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PATHOGENESIS OF THE INITIAL STAGES OF SEVERE COVID-19

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Since SARS-CoV-2 first appeared in humans, the scientific community has tried to gather as much information as possible in order to find effective strategies for the containment and treatment this pandemic coronavirus. We reviewed the current published literature on SARS-CoV-2 with an emphasis on the distribution of SARS-CoV-2 in tissues and body fluids, as well as data on the expression of its input receptors on the cell surface. COVID-19 affects many organ systems in many ways. These varied manifestations are associated with viral tropism and immune responses of the infected person, but the exact mechanisms are not yet fully understood. We emphasize the broad organotropism of SARS-CoV-2, as many studies have identified viral components (RNA, proteins) in many organs, including immune cells, pharynx, trachea, lungs, blood, heart, blood vessels, intestines, brain, kidneys, and male reproductive organs. Viral components are present in various body fluids, such as mucus, saliva, urine, cerebrospinal fluid, semen and breast milk. The main SARS-CoV-2 receptor, ACE2, is expressed at different levels in many tissues throughout the human body, but its expression levels do not always correspond to the detection of SARS-CoV-2, indicating a complex interaction between the virus and humans. We also highlight the role of the renin-angiotensin aldosterone system and its inhibitors in the context of COVID-19. In addition, SARS-CoV-2 has various strategies that are widely used in various tissues to evade innate antiviral immunity. Targeting immune evasion mediators of the virus can block its replication in COVID-19 patients. Together, these data shed light on the current understanding of the pathogenesis of SARS-CoV-2 and lay the groundwork for better diagnosis and treatment of patients with COVID-19.

Keywords: *COVID-19; coronavirus; SARS-CoV-2; ACE2 receptor; antiviral immunity; COVID-19 pathogenesis.*

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List of abbreviations

APV — artificial pulmonary ventilation
ARDS — acute respiratory distress syndrome
RAAS — renin-angiotensin-aldosterone system
ACE2 — angiotensin-converting enzyme 2
Ang — angiotensin
B1R — bradykinin B1 receptor
DABK ([des-Arg973]-bradykinin) — active metabolite of bradykinin
FDA — Food and Drug Administration
FGF — fibroblast growth factor
G-CSF — granulocyte colony-stimulating factor
GM-CSF — granulocyte-macrophage colony-stimulating factor
JAK2 — Janus kinase 2
IFN — interferon
IL — interleukin
IP-10 — interferon-γ-inducible protein 10
IRF1 — interferon regulatory factor
ISG — interferon-stimulated gene
MIP — macrophage inflammatory protein
MCP-1 — monocyte chemoattractant protein 1
NRP1 — transmembrane cell receptor neuropilin 1, product of the NRP1 gene
PDGF — platelet-derived growth factor
STAT1 — signal transducer and activator of transcription 1
Th — T-helper, T lymphocytes
TMPRSS2 (transmembrane protease serine 2) — membrane-bound serine protease, TMPRSS2 gene product
TNF-α — tumor necrosis factor alpha
VEGF — vascular endothelial growth factor

INTRODUCTION

On March 11, 2020, the World Health Organization declared a COVID-19 pandemic. During the COVID-19 pandemic, 167,011,807 people were infected with the SARS-CoV-2 coronavirus worldwide (as of 05/25/2021) with a registered mortality rate of 3,472,068 people [1]. The development of fatal complications of COVID-19 is accelerating due to acute respiratory distress syndrome (ARDS), secondary infections, sepsis, and multiple organ failure [2]. About 5% of COVID-19 patients have severe symptoms, including septic shock, ARDS, and organ dysfunction, while 80% of patients have mild or no symptoms [3].

INFECTIOUSNESS

COVID-19 affects many organ systems in a variety of ways. These varied manifestations are associated with viral tropism and the immune response of the infected person, but the exact mechanisms are not yet fully understood. The broad organotropism of SARS-CoV-2 is indicated by the presence of viral components (RNA, proteins) in many human organs, including the pharynx, trachea, lungs, heart, vessels, blood, intestines, brain, kidneys, liver, and testes, as well as in various fluids of the body such as saliva, mucus, urine, cerebrospinal fluid, semen, and breast milk. The SARS-CoV-2 virus infects cells through angiotensin-converting enzyme-2, a polyfunctional membrane-bound receptor which is present on the cell membranes of all organs and makes tissues vulnerable to viral invasion [4–7]. Cellular serine protease TMPRSS2 (transmembrane protease serine 2) promotes the penetration of the coronavirus by cleaving the viral S-protein and the ACE2 receptor and thereby facilitating the fusion of the virus with the cell membrane and internalization of the virus into the target cell [4]. Expression of ACE2 and TMPRSS2 protease (and possibly other coreceptors) is widespread in cells of many tissues, including nasal goblet secretory cells; cells of the upper respiratory tract; alveolar epithelial cells type 2; epithelial cells of the esophagus; glandular cells of the stomach; intestinal enterocytes; cholangiocytes; macrophages; myocytes of the heart and skeletal muscles; cells of the proximal tubules of the kidneys, bladder, liver, nervous system and retina; parietal cells of the glomeruli; vascular endothelial cells and keratinocytes [8–13]. Cell lines of various tissue origins with ACE2 overexpression can have different levels of SARS-CoV-2 infection; in general, the susceptibility of cells to infection correlates with the level of ACE2 expression. When ACE2 and TMPRSS2 are expressed together, most cell lines become highly susceptible to infection [14].

According to the Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) projects, analysis of ACE2 expression in 31 human tissues revealed the highest receptor expression in the small intestine, followed by kidneys, testes, heart and lungs, thyroid gland and adipose tissue, the lowest expression in blood, spleen, bone marrow, blood vessels, and muscles [15]. This distribution is confirmed by S.D. Unudurthi et al. [16]. Moderate levels of ACE2 expression have been established in the lungs, colon, liver, bladder, and adrenal glands. In the human heart, ACE2 expression is low in cardiomyocytes and high in pericytes [17]. ACE2 expression in cardiomyocytes is significantly increased in patients with heart diseases. Lower levels of expression were registered in fibroblasts, endothelial cells, and leukocytes. A wide range of symptoms and complications of COVID-19 is associated with the presence of this receptor, such as anosmia and ageusia, respiratory and gastrointestinal disorders, and multiple organ dysfunction, including damage to the heart, liver, kidneys, and brain [18]. Immunohistochemical studies confirm that the small intestine expresses higher levels of ACE2 mRNA than any other organ. The duodenum, kidneys, and testes also showed strong signals, while the signal in the airways was weak [19].

