses

The first phenomenon observed in the aberrant host immune response against SARS-CoV-2 is an impaired induction of both type I and type III (IFN-λ) IFNs. Type I IFN levels tend to decline rapidly, whereas that of IFN-λ persist longer. Despite the limited ability of COVID-19 patients to produce IFNs, especially at the initial stage of the disease, they robustly express pro-inflammatory cytokines that can be maintained in the serum at high concentrations for a prolonged period of time. Delayed but persistent type I IFN responses may drive the excessive lung infiltration by monocytes/macrophages and neutrophils, as it has been shown for other CoVs (78). These cells can, in turn, produce high amounts of pro-inflammatory cytokines like TNFα, IL-6, IL-1β, as well as chemokines, that amplify the recruitment of immune cells on site and propagate the inflammatory response, thus contributing to cytokine deregulation (79). Findings from different patients suggest that some aspects of the disease, like the myopathy and the Pernio-like lesions (or COVID toes), may result from a type I interferonopathy. It is appreciated now that an initial robust type I IFN response can result in faster and better clearance of SARS-CoV-2, while a muted response, leads to viral persistence and severe disease (11, 80). In support of the above, patients with COVID-19 treated early with IFN-α2b exhibit reduced in-hospital mortality, whereas late application of therapy leads to increased mortality and delayed recovery. This observation highlights the crucial importance regarding the timing of IFN production in COVID-19 (44, 81).

Cytokine Deregulation

The diminished interferon response leads to an ineffective viral clearance and a deregulated activation of the adaptive immunity, which in turn results in enhanced activation of the innate immunity as a compensatory mechanism. This condition further results in an aggressive uncontrolled inflammatory response and the release of a large amount of pro-inflammatory cytokines and chemokines which cause damage in different organs/systems. Circulating IL-6, IL-18, IFN-γ, IL-15, TNFα, IL-1α, IL-1β, IL-2, G-CSF, IP-10 (or CXCL10), MCP1 (or CCL2), MCP3 (CCL7) and MIP-1α (CCL3) are over-expressed in patients with moderate or severe COVID-19 (82). Studies have suggested a direct correlation between cytokine levels and lung injury, multiple organ failure, and an unfavorable prognosis. So far, no specific cytokine or cell has been identified as the driver of this process (83). This state has been referred, by some researchers, as “cytokine storm”. However, this term has been queried because the hyper-inflammatory phenotype seen in severe COVID-19 is different from other hyper-inflammatory conditions, since the cytokine levels are not particularly exacerbated (84).

Different inherent or acquired conditions including gender differences (female vs male) (85), inflamming and other pre-existing conditions such as baseline immunosuppression, immune exhaustion and immunosenescence are additional factors which could affect this delicate balance (56, 86, 87). In elderly patients, inflammaging – a chronic low-grade sterile inflammation characterized by high baseline serum levels of CRP and cytokines like IL-6 and IL-8 (88)– along with the inflammation induced by SARS-CoV-2, can drive the expression of specific transmembrane molecules. Such molecules like the Major histocompatibility complex class I chain-related protein A (MICA) and B (MICB) are also expressed by senescent cells normally found on the respiratory epithelium. As a result, highly-differentiated senescent T-cells with an NK-like phenotype that express NK-receptors (NKG2D) are attracted leading to the subsequent destruction of the epithelial cells (89). Although a clear distinction between factors that drive the aberrant cytokine expression, and factors that are the result of this deregulation is in the majority of cases difficult to be pointed out, we will try to address these two parameters separately.

DRIVERS OF CYTOKINE DeregULATION

Immune-Mediators

As presented earlier in this review, the cytokine over-expression is a result of a deregulated innate and adaptive immune response. In severe COVID-19 macrophages seem to play a central role through the excessive production of pro-inflammatory cytokines and chemokines (90). Macrophages from patients with severe COVID-19 exhibit lower expression of apolipoproteins which correlates with a pro-inflammatory phenotype (91). Resident pro-fibrotic (SPP1high) and inflammatory activated (FABP4+) macrophage populations are dominant in the lungs of patients with severe COVID-19 (92). In addition, activated monocytes/macrophages can express tissue factor (TF) and promote thrombosis (93). Moreover, peripheral blood neutrophils of
severe COVID-19 patients show increased numbers and marked hyper-responsiveness, as attested by the enhanced degranulation, neutrophil extracellular traps (NETs) release and pro-inflammatory cytokine production (94). An increased neutrophils/lymphocytes ratio has been associated with a higher morbidity and mortality of COVID-19 (95). Mast cell activation and the release of various vasoconstrictive or inflammatory mediators, like histamine, tryptase, chymase, IL-1β, IL-6, TNF-α, CCL2, GM-CSF and CXCL10, contribute to local inflammation, tissue destruction, and cytokine imbalance (96). Additionally, the decreased numbers of CD4+ T-cells observed in severe COVID-19 can contribute to hyper-inflammation via the impaired regulatory mechanisms of the inflammatory process or via impaired adaptive T- and B-cell responses (23, 97). Finally, increased IL-17 production by Th17 cells in COVID-19 patients has broad pro-inflammatory effects through the upregulation of pro-inflammatory cytokines like G-CSF, IL-1β, IL-6, TNFα, as well as chemokines like MIP2A, IL-8, IP10, MIP3A, and matrix metalloproteinases (98).

Cellular Stress-Response Mediators

Cellular Senescence

Cellular senescence is a stress response against a plethora of cellular insults, including effects of infectious agents through mechanisms of DNA damage, cell fusion and ER stress (31). It is characterized by 4 interconnected hallmarks of: cell cycle arrest, macromolecular damage, altered metabolism and pro-inflammatory features, called the senescence-associated secretory phenotype (SASP) (99). Such a response is activated upon accumulation of sub-lethal cellular damage that cannot be resolved by the physiological repair mechanisms and acts as fail-safe to prevent propagation of damage – viral dissemination, in the case of viral illnesses (100). in both cellular and tissue level (101). Thus, its homeostatic role is widely accepted in physiology and pathophysiology, with its role in infectious diseases being still ill-defined (102). In some instances, cellular senescence is beneficial to the host since it obstructs viral replication and damage, while in others, it appears to facilitate viral-induced pathology (102, 103). Such a binary mode of action characterizes many facets of cellular senescence. It is likely that within this context, the fitness of the host (102), the timing of initiation of cellular senescence program and its duration, all together play key roles in the outcome of viral diseases like COVID-19. Within this context, we have recently showed that SARS-CoV-2 triggers senescence with various clinical and epidemiological implications, including quasispecies generation (31).

The above findings are of high clinical translational potential, as drugs specifically targeting the elimination of senescent cells, could alter disease clinical course once established, or even be used prophylactically prior to viral exposure. Nonetheless, large clinical trials are necessary for the establishment of the type and timing of such treatment strategies.

Uncontrolled Inflammatory Cell Death – PANoptosis

SARS-CoV-2 can trigger different types of cell death (Figure 5) (33, 104–107). Uncontrolled inflammasome activation and excessive pyroptosis, which is suggested to be the main “death mode” observed in COVID-19, can result in an hyper-inflammatory condition leading to severe disease, particularly in elderly patients (105). NLRP3, the major component of inflammasome, is already activated in older people as well as different pathological states that also serve as risk factors for severe COVID-19, including obesity, hypertension, cardiovascular disease, COPD and diabetes mellitus (108). Given that the threshold of inflammasome activation is very low in such patients, SARS-CoV-2 infection offers a significant additive effect predisposing them to uncontrolled inflammasome activation and subsequent hyper-inflammatory response (109). Interestingly, quite recently, a novel type of programmed inflammatory cell death called PANoptosis, has been described in murine models of COVID-19 exhibiting hypercytokinemia, similar to that described in severe COVID-19 in humans. The term PANoptosis is used to describe the simultaneous activation of Pyroptosis, Apoptosis and Necroptosis in the same cell, leading to its inflammatory death (34). More specifically, increased TNFα/IFN-γ signaling has been shown to activate the STAT1/IRF1/iNOs/NO pathway, which in turn leads to the formation of a cytoplasmic multimeric protein complex called PANoptosome. PANoptosome then acts as a molecular scaffold for the assembly and activation of effector molecules like RIPK1/RIPK3/FADD/caspase 8, subsequently followed by the contemporaneous activation of pyroptosis via GSDME, apoptosis via Caspase 3/7 and necroptosis via MLKL. This inflammatory cell death further enhances the inflammatory state by the release of PAMPs, DAMPs and other proinflammatory molecules (34).

CONSEQUENCES OF CYTOKINE DEREGULATION

The above-described cytokine deregulation, augmented by the deregulated Renin-Angiotensin-Aldosterone System (RAAS) shown in Figure 2, has a series of immunological and physiological effects that eventually lead to multi-organ damage, and if not treated, to multi-organ failure, ARDS and death (Figure 6) (87, 110–112).

Immune Sequelae

Deregulated Innate Immunity

Decreased HLA-DR and increased PD-L1 expression has been documented in COVID-19 monocytes (94). These findings suggest exhaustion of these cells and probably impaired antigen presentation to naïve T cells (113). DCs in COVID-19 patients are reduced, with impaired maturation and function, suggesting also impaired antigen presentation and T cell activation (114). Patients with severe disease display the highest expression of PD-L1 and the lowest expression of the maturation marker CD80 on DCs (94). Activated NK cells expressing KIRs and CD16 are significantly decreased in COVID-19 patients, implying either impaired maturation or increased migration into the target tissues (115). They also...
correlate inversely with IL-6 plasma concentrations (116). Moreover, NK cells also show signs of exhaustion, with increased expression of the inhibitory CD94-NKG2A receptor heterodimer and decreased expression of CD107a, IFN-γ, IL-2 and granzyme B (117). Additionally, a low eosinophil count has been correlated to a worse prognosis in COVID-19 patients (118). Finally, increased numbers of myeloid-derived suppressor cells (MDSCs) – pathologically activated monocytes and neutrophils with potent immunosuppressive activity – have been detected in COVID-19 patients, with their number and activity correlating to disease severity (119–122). Such MDSCs have been shown to exhibit enhanced immunosuppressive activity by suppressing T-cell proliferation and function, as well as increased IFN-γ and TNF-α production, probably contributing to a form of immune-paralysis observed in COVID-19 patients (119–121). Interestingly, during the convalescent phase of the disease, the numbers of MDSCs decline, but still remain increased in patients with severe COVID-19 in comparison to patients with mild disease (at least during the initial phase of convalescence) (119).

Deregulated Adaptive Immunity

**T-Cells**

CD4+ and CD8+ lymphopenia is commonly found in COVID-19 patients, resulting possibly either from direct cytotoxic effects of the virus, or from enhanced T-cell apoptosis due to the dysregulated cytokine milieu or metabolic disorders such as lactic acidemia (123). Decreased T cell counts in severe COVID-19 have been associated with reduced T cell activation and function markers, including TCR subunits (CD3e, CD3γ, CD247, TRAC, TRBC1), surface accessory molecules (CD4, CD8α, CD8β, CD2), T cell migration stimulators (DDP4), TCR signaling kinases (ZAP70, LCK, FYN) and MHC-II molecules (124).
T cells also express high levels of exhaustion markers like PD1, TIM3, and CD57, suggesting a functional immune deficiency (125). In severe COVID-19, T cells also show features of an exhausted phenotype. These cells possibly not only lack markers of clonal expansion, but at the same time produce pro-inflammatory molecules (126). Thus, high levels of IL-1α and TNFα promote Th17 responses, increased vascular permeability and leakage. In turn, Th17 cells produce IL-17, GM-CSF, IL-21, and IL-22 (126). IL-17 has broad chemotactic effects through the upregulation of chemokines such as MIP2A, IL-8, IP10, and MIP3α (127).

Surprisingly, a marked plasma increase of typical Th2 cytokines, including IL-4, IL-10, and IL-13 has been observed in some severe COVID-19 patients. This observation could indicate that the activation of immune system in some severe cases, is not Th1-restricted but possess a much broader activation, involving all cells, similarly to what occurs during bacterial sepsis. Such a condition can lead in a form of immunoparalysis (126, 128).

FoxP3+ Tregs are also elevated in several severe COVID-19 patients. It appears that severe COVID-19 entails a striking induction of Treg activation markers including FoxP3, KLRG1, and PD1 and a reduction of CD45RA, a marker of naïve Tregs. Moreover, severe COVID-19 induces the expression of Tbet that preferentially controls Th1 responses and downregulates the inflammatory cytokines TNFα, IL-1β. These changes suggest that Tregs in severe COVID-19 are shaped towards a super-suppressive phenotype equivalent to that observed in tumor Tregs (129).

B-Cells
Analyses of circulating B cells in patients with severe COVID-19 have shown an expansion of oligoclonal plasmablasts and reduced frequency of memory B cells (47, 130). The antibody sequences of the largest B cell clones isolated from these patients have variable levels of somatic hyper-mutations and VH gene usage, indicative, as anticipated, of a polyclonal B cell response (130). Many COVID-19 patients possess neutralizing antibodies...
minimally mutated, suggesting a limited affinity maturation and, consequently, low binding affinity towards SARS-CoV-2 (131). This disparate antibody response might indicate a failure to develop robust long-lasting protective humoral responses against SARS-CoV-2 in some patients (132).

Early antibody responses against the spike protein have been associated with a favorable outcome, while early responses against the viral nucleocapsid with poor clinical outcomes (133). Detailed analysis of the antibody subtypes has revealed elevated IgG1 antibody production with a significantly increased Fc afucosylation. Afucosylated antibodies possess a much higher affinity for the FcγRIIIa receptor, triggering NK cell activation, the production of pro-inflammatory cytokines from monocytes/macrophages, and the cytotoxic effector cell activities provided by cells bearing FcγRIIIa receptor (134). At the same time, afucosylated antibodies against SARS-CoV-2 might contribute to antibody-dependent enhancement of viral infectivity by promoting viral internalization in different cells expressing Fcγ receptor, as it has been shown in animal studies (135).

**Disease Sequelae**

**Endothelial Activation and Endothelitis**

The high levels of circulating pro-inflammatory cytokines seen in severe COVID-19 are associated with local and systemic endothelial dysfunction and injury. IL-6 and TNFα can increase vascular permeability and activate the endothelium (24). In turn, endothelial activation leads to increased TF expression, downregulation of thrombomodulin expression and loss of heparin sulfate - all protective mechanisms against thrombosis (136). Direct endothelial cell infection by SARS-CoV-2 and subsequent endothelitis can lead to increased vascular permeability, platelet activation, enhanced thrombin generation and decreased fibrinolysis, contributing to a hypercoagulable state (137, 138). Moreover, SARS-CoV-2 has been shown to directly disrupt the endothelial barrier by altering the surface expression of VCAM1 and the tight junction scaffold proteins ZO-1 and ICAM1, resulting in both chemotaxis and extravasation of inflammatory cells in the perivascular space (139). In COVID-19 patients, the perivascular space around the damaged endothelium is infiltrated by increased numbers of CD3+ and CD4+ T-lymphocytes and the production of pro-inflammation as described above (140). It is hypothesised that the endothelial cell dysfunction, when it occurs in COVID-19, characterises the more severe forms of the disease, underpinning the associated complications including the capillary leak syndrome and thrombosis (143). Such a dysfunction can be caused by endothelial cell inflammation, as shown in autopsy studies (144, 145) which can either reflect direct viral cytotoxic effects or a bystander/indirect effect of hyper-cytokinemias (146). Although the latter is generally well accepted in the literature, direct endothelial cell infection by SARS-CoV-2 remains still elusive.

**Immunothrombosis**

The coagulation system and the immune system are two co-workers, complementing each other to provide host defense and limit the dissemination of invading pathogens (147). COVID-19 is characterized by an elevated risk for thrombosis both in macro- and micro-circulation, contributing substantially to morbidity and mortality (148). Many studies suggest that SARS-CoV-2, through various mechanisms, has the ability to affect different cells or systems in both the immune and the coagulation arm of the host defense, amplifying lung or systemic inflammation and subsequently leading to tissue damage and thrombosis in a process known as immunothrombosis (149, 150). The main contributors to this phenomenon - as depicted in Table 2 (93, 151–173) and Figure 7 (149, 161, 163, 174–176) - are: megakaryocyte dyshomeostasis, platelet activation, Neutrophil Extracellular Traps (NETs) formation, complement hyper-activation and coagulation cascade activation as well as endothelial activation/inflammation as described above (149, 150, 163).