The SARS-CoV-2 coronavirus uses the viral spike glycoprotein (S-protein) to attach and penetrate into the cell [20]. The binding affinity of the S (spike) protein of the SARS-CoV-2 virus to ACE2 is 10–20 times higher than that of the S-protein of the SARS-CoV virus [21]. The oral cavity is one of the main sites of SARS-CoV-2 infection and facilitates transmission of the virus through saliva. Analysis of S-protein expression confirmed the virus presence in the salivary glands and oral mucosa. The saliva of patients infected with SARS-CoV-2 contains epithelial cells expressing ACE2 and SARS-CoV-2 RNA. The dynamics of virus shedding and the amount of viral particles in saliva correlate with the severity of COVID-19 symptoms, including ageusia. After recovery, antibodies against the SARS-CoV-2 proteins are found in the saliva. Single cell RNA sequencing (scRNAseq) revealed a unique anatomical and cellular diversity in the oral cavity and oropharynx that affects susceptibility to infection. The secretory epithelium of the salivary glands expresses the main factors of penetration into the epithelium, and the joint expression of ACE2 and TMPRSS2 was found in mucous, ductal, and myoepithelial cells. The extensive expression of viral penetration factors and significant

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infection of the epithelium of the small salivary glands in the tongue, palate, and mucous membranes, which are foci of infection with SARS-CoV-2, have been confirmed. The symptoms of COVID-19 are due to infection of the salivary glands and tongue, such as dry mouth and changes in taste; the absence of such symptoms explains the phenomenon of "silent spreaders" of COVID-19. The presence of SARS-CoV-2 infected squamous epithelial cells in saliva provides a mechanism for the spread of the disease. The detection of an oral source of infection and replication of SARS-CoV-2 in the salivary glands and the natural pathway of the virus through saliva emphasizes the need for simultaneous testing of the oral and nasopharyngeal sites. Given the documented oral infection of SARS-CoV-2 and the ease of transmission through saliva, asymptomatic transmission of SARS-CoV-2 is considered the "vulnerable spot" of this pandemic, since it causes nearly 45% of COVID-19 cases. These data provide strong evidence to support universal public healthcare measures, including wearing masks, social distancing, and washing hands to limit exposure to potentially infectious aerosols and oral cavity fomites [22].

First of all, the SARS-CoV-2 virus infects the lungs, causing ARDS and respiratory failure in some patients, which represent one of the main causes of lethal outcome in COVID-19 patients [23]. However, as tissue samples from infected and/or deceased individuals become available and screened for viral proteins or particles, there is a growing awareness that SARS-CoV-2 can affect extrapulmonary organs. Virus replication was revealed in the intestine [14, 24, 25], lungs, esophagus, liver, and kidneys [14, 26, 27], as well as heart [14, 27, 28], and brain [14, 27, 29]. The maximum number of copies of SARS-CoV-2 per cell is found in the respiratory tract, while lower levels are found in the kidneys, liver, heart, brain, and blood. These data indicate the extensive organotropism of SARS-CoV-2.

Data on the localization of viral receptors can present an idea of the mechanisms of virus penetration, tissue tropism and pathogenesis of the disease; therefore, it is of interest to study the possible correlation of COVID-19 symptoms with the distribution pattern of ACE2. Angiotensin-converting enzyme (ACE) is known for its role in the regulation of blood pressure by converting the decapeptide angiotensin I (AngI) to the active octapeptide angiotensin II (AngII) and cleaving bradykinin and neurotensin. Although ACE and ACE2 have significant sequence homology in the catalytic domains, they act on different peptide substrates of angiotensins. Gene ontology studies have revealed new data on the potential physiological role of ACE2. The five most significant terms in gene ontology include angiogenesis, blood vessel morphogenesis, vasculature development, cardiovascular development, and blood vessel development. Gene ontology analysis also established that ACE2 is involved in the "complement and coagulation cascade" [19]. Indeed, patients with severe COVID-19 often have blood clotting disorders that mimic systemic coagulopathies, such as disseminated intravascular coagulation (DIC) and thrombotic microangiopathy, but COVID-19 has its own distinctive features (stronger increase in D-dimer concentration, moderate decrease in platelet count and an insignificant lengthening of prothrombin time, which may remain unnoticed if expressed as an international normalized ratio) [30]. Thus, angiogenesis and morphogenesis of blood vessels can be considered as another presumed function of ACE2 in addition to its classical role as a key angiotensin (1-7)-forming enzyme [19].

Research of SARS-CoV-2 penetration into cells has focused almost entirely on ACE2. Low levels of ACE2 expression in the cells of the respiratory and olfactory epithelium indicate the participation in this process of cofactors necessary to facilitate the interaction of the virus with such cells. The transmembrane cellular neuropilin receptor-1 (NRP1) represents a factor that enhances the interaction of the virus with ACE2 [31]. The transmembrane protease furin cleaves the full-length S-protein of the SARS-CoV-2 virus into two linked polypeptides (S1 and S2) to form the carboxyterminal amino acid sequence Arg-Arg-Ala-Arg (RRAR) on the S1 subunit, which increases the association of the virus with NPR1 which is expressed in the respiratory and olfactory epithelium and regulates biological processes, including axonal growth, angiogenesis, and vascular permeability [20]. Binding of SARS-CoV-2 to the NPR1 receptor increases its tropism and infectivity in cell cultures and *in vivo*. The S1 subunit of the viral S protein, which carries the receptor binding domain (RBD), binds to ACE2, while the transmembrane S2 subunit facilitates fusion of the viral envelope with the host cell membrane. The combination of a high affinity for the ACE2 receptor and an increased tropism due to the presence of a polybasic cleavage site in its spike protein increases the ability of SARS-CoV-2 to infect extrapulmonary organs compared to other coronaviruses, hence entry receptors and proteases are potential therapeutic targets for combating infection with SARS-CoV-2 [16].