**Autoantibodies and Autoimmunity**

Some researchers have questioned whether the manifestations of COVID-19 are a mere result of the infection per se or a result of autoimmune reactions initiated or exacerbated by the infection (177). Viral infections not only share common immune responses with autoimmune diseases (ADs), but also they can break immune tolerance and cause autoimmunity by a variety of mechanisms such as molecular mimicry, bystander activation and epitope spreading (178–180). The reduction of regulatory T cells and the increased number of Th17 and cytotoxic CD8+ T-cells that are observed in severe COVID-19 (as described above), have also been described in several autoimmune/inflammatory/autoimmune diseases (181, 182).

Additionally, lymphopenia and neutrophilia, as well as extrafollicular B-cell activation that have been observed mainly in severe COVID-19, also consist a common feature between COVID-19 and some ADs (183, 184). Moreover, cytokine deregulation and immune hyper-stimulation with some features of immunoparalysis can break tolerance and trigger the activation of auto-reactive T- and B-cells in autoimmune susceptible individuals in an antigen-independent manner, a phenomenon known as bystander activation (185, 186). Additionally, peptide sharing analyses have identified a massive hexapeptide and heptapeptide sharing between SARS-CoV-2 protein S and human proteins, with more than 460 such examples (187, 188). It has therefore been suggested that antibodies against SARS-CoV-2 peptides or against the viral S protein, could boost immune cross-reactions against human proteins like the pulmonary surfactant, the brainstem respiratory pacemaker transglutaminases 2 and 3, mitochondrial proteins, nuclear antigens etc., thus leading to autoimmune tissue destruction (189). Finally, it has been proposed that the enhanced NETosis observed in COVID-19 could also drive autoimmune phenomena, since excessive NETosis has been implicated in the pathophysiology of other autoimmune diseases (186, 190). Interestingly, enhanced
expression and circulation of autoantigens like MPO or PRTN3 has also been observed in COVID-19 patients, and the levels of expression of some of these autoantigens correlate with disease severity (191).

In COVID-19 patients, a variety of autoimmune diseases/phenomena have been described, reflecting the overall aberrant immune activation observed in these patients:

1) Different autoantibodies have been detected in many COVID-19 patients with no previous autoimmune rheumatic disease, including antinuclear antibodies, p-ANCA, c-ANCA, anti-CCP, anti-RNP, anti-centromere, Rheumatoid Factors, antitopoiso merase I and anti-prothrombin, with no, so far, clear clinical significance. Interestingly, some of these antibodies correlate with high levels of CRP and more severe disease.
However, whether these antibodies are “functional” and have an etiopathogenic relation to COVID-19 manifestations or are just an epiphenomenon, for the majority of cases, remains to be proven (180, 192, 193).

2) Autoimmune thrombocytopenia or hemolytic anemia with the concurrent detection of warm autoantibodies or cold-agglutinins have also been described, and some of these cases have been successfully treated with IVIG (189). As for the hemolytic anemia, molecular mimicry is probably the main pathogenetic mechanism, since for example, the erythrocyte protein Ankyrin-1 shares a common pentamer with SARS-CoV-2 spike protein (194). Interestingly, IgG3 which is associated with worse prognosis in COVID-19, is the only IgG subclass capable of forming cryoglobulins through Fc-Fc interactions (195). On the other hand, some cases of vaccination against SARS-CoV-2 have been associated with a prothrombotic immune thrombocytopenia (VIPIT) attributed to autoantibodies directed against PF4, a situation similar to Heparin-Induced Thrombocytopenia (HIT) (196).

3) Antiphospholipid syndrome or even catastrophic antiphospholipid syndrome with the involvement of at least 2 target-organs have also been described in COVID-19 patients. Interestingly, not all 3 types of antiphospholipid antibodies (lupus anticoagulant and/or anticardiolipin and/or beta2-glycoprotein I) have not been detected in all cases. The levels of antiphospholipid autoantibodies correlated with the CRP levels in serum, D-dimer concentrations in plasma, platelet counts, absolute neutrophil counts, calprotectin in serum (marker of neutrophil activation), and myeloperoxidase–DNA complexes in serum (marker of NETs). Additionally, high levels of such antibodies are associated with more severe disease. However, antiphospholipid antibodies are not always accompanied by thrombosis and in such cases they can possibly be merely the result of the immune hyperactivation and prothrombotic state observed in these patients (171, 192, 197).

4) Some patients have developed different forms of vasculitis (small vessel, middle vessel, large vessel, IgA, cutaneous, CNS vasculitis etc.) in which the inflammation is not limited to the endothelium, but manifests as a peri- and pan-arteritis with immune complex depositions[Reviewed in (186, 198)]. A more severe form of vasculitis is the Kawasaki-like disease (KD-like) or Multisystem Inflammatory Syndrome in children with COVID-19. During the pandemic, a striking rise in KD-like cases has

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**FIGURE 7** SARS-CoV-2-triggered NETs induce thromboinflammation through interactions with complement system, platelets and the coagulation cascade (161, 163). NETs provide a platform for expression-activation of coagulation factors such as TF and FXII. They also interact with vWF released by endothelial cells and platelets, leading to platelet adhesion and fibrin formation (174). Additionally, NET-derived histones and TF can induce endothelial cell activation and thrombogenicity. Furthermore, NET formation also enhances thrombus stability and lytic resistance, while through the release of neutrophil elastase, degrades TFPI and thrombomodulin and thus dampens anticoagulation (174). Probably, in COVID-19, increased levels of platelet-derived thrombin and factors such as polyphosphate (polyP), high mobility group box 1 (HMGB1), PF4, RANTES, as well as complement and IL-1, IL-6 and IL-8 can trigger NETosis, in which NET formation also enhances thrombus stability and lytic resistance, while through the release of neutrophil elastase, degrades TFPI and thrombomodulin and thus dampens anticoagulation (174). Probably, in COVID-19, increased levels of platelet-derived thrombin and factors such as polyphosphate (polyP), high mobility group box 1 (HMGB1), PF4, RANTES, as well as complement and IL-1, IL-6 and IL-8 can trigger NETosis. It is possible that the thromboinflammation is triggered by the release of NETs. The interaction between platelets and NETs may play a central role in the immunothrombosis and ARDS observed in COVID-19 (161, 163, 176). ARDS, acute respiratory distress syndrome; FXII, coagulation factor XII; IL, interleukin; NET, neutrophil extracellular trap; PF4, platelet factor 4; RANTES, Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted; TF, tissue factor; TFPI, tissue factor pathway inhibitor; vWF, von Willebrand factor.
been reported. This disease affects mainly children and targets preferentially the heart, the intestine, and the brain. The pathogenetic cause of this disease seems to be an autoimmune vasculitis, since different autoantibodies have been described in these patients, like antibodies against endoglin, MAP2K2 and members of the Casein Kinase Family, with a distinct inflammatory profile from that of COVID-19 cytokine deregulation (199).

5) Other patients have developed an acute inflammatory immune-mediated progressive polyradiculoneuropathy presenting with tingling, weakness, autonomic dysfunction, and pain, resembling Guillain-Barre syndrome or its variant Miller-Fisher syndrome. Some of these patients have been successfully treated with IVIG or plasmapheresis (189).

6) Cases of inflammatory arthritis or spondyloarthritis, usually 1-3 weeks after the infection have also been recorded (198).

7) Some COVID-19 patients have developed a new systemic connective tissue disease like Systemic Lupus Erythematosus (SLE). On the other hand, a flare of a preexisting autoimmune disease after COVID-19 has also been described (200–204).

8) Case reports of autoimmune myositis or glomerulonephritis, as well as autoimmune endocrinopathies (thyroiditis, Grave’s disease, type I diabetes mellitus) have also been described in the context of COVID-19 [reviewed in (186, 198)].

9) Some researchers also believe that anosmia (loss of smell) that develops in many COVID-19 patients (186), could also be an autoimmune phenomenon, since such olfactory disturbances have also been described in other autoimmune conditions (205).

10) Based on histopathological, radiological and serological similarities between COVID-19-related ARDS and acute exacerbation of connective tissue disease-induced interstitial lung disease, some researchers suggest that lung involvement in COVID-19 in predisposed patients might be a type of organ-specific autoimmune reaction (206).

COVID-19 in patients already suffering from an AD – either clinically or subclinically – may be affected by this AD or the medications used for it and, to the contrary, may influence this AD. On the other hand, patients without any AD face the danger of developing a new AD as a result of COVID-19 (207). Theoretically, patients with autoimmune diseases are at increased risk for infections and thus increased risk for COVID-19, due to deregulated adaptive immunity (cellular and/or humoral) (208). However, different studies addressing this issue have not clearly established such an increased risk (189, 209, 210). To the contrary, some patients with prior autoimmune/autoinflammatory conditions infected with SARS-CoV-2 have shown better outcomes, probably due to baseline immunomodulatory medications (211–213). For example, the use of TNF inhibitors has been associated with protection from severe COVID-19, while most of the disease-modifying anti-rheumatic drugs (DMARDs) do not seem to confer increased risk (214). On the other hand, patients treated with Rituximab or Secukinumab exhibit high concentrations of IL-6 and seem to have a worse prognosis. CD20 blockers, in particular, could impair B-cell function and therefore the production of neutralizing antibodies against SARS-CoV-2 (215). Chronic sulfasalazine use has also been associated with worse prognosis (216). Moreover, it seems that co-administration of conventional synthetic DMARDs with biologic treatments puts patients at increased risk for severe COVID-19 (217). Finally, chronic treatment with glucocorticoids has been associated with poorer outcomes (218).

Genes associated with genetic susceptibility for autoimmune diseases (like TLR7, MHC region, PTEN22, TYK2 and IL6R) are being assessed in an effort to explain heterogeneity in COVID-19 severity and organize treatment decisions in a personalized way, but the results are still inconclusive. For example, genetic variations in IL-6 signalling could affect both the presentation of COVID-19 and the response to an anti-IL6 treatment (68).

**DISCUSSION**

It has been more than 1,5 years now since the initial declaration of COVID-19 as a pandemic. During this time, a great amount of knowledge has been gathered as a result of intensive research from many labs and hospitals around the world. An important message coming out of these studies is that many of the detrimental effects of COVID-19 in end-organ damage/pathology are caused by insults directly attributed to the virus per se, but are also secondary to the immune deregulation (219).

In this review, we attempted to summarize in a stepwise approach, the key pathophysiological processes that take place from viral inhalation to disease establishment, with emphasis on the immunopathology of COVID-19 (ranging from immunity to autoimmunity) and its heterogeneous clinical outcomes. Intensive ongoing research all over the world, makes the need for continuous update of the acquired knowledge more than essential since new concepts constantly arise (for example PANoptosis or cellular senescence as features of COVID-19 pathobiology). Figure 8 depicts the critical steps for viral clearance or alternatively for viral persistence and aberrant/sustained immune responses resulting in severe disease and/or death.

Many researchers nowadays suggest, that the deregulated immune response against SARS-CoV-2, as it is presented in this review, has some characteristic features of autoinflammation and autoimmunity and could therefore be used as a disease-model for this cluster of diseases of unknown etiology (192). A single virus – SARS-CoV-2 – has been shown to possess the capability to drive such a robust hyper-inflammatory immune response that even self-tolerance can be disrupted. As a consequence, and probably through other mechanisms like molecular mimicry, autoantibodies can be produced and result in different autoimmune phenomena and end-organ damage (220). Many researchers believe that the main manifestations of COVID-19 might be attributed to such autoimmune phenomena and not to the infection per se. For example the time needed for the full panel of symptoms to arise (1-2 weeks), coincides with the time needed for the production of autoantibodies (220, 221). Persistence of these autoantibodies and/or the accompanying...
inflammation after the resolution of the acute phase of COVID-19 has also been proposed as a possible mechanism leading to the so-called “long-COVID” (222–224). On the other hand, it is of interest, that, some of the main drugs used in patients hospitalized with COVID-19 (like corticosteroids, IL6 inhibitors, Jak inhibitors etc.) are immunosuppressive/immunomodulatory drugs commonly used in autoimmune/autoinflammatory diseases (225–229).

Many data that are now available strongly support the concept of COVID-19 being more than an infectious disease, with autoimmunity playing a pivotal role. However, many questions regarding COVID-19 and autoimmunity remain unanswered. On the one hand, complete understanding of the pathogenetic mechanisms underlying COVID-19, will give the opportunity for new, more targeted – thus more effective and less harmful – treatments. For example, blockade of inflammatory cell death (like pyroptosis and PANoptosis) could offer an alternative drug-target for COVID-19 and also help in the treatment of autoinflammatory diseases. Additionally, the temporal distribution of the different immunopathological mechanisms that drive COVID-19 pathogenesis, could help identify the right timing for specific therapies (like interferons). Moreover, if COVID-19 is not dealt with as a purely infectious disease, then other therapies could be implicated in its treatment (like anti-CD20 for the elimination of B-cells, IVIG or plasmapheresis for the neutralization or removal of pathogenic autoantibodies etc.). Detection of susceptibility genes/polymorphisms could also help identify patients at risk for developing severe COVID-19 and autoimmune manifestations and guide treatment in a personalized manner.

On the other hand, it is not known whether all autoantibodies that are created in autantibody-naïve patients have an “active” role, or some of them represent an epiphenomenon. Additionally, neither what happens with these antibodies as time passes after the infection resolution, nor the mechanisms regulating their disappearance or preservation are known. Such knowledge could offer new insights into the treatment of autoimmune diseases. Moreover, since the cases of long-COVID have started to increase, it is essential to understand the pathogenetic mechanisms underlying this condition, to treat it in a more targeted-manner. Such knowledge could probably offer new ideas about the treatment of other chronic conditions like chronic pain or fibromyalgia.

Another promising concept regarding COVID-19 pathobiology and autoimmunity, is the concept of cellular senescence. It has been shown, that cellular senescence,
and especially immunosenescence, is associated with the development of autoimmunity (230, 231). Could cellular senescence that precedes COVID-19 (e.g. in the elderly) or is triggered by the disease, put patients at higher risk for developing autoimmunity?

Lymphopenia is regarded as a hallmark of COVID-19 [according to the study and the disease severity, frequency can reach even 85% (232, 233)], with redistribution to sites of active infection and enhanced destruction within the context of cytolikemia being proposed mechanisms. Yet the exact aetiology behind low lymphocyte count is still unknown as well as whether direct SARS-CoV-2 tropism to such cells exists (232). Additionally, studies have demonstrated the presence of spike viral antigen in lymphocytes by immunohistochemistry, while isolation of viral RNA extends beyond lymphocytes to include almost all leukocyte types (234). As a result, leukocytes could be hypothesized to act as a trojan horse facilitating immune evasion and potential viral dissemination systemically. However, appropriate experiments are still lacking to support this notion. In line with that, the functional outcome of direct viral replication within leukocytes would need to be investigated and the fate of those cells to be determined. Could exhaustion of the infected cells take place or immunosenescence be triggered as a response? What is driving immune exhaustion in COVID-19 and what differences exist between mild and severely infected individuals? What is the role of immunosenescence in the establishment of the cytokine deregulation and how does it correlate with clinical outcomes? Does the degree of immunosenescence differ in mild vs severe disease, and if so, how?

All the above are some questions that if answered, they would deepen our understanding of the immunological phenomena in COVID-19 and possibly widen our currently limited-treatment options against it. Scientists are eagerly seeking for new therapeutic agents (235) and drug repurposing tailored to COVID-19 is actively being investigated (236). In the light of findings pinpointing cellular senescence as an important disease modulator, especially in high-risk populations (31, 237, 238), such efforts could be expanded to include experimental use of senolytic drugs as well. This group of medicines act either by apoptosis-induced elimination of senescent cells (senolytics) or inhibiting their SASP-mediated effects (senomorphics) (239). Irrespective of their mode of action, senolytic drugs have tremendous translational potential not only in age-related diseases but in the treatment of infectious diseases too (240). As a stress response triggered by viral infections, senescent cells become tangible targets for elimination with these drugs potentially altering disease course by disrupting viral replication within them and clearing their associated inflammatory properties. Their use, either upon disease establishment or even prophylactically in high-risk populations harbouring a high-burden of these cells, is promising (241). Nonetheless, as these cells are important to normal homeostasis and physiology (99, 101), their destruction comes at a fair cost. To illustrate the latter, the use of senolytics has been associated with reactivation of latent viral infections (242), systemic toxicity (243) and aberrant wound healing (244). Formal studies and clinical trials to assess their clinical efficacy are required prior to their consideration as therapeutic agents. As cellular senescence has a bright and a dark side, depending, at least in part, on the duration of its effects (245), clarifying the specifics of the timing of their interventional use should be a priority.