The presence of a furin-type cleavage site (RRAR) at the junction of the S1 and S2 subunits in the S-pro-

tein of the SARS-CoV-2 virus, which is absent in SARS-CoV, explains the accelerated spread of SARS-CoV-2. Similar sequences have been found in the S proteins of many other human pathogenic viruses, including Ebola virus, HIV-1, and highly virulent strains of avian influenza. The presence of the RRAR cleavage site in SARS-CoV-2 leads to increased pathogenicity due to the activation of binding to cell surface receptors. Furin-mediated processing affects SARS-CoV-2 tropism and increases infectivity. By binding substrates cleaved by furin, NRP1 enhances significantly the cytopathic activity of the SARS-CoV-2 virus, however, the infectivity of mutant SARS-CoV-2 with an altered furin cleavage site does not depend on NRP1 [31]. Proteins S1 and S2 formed during cleavage remain non-covalently bound to the serine protease TMPRSS2. Blocking these interactions, including inhibition of furin, with selective inhibitors reduces the penetration of SARS-CoV-2 into cells. Thus, the NRP1 receptor serves as a cofactor for the interaction of the virus with ACE2 and a potential therapeutic target for COVID-19. By sequential staining using antibodies against SARS-CoV-2 proteins (to distinguish between extracellular and intracellular viral particles), it was revealed that NRP1 depletion does not affect the binding of SARS-CoV-2 to the cell surface, but reduces the virus internalization [20].

The expression of RNA of NRP1 and its homologue NRP2 is increased in SARS-CoV-2-positive cells compared to neighboring uninfected cells in the bronchoalveolar lavage fluid of critically ill patients with COVID-19. In lung tissue and olfactory epithelium, ACE2 is expressed at very low levels, and NRP1 and NRP2 are abundantly expressed in almost all lung and olfactory cells, with the highest expression levels in endothelial cells. Due to the impaired olfaction in a significant number of patients with COVID-19 and the presence of NRP1 in the olfactory epithelium, an immunohistochemical analysis of autopsy samples of COVID-19 patients was performed. Immunoreactivity of NRP1 was revealed in the surface layer of the human respiratory and olfactory epithelium. ACE2 was almost not detected in these tissues. Inside the olfactory epithelium, NRP1 was registered in cells positive for the oligodendrocyte transcription factor 2 (OLIG2) which is mainly expressed by the progenitors of olfactory neurons [31].

The genomic editing system CRISPR (clustered regularly interspaced short palindromic repeat) has been used by a group of specialists from a number of major USA medical centers as a tool for screening the genomes of infected cells. In addition to the genes *ACE2*, *TMPRSS2*, and *CTSL*, encoding such well-known infectivity factors as the ACE2 receptor, TMPRSS2 proteases, and cathepsin L, other genes have been found that affect the infectivity of SARS-CoV-2, including HMGB1 (high-mobility group box 1). The pleiotropic nuclear protein HMGB1, which binds nucleosomes, transports genetic material and functions as a secreted alarmin in response to viral infection, regulates ACE2 expression, and is critical for penetration of SARS-CoV and SARS-CoV-2 into the cell. It is noteworthy that HMGB1 regulates ACE2 expression intracellularly rather than through its cytokine or alarmin function, suggesting a distinct mechanism of action for HMGB1 in SARS-CoV-2 infection. Low molecular weight antagonists of the identified gene products inhibit infection with SARS-CoV-2 virus in human and monkey cells, demonstrating the conservative role of these genetic matches in different species. This approach identifies cellular infectivity factors that regulate susceptibility to coronaviruses and identifies SARS-CoV-2 specific therapeutic targets [32].

ACE and ACE2 function as zinc metallopeptidases, performing a balancing function in the renin-angiotensin-aldosterone system (RAAS), but have different substrate specificities that determine their different roles in the RAAS. Most of ACE2 is in an insoluble form associated with cell membranes. In both soluble and insoluble form, ACE2 converts AngI to angiotensin-(1-9) and AngII to angiotensin-(1-7); ACE2, unlike vasoactive ACE, does not degrade bradykinin and is insensitive to conventional ACE inhibitors. Angiotensin-(1-7) controls various hazardous processes in the body, such as inflammation, angiospasm, and thrombosis, counteracts the vasoconstrictor effect of AngII, exhibits anti-fibrotic, antioxidant, and antihypertrophic protective properties and promotes the development of angiogenesis in damaged tissue during myocardial infarction and stroke [19]. Expression of ACE2 appears to protect the lungs from damage. Low ACE2 activity in the lungs promotes their inflammation and rapid neutrophil infiltration, which increases inflammation. Angiotensin-(1-9) has shown a protective effect on cardiac and vascular remodeling in COVID-19 [10].

Proteins ACE2 and TMPRSS2 are expressed in endothelial cells, which makes them susceptible to SARS-CoV-2 as target cells. ACE2 is the key negative regulator of the RAAS [10, 18]. After penetration into the cell, the virus removes ACE2 from the cell surface, weakens the activity and protective functions of the receptor, which results in the RAAS imbalance. Therefore, in addition to pulmonary sequelae such as ARDS,

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inactivation of ACE2 has great potential to impair cardiovascular system health [33, 34]. Low expression of ACE2, caused by various aspects (old age, diabetes, hypertension), increases the SARS-CoV-2 infection severity [35]. This is consistent with epidemiological statistics showing that the majority of patients with serious and fatal manifestations of COVID-19 are the elderly individuals and patients with cardiovascular diseases [36]. ACE2 deficiency has been revealed in SARS-CoV-2 infected patients with various risk factors such as hypertension, cardiovascular diseases, diabetes, and old age. Thus, suppression of ACE2 activity by the virus may be particularly detrimental to patients with an underlying ACE2 deficiency. Further decrease in ACE2 expression after infection with SARS-CoV-2 may exacerbate the imbalance between the protective and adverse roles of the RAAS. Dysregulation of this axis in the lungs leads to thrombotic and inflammatory conditions [35]. Increased potassiumuresis as a marker of RAAS activation may be associated with high AngII levels in COVID-19 patients [37].