Concurrently to human studies, a series of pre-clinical animal models (mainly non-human primates, ferrets, Syrian hamsters, and genetically modified mice) have also been used [reviewed in (246–248)]. Unfortunately, these disease-models do not mimic human disease in its entirety. One important difference between humans and animal models of COVID-19, is the fact that most of the animals develop only mild-moderate disease that is limited to the lungs. In most of these cases, the initial response to SARS-CoV-2 consists of a local innate immune activation (macrophages, mononuclear cells, NK cells, neutrophils) in the lungs with the production of inflammatory cytokines like IFN-γ and IL-6 and in some cases with the additional help of residual lymphocytes – especially cytotoxic CD8+ T-cells. Moreover, in some of these models, type I and III IFNs have been shown to play a protective role. However, in contrast to humans, no severe cytokine deregulation with robust hyper-cytokinemia (and their consequences) has been described, and the initial phase of immune activation is usually followed by a more anti-inflammatory 2nd phase and disease resolution. These observations can be attributed either to an “inherent weakness” of each animal model (e.g., lack of enough ACE2 receptors for the initial infection to take place at sufficient levels) or to the local immune responses developed in these animals, that manage to eliminate the virus and prevent systemic disease. Interestingly, this milder disease phenotype can be overridden with the use of older animals or use of a higher viral inoculum [reviewed in (249, 250)]. This observation suggests that the immune system has finite “capacity” of dealing with an intruder, and this capacity might decline with age. Further studies in animal models of COVID-19 and interpretation of their results in the light of comparative immunology might give us some new information about the crucial initial immune response that could block SARS-CoV-2 on site without evolving into a severe systemic disease.

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AK and KB wrote the review. PS, VG, PV and AT substantially contributed to the discussion of the content, reviewed and edited the manuscript before submission. Additionally, KB, PS and PV created the images presented in this manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Wadman M, Couzin-Frankel J, Kaiser J. Mastic C. A Rampage Through the Body. Science (2020) 368(6489):356–60. doi: 10.1126/science.368.6489.356
2. Abebe EC, Dejenie TA, Shiferaw MY, Malik T. The Newly Emerged COVID-19 Disease: A Systemic Review. Viral J (2017) 17(1):96. doi: 10.1016/j.virj.2020.01363-5
3. Gerotziafas GT, Catalano M, Colgan MP, Pecsvarady Z, Wautrecht JC, Fazeli B, et al. Guidance for the Management of Patients With Vascular Disease or Cardiovascular Risk Factors and COVID-19: Position Paper From VAS-European Independent Foundation in Angiology/Vascular Medicine. *Thromb Haemost* (2020) 120(12):1597–628. doi: 10.1055/s-0040-1715798
4. Nishigami M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and Its Mechanisms. *Pathogens* 9(5):331. doi: 10.3390/pathogens9050331
5. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, et al. The ACE2/Angiotensin-(1-7)/Mas Axis of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. *Rev Esp Med Nucl Oncol* (2020) 41(5):355–9. doi: 10.1016/j.ijmed.2020.04.037
6. Ikeda T, Shindo F, Kozono K, Ishikawa M, et al. ACE2 Depletion in Mouse Cardiac Myocytes and an Inflammatory Responses and Cell Death Through Caspase-8 Activation. *Cell Death Discov* (2021) 7(1):43. doi: 10.1038/s41420-021-00428-w
7. Sen CK, Singh H, Kaur H, Singh K, et al. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System in COVID-19: A Novel Hypothesis for Clinically Suspected Hypercoagulopathy and Microvascular Immorthrombosis. *Clin Chim Acta* 507:167–73. doi: 10.1016/j.cca.2020.04.027
8. Teuwen LA, Gelfand V, Pasut A, Carmeliet P. COVID-19: The Vasculature Unleashed. *Nat Rev Immunol* (2020) 20(7):389–91. doi: 10.1038/s41577-020-0343-0
9. Kuba K, Imai Y, Penninger JM. Multiple Functions of Angiotensin-Converting Enzyme 2 and Its Relevance in Cardiovascular Diseases. *Circ J* (2013) 77(2):301–8. doi: 10.1253/circj.CJ.12-1544
10. Bernstein KE, Khan Z, Gian JF, Cao DY, Bernstein EA, Shen XZ. Angiotensin-Converting Enzyme in Innate and Adaptive Immunity. *Nat Rev Nephrol* (2018) 14(5):325–36. doi: 10.1038/nrneph.2018.15
11. Pal R, Bhansali A. COVID-19, Diabetes Mellitus and ACE2: The Comundrum. *Diabetes Res Clin Pract* (2020) 162:108132. doi: 10.1016/j.diabres.2020.108132
12. Tikellis C, Thomas MC. Angiotensin-Converting Enzyme 2 (ACE2) Is a Key Modulator of the Renin Angiotensin System in Health and Disease. *Int J Peptides* 2012:256294. doi: 10.1155/2012/256294
13. Li S, Zhang Y, Guan Z, Li H, Ye M, Chen X, et al. SARS-CoV-2 Triggers CoV-2: Evasion Strategies and Activation Trigger Hypoxia and Vascular Disease: From Basic Mechanisms to Clinical Perspectives. *Nat Rev Cardiol* (2020) 17(9):543–58. doi: 10.1038/s41569-2020-00356-5
14. Weibiao Zeng KQ, Ye M, Zheng L, Liu X, Hu S, Zhang W, et al. COVID-19-Associated Acute Kidney Injury: Consensus Report of the Renin-Angiotensin-Aldosterone System in COVID-19: A Systematic Review. *Adv Wound Care* (2021). doi: 10.1016/j.adwcc.2020.08.028
15. Singh H, Kaur H, Singh K, Sen CK. Cutaneous Manifestations of COVID-19: A Systematic Review. *Adv Wound Care* (2021) 10(2):51–80. doi: 10.1089/wound.2020.1309
16. Xie X, Chen J, Wang X, Zhang F, Liu Y. Age- and Gender-Related Difference in the Expression Profi le of ACE2 in Rat Lung. *Peptides* (2012) 2012:256294. doi: 10.1155/2012/256294
17. Tikellis C, Thomas MC. Angiotensin-Converting Enzyme 2 (ACE2) Is a Key Modulator of the Renin Angiotensin System in Health and Disease. *Int J Peptides* 2012:256294. doi: 10.1155/2012/256294
18. Steinberg A, Ibrahim F, Khorrami M, et al. COVID-19 Disease: A Systemic Review. *Nat Rev Nephrol* 2020) 16(12):747–64. doi: 10.1038/s41581-2020-00356-5
19. Moutsopoulos HM. Moutsopoulos for his contribution in reviewing the manuscript before submission.
Kaklamanos et al. COVID-19 Immunobiology

Damage. *Clin Exp Immunol* (2020) 202(2):193–209. doi: 10.1111/cei.13525

36. Zhao Y, Kuang M, Li J, Zhu L, Jia Z, Guo X, et al. SARS-CoV-2 Spike Protein Interacts With and ActivatesTLR4. *Cell Res* (2021) 31(7):818–20. doi: 10.1038/s41422-021-00959-9

37. Kawai T, Akira S. The Roles of TLRs, RLRs and NLRs in Pathogen Recognition. *Int Immunol* (2009) 21(4):317–37. doi: 10.1093/immun/ dpx017

38. Perry AK, Chen G, Zheng D, Tang H, Cheng G. The Host Type I Interferon Response to Viral and Bacterial Infections. *Cell Res* (2005) 15(6):407–22. doi: 10.1038/sj.cr.7290250

39. McNab F, Mayer-Barber K, Sher A, Wack A, O’Garra A. Type I Interferons in Infectious Disease. *Nat Rev Immunol* (2015) 15(2):87–103. doi: 10.1038/ nri3787

40. Sa Ribeiro M, Jovenet N, Drexux M, Nisole S. Interplay Between SARS-CoV-2 and the Type I Interferon Response. *PloS Pathog* (2020) 16(7):e1008737. doi: 10.1371/journal.ppat.1008737

41. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* (2020) 181(5):1036–45.e9. doi: 10.1016/j.cell.2020.04.026

42. Galani IE, Rovina N, Lampropoulou V, Triantafyllia V, Mavroudis M, Pavlos E, et al. Untuned Antiviral Immunity in COVID-19 Revealed by Temporal Type I/III Interferon Patterns and Flu Comparison. *Cell Host Microbe* (2021) 22(1):32–40. doi: 10.1016/s41590-020-00840-x

43. Taefehshokr N, Taefehshokr S, Hemmat N, Heit B. Covid-19: Perspectives on Innate Immune Evasion. *Front Immunol* (2020) 11:580641. doi: 10.3389/ fimmu.2020.580641

44. Zhang Y, Zhang J, Chen Y, Luo B, Yuan H, Huang F, et al. The ORF8 Protein of SARS-CoV-2 Mediates Immune Evasion Through Potentially Downregulating MHC-I. (2020). Available at: https://www.biorxiv.org/content/101101202005241183v1

45. Wilk AJ, Rustagi A, Zhao NQ, Roque J, Martínez-Colon GJ, McKechnie JL, et al. A Single-Cell Atlas of the Peripheral Immune Response in Patients With Severe COVID-19. *Nat Med* (2020) 26(7):1070–6. doi: 10.1038/s41591-020-0944-y

46. Giamaurellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglu K, Antoniadou A, Antonakos N, et al. Complex Immune Dysregulation in COVID-19 Patients With Severe Respiratory Failure. *Cell Host Microbe* (2020) 27(6):992–1000.e3. doi: 10.1016/j.chom.2020.04.009

47. Gordon DE, Jang GM, Bouchaddou M, Xu J, Obernier K, White KM, et al. A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing. *Nature* (2020) 583(7816):459–68. doi: 10.1038/s41586-020-2286-9

48. Lauro R, Irrera N, Eid AH, Bitto A. Could Antigen Presenting Cells Represent a Protective Element During SARS-CoV-2 Infection in Children? *Pathogens* (2021) 10(4):476. doi: 10.3390/pathogens10040476

49. Sette A, Croatti S. Adaptive Immunity to SARS-CoV-2 and COVID-19. *Cell* (2021) 184(4):861–80. doi: 10.1016/j.cell.2021.01.007

50. Shibabaw T, Molla MD, Tefert B, Ayelign B. Role of IFN and Complements in Infectious Disease. *Cell Rep* (2020) 30:507–18. doi: 10.1016/j.celrep.2020.04.015

51. Belogiannis K, Florou VA, Fragkou PC, Ferous S, Chatzis L, Polyzou A, et al. SARS-CoV-2 Antigenicity as a Confounding Factor in Immunodiagnostic Assays: A Case Study. *Viruses* (2021) 13(6):1143. doi: 10.3390/v13061143

52. Shibabaw T, Molla MD, Teferi B, Ayelign B. Role of IFN and Complements in Infectious Disease. *Cell Rep* (2020) 30:507–18. doi: 10.1016/j.celrep.2020.04.015

53. Bastard P, Liao P, Liu Z, Le Pen J, Moncada Velez M, Chen J, et al. Inborn Errors of Type I IFN Immunity in Patients With Life-Threatening COVID-19. *Science* (2020) 370(6515):eabd4570. doi: 10.1126/science.abd4570

54. Bastard P, Rosen LB, Zhang Q, Michalidis E, Hofmann HH, Zhang Y, et al. Autoantibodies Against Type I IFNs in Patients with Life-Threatening COVID-19. *Science* (2020) 370(6515):eabd4585. doi: 10.1126/science.abd4585

55. Karaderi T, Bareke H, Kunter I, Seytanoglu A, Cagnan I, Balci D, et al. Host Genetics at the Intersection of Autoimmunity and COVID-19: A Potential Key for Heterogeneous COVID-19 Severity. *Front Immunol* (2020) 11:586111. doi: 10.3389/fimmu.2020.586111

56. Su Y, Chen D, Yuan D, Lusted C, Choi J, Dai CL, et al. Multi-Omics Resolves a Sharp Disease-State Shift Between Mild and Moderate COVID-19. *Cell* (2020) 183(6):1479–95.e20. doi: 10.1016/j.cell.2020.10.037

57. Overmyer KA, Shishkova E, Miller IJ, Balnis J, Bernstein MN, Peters-Carehouse TM, et al. Large-Scale Multi-Omic Analysis of COVID-19 Severity. *Cell Syst* (2021) 12(1):23–40.e7. doi: 10.1016/j.cels.2020.10.003

58. Schlüte-Schrepping J, Reussch, Packid D, Bassler K, Schlückeser S, Zhang B, et al. Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. *Cell* (2020) 182(6):1419–40.e23. doi: 10.1016/j.cell.2020.08.001

59. Silvain A, Chaupis N, Dunsmore G, Goubet AG, Dubuisson A, Derosa L, et al. Elevated Calprotectin and Abnormal Myeloid Cell Subsets Discriminate Severe From Mild COVID-19. *Cell Rep* (2020) 182(6):1401–18.e18. doi: 10.1016/j.celrep.2020.08.002

60. Chevrier S, Zurbuchen Y, Cervia C, Adams S, Raaber ME, de Souza N, et al. A Distinct Innate Immune Signature Marks Progression From Mild to Severe COVID-19. *Cell Rep Med* (2021) 2(1):100166. doi: 10.1016/j.xcrm.2020.10.001

61. Lu A, Wang X, Yu C, Zhang L, Xia L, Yang H, et al. Clinical Characteristics of 148 UK Patients With COVID-19. *Clin Infect Dis* (2020) 71(15):762–8. doi: 10.1093/cid/ciaa248

62. Uhl S, Hoagland D, Moller R, et al. Repurposing. *Cell Syst* (2021) 2(1):100166. doi: 10.1016/j.xcrm.2020.10.001
in SARS-CoV-2- Reactive CD8(+) T Cells. Sci Immunol (2021) 6(55): eabf4782. doi: 10.1126/sciimmunol.abe8757.

76. Erichrander S, Lee Y, Grubbs G, Coyle LM, Klenov L, Akasaka O, et al. Longitudinal Antibody Repertoire in “Mild” Versus “Severe” COVID-19 Patients Reveals Immune Markers Associated With Disease Severity and Resolution. Sci Adv (2021) 7(1):eabf2467. doi: 10.1126/sciadv.eabf2467.

77. Zohar T, Loos C, Fischinger S, Attye C, Wang C, Steind MF, et al. Compromised Humoral Functional Evolution Tracks With SARS-CoV-2 Mortality. Cell (2020) 183:6(19):15-19.e2. doi: 10.1016/j.cell.2020.10.052.

78. Gomez-Rial J, Rivero-Calle I, Salas A, Martinon-Torres F. Role of Monocytes/Macrophages in Covid-19 Pathogenesis: Implications for Therapy. Infect Drug Resist (2020) 13:2485–93. doi: 10.2147/IDR.S258639.

79. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson J, et al. COVID-19 Consider Cytokine Storm Syndromes and Immunosuppression. Lancet (2020) 395(10223):1033–4. doi: 10.1016/S0140-6736(20)30628-0.

80. Damsky W, Peterson D, King B, When Interferon Tiptoes Through COVID-19: Pernio-Like Lesions and Their Prognostic Implications During SARS-CoV-2 Infection. J Am Acad Dermatol (2020) 83(3):e269–70. doi: 10.1016/j.jaad.2020.06.052.

81. Wang N, Zhan Y, Zhu L, Hou Z, Liu F, Song P, et al. Retrospective Multicenter Cohort Study Shows Early Interferon Therapy Is Associated With Favorable Clinical Responses in COVID-19 Patients. Cell Host Microbe (2020) 28(3):455–62.e2. doi: 10.1016/j.chom.2020.07.005.

82. Perico I, Benigni A, Casarighi F, Ng LF, Renia L, Remuzzi G. Immunity, Endothelial Injury and Complement-Induced Coagulopathy in COVID-19. Nat Rev Nephrol (2021) 17(1):46–64. doi: 10.1038/s41581-020-00357-4.

83. Lucas C, Wong P, Klein J, Castro TBR, Silva J, Sundaram M, et al. Longitudinal Analyses Reveal Immunological Misfiring in Severe COVID-19. Nature (2020) 584(7821):463–9. doi: 10.1038/s41586-020-2588-y.

84. Sinha P, Matthey MA, Calfee CS. Is a “Cytokine Storm” Relevant to COVID-19? JAMA Internal Med (2020) 180(9):1152–4. doi: 10.1001/jamainternmed.2020.3313.

85. Peckham H, de Gruijter NM, Raine C, Radziszewska A, Ciurtin C, Zohar T, Loos C, Fischer S, Attye C, Wang C, Steind MF, et al. Disharmonic Inflammation, Neutrophils, and Cytokine Storms in COVID-19: Augmented Neutrophils’ But Impaired Monocytes’ and Dendritic Cells’ Responsiveness. Cells (2020) 9(10):2206. doi: 10.3390/cells9102206.