In addition to the RAAS, ACE2 is associated with the kallikrein-kinin system, the activation of which leads to the release of bradykinin and is significant in the inflammatory process. One of the functions of ACE2 is the hydrolysis of the active metabolite of bradykinin ([des-Arg973]-bradykinin, DABK). After SARS-CoV-2 binds to the ACE2 receptor to enter the cell, the loss of ACE2 function occurs, caused by endocytosis and activation of proteolytic cleavage and processing of ACE2 [4, 10]. Invasion of SARS-CoV-2 reduces the expression of ACE2, suppresses its protective functions, including anti-inflammatory ones, and enhances the effects of AngII in infected patients [35, 38], leading to pulmonary inflammation, thrombosis, and acute kidney damage [3]. Decreased levels and weakening of ACE2 activity due to viral infection leads to impaired DABK inactivation, increased signaling through the bradykinin B1 receptor (B1R) and activation of the kallikrein-kinin system through the B1R, which in turn increases leukocyte involvement and fluid extravasation in the lungs. Blocking the production of bradykinin or its receptors may open a new therapeutic window for the treatment of COVID-19-associated ARDS, especially before the disease becomes irreversible [39].

Kallikreins are found in many human tissues, including the epithelium of the upper and lower respiratory tract. There are two classical ways of kinin formation, plasma and tissue. The substrate of plasma kallikrein is bradykinin, and the substrate of tissue kallikreins is low molecular weight kininogen which leads to the formation of the decapeptide lysylbradykinin (kallidin). After activation of factor FXIIa, plasma kallikrein cleaves bradykinin, releasing the nonapeptide bradykinin-1-9. During inflammation, plasmin enhances the cleavage of bradykinin by plasma kallikrein. Kallidin and its active metabolite DABK bind to two different receptors, kallidin is a ligand of the B2 receptor (B2R), and DABK is the main agonist of the B1R receptor. B2R is constitutively expressed in mammalian cells (e.g., endothelial and smooth muscle cells), whereas B1R expression is mainly induced by cytokines during infections and immunopathology. Through B2R, bradykinin activates signaling pathways, which results in expansion and increase in vascular permeability, the development of edema, hypotension, pain, and fever which are typical clinical signs of COVID-19. Bradykinin is one of the most potent inflammatory mediators capable of stimulating the production of superoxide radicals, nitric oxide, histamine, arachidonic acid, prostaglandin E2, prostacyclin, inflammatory cytokines such as interleukins 1 and 6 (IL-1, IL-6), tumor necrosis factor alpha (TNF-α), and tissue plasminogen activator (t-PA). The close relationship between the kallikrein-kinin system and the RAAS is evidenced by the fact that B2R forms homo- and heterodimers with several RAAS receptors involved in the regulation of some physiological functions, including the regulation of the risk of thrombosis. B1R mediates several reactions, including vasodilation, hypotension, and increased vascular permeability, all of which are typical aspects of COVID-19. In patients infected with the SARS-CoV-2 virus, the expression of genes for kallikreins and bradykinin receptors is increased [40].

The role of bradykinin in the pathogenesis of COVID-19 is indicated by some clinical aspects noted in patients. F.L. van de Veerdonk et al. [41] believe that lung angioedema due to the activation of bradykinin B1R and B2R receptors on lung endothelial cells is a significant trait of COVID-19 and that blocking of these receptors and inhibiting plasma kallikrein activity in the early stages of the disease can prevent ARDS. Unlike B2R, the B1R receptor on endothelial cells is activated by proinflammatory cytokines. Loss of ACE2 activity in acute lung injury leads to increased B1R-dependent signaling, increased vascular permeability, and angioedema. Angioedema is a symptom of an early stage of the disease and may explain the typical CT scans and the patient's drowning sensation. In some patients, this is accompanied by a clinical exacerbation of the disease approximately on day 9 due to the formation of antibodies against the S-protein of the coronavirus,

which can contribute to the progression of the disease due to the influx of immune cells and pro-inflammatory cytokines. In parallel, the inflammation induces an increase in B1R expression, which leads to continued dysfunction of ACE2 in the lungs due to the virus persistence. In this case, the key role is assigned to bradykinin and DABK. The RAAS controls strictly the kinin system. ACE cleaves bradykinin and ACE2 cleaves DABK. Thus, ACE and ACE2 function as regulatory brakes in the kinin system. When ACE2 binds to SARS-CoV-2 and is internalized into the cell during infection, the extracellular levels and functions of the enzyme are reduced, resulting in bradykinin signaling transfer into overload mode [40].

This molecular mechanism of COVID-19, named the "bradykinin storm", provides points of therapeutic intervention, which can be addressed using existing FDA-approved (Food and Drug Administration) pharmaceuticals. For example, Icatibant (trade name Firazyr) represents a bradykinin receptor antagonist. Ecallantide, which targets kallikrein, inhibits the production of bradykinin. Analysis of gene expression in bronchoalveolar lavage cells of COVID-19 patients reveals a critical imbalance in the RAAS, represented by a decrease in ACE expression in combination with an increase in the expression of ACE2, renin, angiotensin, key RAAS receptors and kallikrein enzymes, as well as both bradykinin receptors. This atypical RAAS pattern increases bradykinin levels in many tissues and organs and causes hypotension, vasodilation, and increased vascular permeability. The release of fluid into the lungs caused by the bradykinin storm, combined with excess hyaluronic acid, results in the formation of a gel-like substance that disrupts gas exchange in the lungs of patients with severe COVID-19. Thus, a bradykinin storm may be responsible for the most severe symptoms of COVID-19. The authors believe that the pathology of COVID-19 results from a bradykinin rather than a cytokine storm (although these two phenomena are not mutually exclusive) [42].

IMMUNE SYSTEM

Impaired regulation of immune responses in COVID-19

SARS-CoV-2 can induce an immune response in two phases, namely (1) an early specific acquired immune response to destroy the virus and suppress the disease progression, and (2) uncontrolled inflammation as a mechanism responsible for the development of ARDS. SARS-CoV-2 infection triggers a local immune response, including involvement of innate immunity cell populations and the generation of virus-specific adaptive B- and T-cell responses, which resolve in most cases the infection with minimal inflammation and lung damage. Within three weeks after the onset of symptoms, all patients test positive for antiviral immunoglobulin G (IgG); seroconversion to IgM and IgG can occur sequentially or simultaneously [12]. Most patients recover quickly from COVID-19 by developing antibodies to the infection. However, the slow response of the immune system can be fatal, therefore a humoral response does not rule out lethal outcome. The rate of infection and the immune response is of decisive importance for the prognosis [5].