86. Bonafe M, Prattichizzo F, Giuliani A, Storci G, Sabbatinelli J, Olivieri F. Multicenter Cohort Study Shows Early Interferon Therapy Is Associated With Favorable Clinical Responses in COVID-19 Patients Reveals Immune Markers Associated With Disease Severity and Resolution. Sci Rep (2020) 10:15838. doi: 10.1038/s41598-020-77218-9.

87. Theoharides TC. COVID-19, Pulmonary Mast Cells, Cytokine Storms, and Beneficial Actions of Luteolin. BioFactors (2020) 46(3):306–8. doi: 10.1002/biof.1633.

88. Channappanavar R, Perlman S. Pathogenic Human Coronavirus Infections: Causes and Consequences of Cytokine Storm and Immunopathology. Semin Immunopathol (2017) 39(5):529–39. doi: 10.1007/s00281-017-0629-x.

89. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

90. Pernio-Like Lesions and Their Prognostic Implications During SARS-CoV-2 Infection. J Am Acad Dermatol (2020) 83(3):e269–70. doi: 10.1016/j.jaad.2020.06.052.

91. Kelley WJ, Zemans RL, Goldstein DR. Cellular Senescence: Friend or Foe to Reproductive Viral Infections? Eur Respir J (2020) 56(6):2002708. doi: 10.1183/13993003.02708-2020.

92. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

93. Kelley WJ, Zemans RL, Goldstein DR. Cellular Senescence: Friend or Foe to Reproductive Viral Infections? Eur Respir J (2020) 56(6):2002708. doi: 10.1183/13993003.02708-2020.

94. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

95. Kelley WJ, Zemans RL, Goldstein DR. Cellular Senescence: Friend or Foe to Reproductive Viral Infections? Eur Respir J (2020) 56(6):2002708. doi: 10.1183/13993003.02708-2020.

96. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

97. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

98. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

99. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.

100. Wu D, Yang XO. TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib. J Microbiol Infect Dis (2020) 53(3):368–70. doi: 10.1016/j.jmiid.2020.03.005.
Levi M, van der Poll T, Buller HR. Bidirectional Relation Between Inflammation and Coagulation. Circulation (2004) 109(22):2698–704. doi: 10.1161/01.CIR.0000136660.51520.9A

138. Jin Y, Ji W, Yang H, Chen S, Zhang W, Duan G. Endothelial Activation and Dysfunction in COVID-19: From Basic Mechanisms to Potential Therapeutic Approaches. Signal Transduct Target Ther (2020) 5(1):293. doi: 10.1038/s41392-020-00454-7

139. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial Cell Injury and Endothelitis in COVID-19. Lancet (2020) 395(10234):1417–8. doi: 10.1016/S0140-6736(20)30937-5

140. Buzhdagyan TP, DeOre BJ, Baldwin-Leclair A, Bullock TA, McGary HM, Khan JA, et al. The SARS-CoV-2 Spike Protein Alters Barrier Function in 2D Static and 3D Microfluidic In-Vitro Models of the Human Blood-Brain Barrier. Neurobiol Dis (2020) 146:105131. doi: 10.1016/j.nbd.2020.105131

141. Akyeampong A, Verdenel SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in COVID-19. N Engl J Med (2020) 383(2):120–8. doi: 10.1056/NEJMoa2015432

142. Kempuraj D, Selvakumar GP, Ahmed ME, Thangavel R, Khan A, et al. COVID-19, Mast Cells, Cytokine Storm, Psychological Stress, and Neuroinflammation. Neurosci (2020) 26(5-6):402–14. doi: 10.1177/10738849147

143. Libby P, Luscher T. COVID-19 Is, in the End, an Endothelial Disease. Eur Heart J (2020) 41(32):3038–44. doi: 10.1093/eurheartj/ehaa623

144. Bryce C, Grimis Z, Pujadas E, Ahuja S, Beasley MB, Albrecht R, et al. Pathophysiology of SARS-CoV-2: The Mount Sinai COVID-19 Autopsy Experience. Mod Pathol (2021) 34(8):1456–67. doi: 10.1038/s41379-021-00793-y

145. Malas MB, Naazie IN, Elsayed N, Marmor R, Clary B, et al. Autopsy Findings in COVID-19-Related Deaths: A Literature Review. Forensic Sci Med Pathol (2020) 17:279–96. doi: 10.1002/fsmp.200310-8

146. Gupta A, Madhavan MV, Sehgal K, Nair M, Mahajan S, Shewrat TS, et al. Extrapulmonary Manifestations of SARS-CoV-2. Nat Med (2020) 26(7):1017–32. doi: 10.1038/s41591-020-0968-3

147. Engelmann B, Massberg S. Thrombosis as an Intravascular Effector of Inflammatory Immunity. Nat Rev Immunol (2013) 13(1):34–45. doi: 10.1038/nri3345

148. Malas MB, Naazie IN, Elsayed N, Mathloothi A, Marmor R, et al. Pathophysiology of SARS-CoV-2: The Mount Sinai COVID-19 Autopsy Experience. Mod Pathol (2021) 34(8):1456–67. doi: 10.1038/s41379-021-00793-y

149. Bonaventura A, Vecchie A, Dagna L, Martinod K, Dixon DL, Van Tassell–Wittmer P, et al. Autopsy Findings in COVID-19-Related Deaths: A Literature Review. Forensic Sci Med Pathol (2020) 17:279–96. doi: 10.1002/fsmp.200310-8

150. Gupta A, Madhavan MV, Sehgal K, Nair M, Mahajan S, Shewrat TS, et al. Extrapulmonary Manifestations of SARS-CoV-2. Nat Med (2020) 26(7):1017–32. doi: 10.1038/s41591-020-0968-3

151. Bronfman L, Ligabue G, Nasillo V, Lusenti B, Gennari W, Fabbiani L, et al. Longitudinal Multi-Omics Analyses Identify Responses of Megakaryocytes, Erythroid Cells, and Plasmablasts as Hallmarks of Severe COVID-19. Immunity (2020) 53(6):1296–314.e9. doi: 10.1016/j.immuni.2020.11.017

152. Roncati L, Ligabue G, Nasillo V, Lusenti B, Gennari W, Fabbiani L, et al. A Proof of Evidence Supporting Abnormal Immunothrombosis in Severe COVID-19: Naked Megakaryocyte Nuclei Increase in the Bone Marrow and Lungs of Critically Ill Patients. Platelets (2020) 31(8):1085–9. doi: 10.1080/08051071.2020.1810224

153. Thaci J, Lismam T. Pulmonary Megakaryocytes in Coronavirus Disease 2019 (COVID-19): Roles in Thrombi and Fibrosis. Semin Thromb Hemost (2020) 46(7):831–4. doi: 10.1055/s-0040-1714274

154. Marra BK, Deneuve F, Middleton EA, Portier I, Rowley JW, Stubben C, et al. Platelet Gene Expression and Function in Patients With COVID-19. Blood (2020) 136(11):1317–29. doi: 10.1182/blood.202000721

155. Zaid Y, Puhm F, Alassy L, Naya A, Oudghiri M, Khalki L, et al. Platelets Can Associate With SARS-CoV-2 RNAs, in Are Hyperactivated in COVID-19. Circ Res (2020) 127:1404–18. doi: 10.1161/CIRCRESAHA.120.317073

156. Taus F, Salvagno G, Cane S, Fava C, Mazzaferri F, Carrara E, et al. Platelets Promote Thromboinflammation in SARS-CoV-2 Pneumonia. Arterioscler.
234. Ren X, Wen W, Fan X, Hou W, Su B, Cai P, et al. COVID-19 Immune Features Revealed by a Large-Scale Single-Cell Transcriptome Atlas. Cell (2021) 184(7):1895–913.e19. doi: 10.1016/j.cell.2021.01.053

235. Garcia G Jr., Sharma A, Ramiah A, Sen C, Purkayastha A, Kohn DB, et al. Antiviral Drug Screen Identifies DNA-Damage Response Inhibitor as Potent Blocker of SARS-CoV-2 Replication. Cell Rep (2021) 35(1):108940. doi: 10.1016/j.celrep.2021.108940

236. Dittmar M, Lee JS, Whig K, Segrist E, Li M, Kamalia B, et al. Drug Repurposing Screens Reveal Cell-Type-Specific Entry Pathways and FDA-Approved Drugs Active Against SARS-CoV-2. Cell Rep (2021) 35(1):108959. doi: 10.1016/j.celrep.2021.108959

237. Camell CD, Yousefzadeh MJ, Zhiu Y, Langhi Prata LGP, Huggins MA, Pierson M, et al. Senolytics Reduce Coronavirus-Related Mortality in Old Mice. Science (2021) 373(6552):eabe4832. doi: 10.1126/science.abe4832

238. Nehme J, Borghesan M, Mackedenski S, Bird TG, Demaria M. Cellular Senescence as a Potential Mediator of COVID-19 Severity in the Elderly. Aging Cell (2020) 19(10):e13237. doi: 10.1111/acel.13237

239. Myrianthopoulos V, Evangelou K, Vasileiou PVS, Cooks T, Vassilakopoulos TP, Pangalis GA, et al. Senescence and Senotherapeutics: A New Field in Cancer Therapy. Pharmacol Ther (2019) 193:31–49. doi: 10.1016/j.pharmthera.2018.08.006

240. Cox LS, Lord JM. Targeting Aging Cells Improves Survival. Science (2021) 373(6552):281–2. doi: 10.1126/science.ab4474

241. Sargiacomo C, Sotgia F, Lisanti MP. COVID-19 and Chronological Aging: Senolytics and Other Anti-Aging Drugs for the Treatment or Prevention of Corona Virus Infection? Aging (2020) 12(8):6511–7. doi: 10.18632/aging.103001

242. Seoane R, Vidal S, Bouzaher YH, El Motiam A, Rivas C. The Interaction of Viruses With the Cellular Senescence Response. Biology (2020) 9(12):455. doi: 10.3390/biology9120455

243. Kaur A, Macip S, Stover CM. An Appraisal on the Value of Using Nutraceutical Based Senolytics and Senostatics in Aging. Front Cell Dev Biol (2020) 8:218. doi: 10.3389/fcell.2020.00218

244. Carpenter VJ, Salch T, Gewirtz DA. Senolytics for Cancer Therapy: Is All That Glitters Really Gold? Cancers (2021) 13(4):723. doi: 10.3390/cancers13040723

245. Coppe JP, Desprez PY, Krtolica A, Campisi J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. Ann Rev Pathol (2010) 5:99–118. doi: 10.1146/annurev-pathol-121808-102144

246. Casel MAB, Rollon BG, Choi YK. Experimental Animal Models of Coronavirus Infections: Strengths and Limitations. Immune Netw (2021) 21(2):e12. doi: 10.4110/in.2021.21.e12

247. Jia W, Wang J, Sun B, Zhou J, Shi Y, Zhou Z. The Mechanisms and Animal Models of SARS-CoV-2 Infection. Front Cell Dev Biol (2021) 9:578825. doi: 10.3389/fcell.2021.578825

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| Acronym/Abbreviation | Full Form |
|----------------------|-----------|
| anti-RNP             | Anti-ribonucleoprotein |
| anti-CCP             | Anti-cyclic citrullinated peptide |
| APOBE1               | Apolipoprotein B mRNA-editing enzyme catalytic polypeptide 1 |
| ARDS                 | Acute respiratory distress syndrome |
| c-ANCA               | Cytoplasmic antineutrophil cytoplasmic antibody |
| CCL                  | Chemokine (C-C motif) ligand |
| CCR                  | Chemokine (C-C motif) receptor |
| CD                   | Cluster of differentiation |
| CD3d, CD3e           | T-cell surface glycoprotein CD3 delta chain, epsilon chain |
| CD16                 | Fc fragment of IgG, low affinity receptor III (FcγRIII) |
| CD4                  | Killer cell lectin-like receptor subfamily D, member 1 (KLRD1) |
| CD107a               | Lymphosomial-associated membrane protein 1 (LAMP-1) |
| CD169                | sialoadhesin |
| CD247                | T-cell surface glycoprotein CD3 zeta chain (CD3ζ) |
| CNS                  | Central nervous system |
| COPD                 | Chronic obstructive pulmonary disease |
| CRP                  | C-reactive protein |
| CXCL                 | Chemokine (C-X-C motif) ligand |
| CXCR                 | Chemokine (C-X-C motif) receptor |
| DC-SIGN              | Dendritic Cell-Specific Intercellular adhesion molecule 3-Grabbing Non-integrin |
| DDP4                 | Dipeptidyl peptidase-4 inhibitor |
| DNA                  | Deoxyribonucleic acid |
| dsRNA                | Double-stranded ribonucleic acid |
| ER                   | Endoplasmic Reticulum |
| FABP4                | Fatty-acid binding protein 4 |
| FADD                  | Fas-associated protein with death domain |
| Ficolins             | Oligomeric lectins with subunits consisting of both fibronectin (Fn)-like globular domains and collagen (Col)-like long thin stretches with lectin (Lin) activity |
| FcγRIII               | Fc fragment of IgG, low affinity receptor III (CD16) |
| Foxp3                | Forkhead box P3 |
| FX                   | Coagulation factor X |
| FXIII                | Coagulation factor X |
| G-CSF                | Granulocyte colony-stimulating factor |
| GM-CSF               | Granulocyte-monocyte colony-stimulating factor |
| GSDME                | Gasdermin E |
| HDAC2                | Histone deacetylase 2 |
| HLA                  | Human leukocyte antigen |
| IACM1                | Intracellular adhesion molecule-1 |
| IFIT                 | Interferon-induced proteins with Tetraticopeptide repeats, |
| IFN                  | Interferon |
| IFNAR                | Interferon-α/β receptor |
| IL                   | Interleukin |
| INOs                 | Inducible nitric oxide synthase |
| IP-10 (or IP-10)     | Interferon gamma-induced protein 10 |
| CXCL10               | Chemokine (C-X-C motif) ligand |
| IRF                  | Interferon regulatory factor |
| ISGs                 | Interferon-stimulated genes |
| KIRs                 | Killer-immunoglobulin like receptors |
| KLRG1                | Killer cell lectin-like receptor subfamily G member 1 |
| LAG3                 | Lymphocyte activation protein 3 |
| LCK                  | Lymphocyte-specific protein tyrosine kinase |
| MAC                  | Membrane attack complex |
| MAP2K2               | Dual specificity mitogen-activated protein kinase 2 |
| MASP                  | Mannan-binding lectin serine protease |
| MBP                  | Mannose-binding protein |
| MCP                  | Monocyte chemotactic protein |
| MD-5                 | Melanoma differentiation-associated gene 5 |
| MHC                  | Major histocompatibility complex |
| MIP-1                | Macrophage inflammatory protein 1 |
| MLKL                  | Mixed lineage kinase domain like |
| MPO                  | Myeloperoxidase |
| MX1                  | Myxovirus resistance 1 |
| NETs                 | Neutrophil extracellular traps |
| NK cells             | Natural killer cells |
| NKG2A-NKG2D          | NKG2A-NKG2D (NKG2-A, NKG2-D) |
| NLRP3                | NLR family pyrin domain containing 3 |
| NLRs                 | Nucleotide-binding Oligomerization Domain (NOD)-Like Receptors |
| NO                   | Nitric oxide |
| nsp                  | Non-structural protein |
| p-ANCA               | Perinuclear antineutrophil cytoplasmic antibody |
| PAI                  | Plasminogen activator inhibitor |
| PAR                  | Protease activated receptor |
| PD1                  | Programmed cell death protein 1 |
| PD-L1                | Programmed death ligand 1 |
| PF4                  | Platelet-factor 4 |
| PRPs                 | Pattern Recognition Receptors, PRTN3: proteinase 3 (PR3) |
| RIPK                 | Receptor-interacting serine/threonine-protein kinase |
| RLRs                 | Retinoic acid-inducible Gene-I-Like Receptors |
| SPP1                 | Secreted Phosphoprotein 1 (Osteopontin) |
| STAT                 | Signal transducer and activator of transcription |
| Tbx1                  | T-box protein expressed in T cells |
| TAK1                 | TAK1-binding kinase 1 |
| TCR                  | T-cell receptor |
| Th                   | T-helper |
| TIM3                 | T-cell immunoglobulin mucin-3 |
| TLR                  | Toll-like receptor |
| TNF-x                | Tumor necrosis factor x |
| TRAC                  | T-cell receptor alpha constant |
| TRBC                  | T-cell receptor beta constant |
| TRIM                  | Tripartite motif-containing |
| UNC93B1               | Unc-93 Homolog B1 |
| VCAM1                | Vascular cell adhesion protein-1 |
| VWF                  | Von Willebrand factor |
| ZAP70                | Zeta-chain-associated protein kinase 70 |
| ZO-1                 | Zonula occludens-1 |

(Continued)