The immune system activation and the production of inflammatory cytokines are required for natural antiviral immune responses. The virus replicates and infects tissues with ineffective immune responses. Binding of SARS-CoV-2 to the ACE2 receptor and viral replication in lung cells lead to apoptosis of epithelial and endothelial cells, pyroptosis of lymphocytes and macrophages, extravasation and inflammation in the lower respiratory tract, which is triggered by antigen-presenting cells [43]. The virus enters the macrophages, and they present the viral antigen to CD4+ T lymphocytes, releasing IL-12 for further activation of T helper 1 (Th1) cells. Activated Th1 cells stimulate B lymphocytes to produce antigen specific antibodies and cytotoxic CD8+ killer T cells against infected target cells containing a viral antigen [44]. CD8+ T cells have a cytotoxic effect, both directly and through the production of proinflammatory cytokines. In COVID-19 patients, levels of cytokines and chemokines are elevated. However, hyperactivation of the immune system during COVID-19 infection leads to a sharp increase in circulating levels of proinflammatory cytokines, namely a cytokine storm that is clinically characterized by systemic inflammation, hyperferritinemia, hemodynamic impairment, and multiple organ failure [3]. Cytokine storm stimulates necrosis or apoptosis of T-lymphocytes, especially in cases of severe disease. Therefore, unrestricted inflammation impairs viral clearance, contributing to the depletion of CD4+ and CD8+ T cells [45, 46]. SARS-CoV-2 can directly infect and replicate in B- and T-lymphocytes, triggering apoptosis in them [47], which is more pronounced in CD8+ T-cells [48]. During SARS-CoV-2 infection, patients have systemic symptoms of varying severity, which are associated with an aggressive inflammatory response and the release of large amounts of pro-inflammatory cytokines. In patients with severe and moderate forms of COVID-19, the levels of circulating IL-1β, IL-2, IL-6, IL-7, IL-8, IL-9,

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IL-10, IL-15, IL-18, FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF, TNF-α, and VEGF are increased. Studies analyzing cytokine profiles in COVID-19 patients have revealed a direct correlation between cytokine storm and lung damage, ARDS, multiple organ failure, and poor prognosis [49–53].

In dysregulated immune responses, the direct cytopathic effect of SARS-CoV-2 can cause pyroptosis as the most immunogenic and highly inflammatory form of programmed death of cell with the release of endogenous danger signals (for example, viral RNA), which are recognized by macrophages, epithelial and endothelial cells. A cascade of local inflammation arises, characterized by the secretion of proinflammatory cytokines and chemokines and the involvement of monocytes, macrophages, and T-lymphocytes, which mediate extensive pathology culminating in ARDS [12]. Cytokine storm enters the bloodstream, as evidenced by high serum levels of inflammatory cytokines IL-6, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α, TNFα, and chemokines CCL2, CCL3, CXCL10 in patients with a severe form of COVID-19, which can lead to septic shock and multiple organ failure [48]. Proinflammatory cytokines can also cause capillary leak syndrome, thrombosis, and disseminated intravascular coagulation [54]. The activation of macrophages by cytokines leads to erythrophagocytosis, anemia, impaired vascular hemostasis and multiple organ failure [55]. Severe lung damage in some COVID-19 patients is attributed to hyperactivation of proinflammatory Th17, high cytotoxicity of CD8+ T cells, and low interferon response to SARS-CoV-2 [49, 56]. Regardless of whether the cytokine storm is a part of cytokine release syndrome (CRS) or secondary hemophagocytic lymphohistiocytosis, also called macrophage activation syndrome, the result is a persistent increase in inflammatory cytokine levels [52].

It is believed that both cytotoxic and humoral adaptive responses are required for effective control of SARS-CoV-2 infection. Virus-specific T cells are present in most patients, and the magnitude of antigen-specific T-cell responses is not related to the disease severity. However, in some patients, an excessive release of cytokines is induced for reasons currently unknown, which causes a cytokine storm resulting in severe lung damage and ARDS [57]. Ultimately, in 70% of lethal outcomes, death is caused by respiratory failure due to ARDS and in 28% by multiple organ failure associated with a sepsis-like cytokine storm [58].

Cytokine storm is associated with hyperinflammatory organ damage during COVID-19. Studies of COVID-19 patients have revealed a relationship between the disease severity and the influx of innate immune cells and inflammatory cytokines [50, 56, 59]. In addition, SARS-CoV-2 infected peripheral blood mononuclear cells from healthy donors showed increased production of pro-inflammatory cytokines. Understanding the molecular pathway by which SARS-CoV-2 induces an overactive inflammatory response is of critical importance for developing effective therapeutic approaches. None of the cytokines individually induces the death of macrophages even at a 10-fold concentration. However, cytokine cocktail-1 (IL-6, IL-18, IFN-γ, IL-15, TNF-α, IL-1α, IL-1β, and IL-2) induces it. This means that synergistic transmission of cytokine signals is required for cell death. Various combinations of the two cytokines have been tested to identify the cytokines involved in this synergy. Out of the 28 pairs tested, only the combination of TNF-α and IFN-γ caused cell death to the same extent as cocktail-1. Treatment of cells with cocktail-2 free of TNF-α and IFN-γ does not lead to similar levels of cell death. TNF-α and IFN-γ can interact synergistically with cocktail-2 to induce cell death. However, the addition of TNF-α or IFN-γ alone to cocktail-2 did not result in cell death, further confirming that synergy between TNF-α and IFN-γ is critical for inducing cell death. The combination of TNF-α with IFN-α, IFN-β or IFN-λ did not cause high levels of cell death, i.e. for coordination with TNF-α, it is type II IFN signaling that is important. The kinetics of cell death induced by costimulation of TNF-α and IFN-γ is proportional to their concentrations. The combined production of TNF-α and IFN-γ induces the death of inflammatory cells by pyroptosis, apoptosis, and necroptosis. Signal axis STAT1 (signal transducer and activator of transcription 1)/IRF1 (interferon regulatory factor), activated by the combined action of TNF-α and IFN-γ, activates the expression of inducible nitric oxide synthase iNOS (nitric oxide synthase 2, NOS2) and the production of nitric oxide (NO). Pharmacological and genetic deletion of this pathway inhibits pyroptosis, apoptosis, and necroptosis in macrophages and protects them from lethal cytokine shock induced by TNF-α and IFN-γ.

In vivo neutralization of TNF-α and IFN-γ in cytokine storm disease models provides significant protection against SARS-CoV-2 infection, sepsis, and hemophagocytic lymphohistiocytosis. Blockade of signaling pathway of the SARS-CoV-2-induced inflammatory cell death may benefit patients with COVID-19 and cytokine storm syndromes by limiting inflammation and tissue damage. The data obtained provide a molecular

description of the term “cytokine storm” and open up new possibilities for the treatment of other infectious, autoimmune, and oncological diseases, in which the synergy of TNF-α and IFN-γ plays a key pathological role. These data have been confirmed experimentally. In a mouse model of TNF-α + IFN-γ-induced shock that mimics the symptoms of COVID-19, administration of not one but only a combination of TNF-α and IFN-γ results in synergistic mortality. These mice showed an increased influx of inflammatory cells into the intestinal lamina propria and the accumulation of neutrophils in the lung capillaries. The combination of TNF-α and IFN-γ causes lung and intestinal damage by activating mediators of apoptosis in the intestinal crypts and lungs of mice. Cell death mediated by caspase-8 and STAT1 is the driving force behind lethality in mice subjected to TNF-α + IFN-γ-induced cytokine shock. Suppression of inflammatory cell death by the controlled STAT1/caspase-8 axis prevents TNF-α + IFN-γ-mediated pathology and death *in vivo*. Mice injected with neutralizing antibodies against TNF-α and IFN-γ were 100% protected from death during TNF-α + IFN-γ-induced shock, i.e. these antibodies neutralized effectively TNF-α and IFN-γ *in vivo* in the COVID-19 model [60].

One of the mechanisms associating the cytokine storm with organ damage is cell death through pyroptosis, apoptosis, and necroptosis. Pyroptosis is implemented by proteins of the gasdermin family through caspase-mediated 1/4/5/8/11 cleavage of the pore-forming gasdermin D molecule (GSDMD); or granzyme A-mediated cleavage of gasdermin B (GSDMB). Apoptosis is mediated by caspases 3/7 after activation of initiator caspases 8/9/10. Necroptosis is mediated by RIPK3 (receptor-interacting serine/threonine protein kinase)-mediated oligomerization of another pore-forming molecule MLKL (mixed lineage kinase domain-like), a component of the necrosome (a protein complex that triggers TNF-induced cell death). Pyroptosis and necroptosis have been identified as lytic forms of inflammatory cell death that release cytokines and other cellular factors to induce inflammation and alert immune cells to pathogenic or sterile damage, while classical apoptosis has historically been considered non-immunogenic, allowing cellular contents to be absorbed and reused by others cells. However, depending on the stimulus, extensive cross-interactions can be formed between pyroptosis, apoptosis, and necroptosis, collectively called PANoptosis, which is inflammatory in nature. That is why the determination of the nature of cell death induced by the combination of TNF-α and IFN-γ, and the use of genetic data for the analysis of activated signaling pathways and molecular mechanisms are important for the determination of therapeutic targets. In patients with severe COVID-19 and in macrophages treated *in vitro* with TNF-α and IFN-γ, the genes encoding the factors IRF1, IRF5, IRF7, and the signaling protein kinase JAK2 (Janus kinase 2) are activated. JAK2 kinase is known to transmit a signal to the transcription factors STAT1 and IRF1, which activate the transcription of IFN-γ-inducible genes. Macrophages lacking STAT1 and IRF1 are protected from death, since activation of apoptotic caspases 3/7/8, pyroptosis GSDME molecule, necroptosis MLKL molecule, as well as iNOS expression and NO production are impaired in them [60].

R. Karki et al. [60] proposed a new paradigm for determining the cytokine storm mechanism. Among the COVID-19-induced cytokines, TNF-α and IFN-γ play a key role in damage to vital organs. Blocking IL-6 produces ambiguous clinical results; blocking other cytokines may also be ineffective. Although suppression of cell death in the context of viral infection carries the risk of increasing the virus release from infected cells, excessive cell death contributes more to the disease pathology than the virus titer. Peak titers of SARS-CoV-2 virus in the respiratory tract can occur even before the onset of pneumonia symptoms. Research by R. Karki et al. [60] outlined the signaling pathway involved by the cytokines TNF-α and IFN-γ, emphasizing a number of additional potential therapeutic targets, namely protein kinase JAK2 and its effectors STAT1, IRF1, iNOS, and NO. Targeting of cytokines which stimulate PANoptosis, together or separately, is the most direct therapeutic strategy, since antibodies against TNF-α and IFN-γ have already been approved for clinical use [60]. For example, emapalumab, an antibody against IFN-γ, is approved by the FDA for the treatment of refractory, recurrent, or progressive hemophagocytic lymphohistiocytosis, in which the pathology mediated by the combination of TNF-α and IFN-γ is decisive [61]. Overall, the identification of this critical pathway for TNF-α + IFN-γ-induced inflammatory cell death provides a variety of drug targets that can be analyzed for efficacy of their inhibiting in COVID-19, as well as other infectious and inflammatory diseases that include TNF-α + IFN-γ-induced cytokine storm, and is the basis for development of evidence-based therapeutic strategies to overcome the ongoing public healthcare crisis [60].

Since the cytokine storm is described by many authors as a hallmark of the COVID-19 pathology, a number of clinical trials are being conducted to assess

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the efficiency of cytokine blockade using inhibitors of IL-6, IL-18, IL-1α, and IL-1β [62–64]. However, blocking pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 has mixed success in the treatment of cytokine storm-associated diseases. In addition, some authors question whether the phenomenon noted in COVID-19 is a cytokine storm indeed, since the levels of proinflammatory cytokines in these patients are lower than in patients with other conditions associated with cytokine storm, such as sepsis [65, 66].

The description of a cytokine storm in COVID-19 patients prompted a direct comparison of COVID-19 with other critical illnesses characterized by elevated cytokine concentrations. M. Kox et al. [65] revealed that, despite the presence of severe pulmonary injury, the cytokine storm is not always registered in COVID-19 patients. The levels of inflammatory cytokines TNF, IL-6, and IL-8 in patients with COVID-19-associated ARDS, who were in the intensive care unit and received artificial pulmonary ventilation (ALV) were significantly lower than in patients with bacterial septic shock and ARDS [66]. The study by D.E. Leisman et al. [66] included groups of patients with COVID-19 (n = 1245), as well as non-COVID-19 conditions such as sepsis (n = 5320), cytokine release syndrome (n = 72), and ARDS (n = 2767). In patients with severe or extremely severe COVID-19, the mean serum IL-6 concentration was 37 pg/ml, while in patients with non-COVID-19 cytokine release syndrome, it was almost 100 times higher (3110 pg/ml, $p < 0.0001$), in patients with sepsis unrelated to COVID-19, it was 27 times higher (984 pg/ml, $p < 0.0001$), and in patients with non-COVID-19 ARDS, it was 12 times higher (460 pg/ml, $p < 0.0001$). These findings call into question the role of the cytokine storm in organ dysfunction caused by COVID-19. They demonstrate that the cytokine storm does not describe adequately the environment where organ dysfunction caused by COVID-19 occurs. Autopsy reports have consistently noted the widespread occurrence of SARS-CoV-2 in various tissues. In this context, it should be borne in mind that lymphopenia and a predominantly hypoimmune state with subsequent viral tissue damage and dysregulated inflammation are consistent with both clinical and pathological abnormalities in COVID-19 and with high concentrations of circulating acute phase reagents [66].

Macrophages represent another source of cytokine storms. The systemic cytokine profile registered in COVID-19 patients was compared to macrophage activation syndrome, which is usually characterized by uncontrolled macrophage activation and proliferation [67]. In addition to resident lung macrophages, in the bronchoalveolar fluid of patients with severe COVID-19, many pro-inflammatory macrophages, elevated levels of IL-1β and IL-6 cytokines, and chemokine receptors were found, which indicates the attraction of inflammatory monocytes and neutrophils [68]. Hyperactivation of macrophages is responsible for increased expression of the “death receptor” FAS (first apoptosis signal) on T cells and severe lymphopenia, which is recorded in more than 80% of patients [69] and is associated with a risk of ARDS [70]. The depletion of T-lymphocytes and lymphopenia is caused by the increased expression of the protein PD-1 (programmed cell death protein 1) on T cells in COVID-19 patients, indicating a quantitative and functional (anergy) depletion of T cells and ineffective immune control over the virus replication that contribute to the disease progression [12, 46, 71]. Post-mortem examination of COVID-19 patients showed massive apoptosis of T cells in the lymph nodes and spleen and the absence of germinal centers necessary for the development of long-term immunity [69, 72, 73].

Proteomic analysis of 17 phenotypically diverse cell lines derived from human lung, liver, intestine, kidneys, heart, and brain revealed universal suppression of interferon signals after infection with SARS-CoV-2. Analysis of the JAK-STAT signaling pathway, as a key component of the interferon response, revealed in a wide range of cell systems that some components of this cascade were attacked by the SARS-CoV-2 virus, which led to desensitization of cells to interferon. The SARS-CoV-2 virus quickly deactivates it and thereby suppresses the immune defense and reduces the sensitivity of cells to interferon treatment. The study of the dynamics of changes revealed that most of the components of the JAK-STAT cascade were depleted at the early stage of infection, which indicates a rapid active suppression of their expression, in contrast to the generalized inhibition of genes, noted at the later stages of infection. These data reveal one of the strategies of SARS-CoV-2 immune evasion and creation of a favorable environment for its replication in various types of tissues. These results indicate that interferon signal suppression is a mechanism widely used by SARS-CoV-2 in various tissues to evade antiviral innate immunity, and that targeting of mediators of viral evasion from immunity can block its replication in COVID-19 patients. In addition, proteomic analysis revealed the differential expression of about 5,000 proteins, hundreds of proteins in each cell line. Protein kinase JAK1 (Janus kinase 1) is a key signaling molecule for interferons

and other cytokines (IL-2, IL-4, IL-6, and IL-7), among cellular proteins which expression is suppressed in all cell lines. Components of the ubiquitin pathway (SP22, UBL5, UBE2C), inflammatory mediators (chemokines CXCL1, CXCL5, CXCL8, and CXCL12), and cell cycle regulating proteins were found among proteins with suppressed expression. Expression of IFI35, a negative regulator of antiviral reactions, is activated by the SARS-CoV-2 virus [14].

One of the earliest and most potent immune responses, IFN signaling, poses an immediate threat to viruses and can quickly eliminate them from infected cells. The interaction of IFN with its receptor complex and subsequent activation of receptor-associated JAK kinases activates the expression of transcription factors of STAT proteins. The SARS-CoV-2 virus inhibits the expression of the IFN receptor and JAK kinase and reduces the sensitivity of infected cells to IFN treatment. The strongest dose-dependent effect is recorded in infected intestinal cells, where STAT phosphorylation is inhibited by more than 90%. Increasing doses of IFN caused an increase in STAT phosphorylation in uninfected cells, and only a minor effect was noted in infected cells. In cells infected with SARS-CoV-2, STAT translocation from the cytoplasm to the nucleus is disrupted and the induction of interferon-stimulated genes (ISG) is completely blocked. These results indicate that SARS-CoV-2 transforms infected cells into a state that is insensitive to interferon [14].

Congenital errors in IFN signaling and the presence of IFN autoantibodies predispose to severe COVID-19 course [74, 75]. SARS-CoV-2 possesses a set of mechanisms to counteract the effector functions of interferon, namely the inhibition of IFN signaling, expression of viral sensors and other signaling molecules. The overall result of this inhibition is the continuous spread of the virus in different tissues. D.Y. Chen et al. [14] showed that by the time a COVID-19 patient receives IFN, a large count of cells are already infected with the virus, and interferon treatment may be ineffective. This is why the timing of IFN therapy will be a key determinant of outcome. Early use of IFN is protective, while its late administration is associated with delayed recovery and increased mortality [76]. The large clinical study SOLIDARITY, conducted by the World Health Organization in 30 countries, revealed no protective effect of IFN in hospitalized patients with advanced stage of COVID-19 [77].

It should be noted that activation of the JAK-STAT pathway stimulates the production of IL-6 and other inflammatory cytokines that attract immune cells to the site of infection in order to destroy the infected cells. Inhibition of the JAK-STAT axis by the SARS-CoV-2 virus suppresses the production of cytokines, thereby disrupting the timely inflammatory response. IFN signaling is involved in the interaction between innate and adaptive immunity. Loss of communication between these two branches of immunity causes severe illness in COVID-19 patients [78, 79]. A study that examined the peripheral blood of patients with COVID-19 of varying severity also revealed a strong correlation between the impaired response to IFN and the clinical outcome of infection [56]. This study reveals the phenomenon of SARS-CoV-2 virus-induced desensitization to IFN as the basis for the pathogenesis of COVID-19 and suppression of innate and adaptive immunity.

ACE2 expression in lymphocytes turns them into potential targets for SARS-CoV-2, which leads to the death of CD4+ and CD8+ T cells, imbalance of both innate and adaptive immune responses, hyperactivation of neutrophils and macrophages, and delayed viral clearance. In the context of COVID-19-associated lymphopenia, there are (1) death of infected lymphocytes and (2) destruction of lymphatic organs by the virus. Postmortem analysis of lymph nodes and spleen confirmed apoptosis of lymphocytes and macrophages CD169+ in COVID-19 patients [3]. Inflammation can enhance the expression of ACE2 on macrophages [48], increasing their susceptibility to the virus. The role of CD169+ macrophages, which contain a viral nucleocapsid protein, as mediators of the death of activated lymphocytes, has been established [12]. Despite the absolute reduction in the total count of T-lymphocytes, including CD4+ and CD8+ T-cells, the main reduction in memory T-helpers and regulatory cells is noted, while the count of naive T-cells and Th17 even increases [73, 80, 81]. In case of a decrease in the absolute count of T-lymphocytes, monocytes, eosinophils, and basophils in severe and extremely severe COVID-19, the neutrophil response increases, which results in an increase in the ratio of neutrophils and lymphocytes [80].

To gain a holistic view of the differences between severe and mild COVID-19 and to integrate the contributions of all major immune cell types, including neutrophils, monocytes, platelets, lymphocytes, and serum, A.J. Combes et al. [82] performed an analysis of whole blood single cells. RNA sequencing of single leukocytes confirmed a positive relationship between the count of neutrophils and the disease severity and an inverse correlation for lymphoid populations. Patients with mild COVID-19 course show a coordinated pattern of ISG expression in all cell populations, and

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these cells are systemically absent in patients with severe disease. Among neutrophils, a population of ISG-expressing cells has been identified, which count varies significantly in patients with mild/moderate and severe COVID-19. The gene signature of ISG includes genes encoding the main antiviral regulators ISG15 and IFITM3 (IFN-inducible transmembrane protein 3) which limit the virus penetration into the cytosol.

Among mononuclear phagocytes (monocytes, macrophages, dendritic cells, and plasmacytoid dendritic cells), 7 clusters of transcriptionally different subpopulations with a heterogeneous amount of ISG and unique molecular identifiers for each cluster were detected. Populations of classic ISG-expressing monocytes and neutrophils have been identified as enriched in mild to moderate COVID-19 patients. The count of plasmacytoid dendritic cells, which are producers of interferon IFN-α, is also usually increased in these patients, although it statistically significant. Expression of genes associated with glycolysis and oxidative phosphorylation by monocytes ISG+ indicates the presence of bacterial sepsis. Differential gene expression analysis demonstrated that ISGs are the dominant genes associated with mild to moderate COVID-19 phenotype. All patients with mild to moderate severity of COVID-19 had a correlation in the frequency of ISG-expressing cells (monocytes, neutrophils, T- and B-lymphocytes). Patients with severe COVID-19 are characterized by a high ratio of S100A12+ neutrophil subpopulations to ISG+ neutrophils [82].

Comparison of cytokine responses elicited by viruses H5N1, H7N9, SARS-CoV, MERS-CoV, and SARS-CoV-2 reveals SARS-CoV-2 specific dysregulation of type I IFN response and its cytokine signatures. Infection with H5N1 and H7N9 influenza viruses, SARS-CoV and MERS-CoV coronaviruses causes early induction of type I IFN, while the type I IFN response to SARS-CoV-2 infection is weak, if ever occurs [83]. A study of serum IFN-α levels revealed that patients with mild to moderate infection produced on average more of this cytokine than those with severe infection, which is consistent with higher ISG+ cell populations, although some patients with severe disease produce high IFN-α levels. ISG-expressing cell populations are associated with mild COVID-19, lower plasma levels of the surfactant SP-D protein (an indicator of alveolar epithelial damage), and only slightly with IL-6 levels. Clustering of most ISG+ cell subpopulations correlates positively with many factors indicating a strong Th1 and ISG response (CXCL1/6/10/11, TNF-β, IL-12B, MCP-2/4), and negatively with others (CCL23, MMP10, HGF). The concentration of serum antibodies against the SARS-CoV-2 Spike and Nucleocapsid proteins became an unexpected anticorrelation of the condition. This anticorrelation was profound, and the authors found it paradoxical that critically ill patients had higher levels of potentially neutralizing antibodies. This clearly contradicts the fact that viral load is associated with severity and mortality in COVID-19. Since high antibody titers and a decrease in viral load represent a sign of later stages of the disease, it has been hypothesized that in these cases, a mild to moderate course of the disease, characterized by a high frequency of ISG signatures, precedes the severe stage of the disease. However, antibody titers in severely ill patients are consistently higher compared to mild/moderate course even 2 weeks after symptom onset, and only 10% of patients with mild/moderate COVID-19 can develop severe illness. Moreover, there is no statistical correlation between the number of days from the onset of the disease and the presence of ISG+ cell populations, which evidences against a simple temporal relationship between mild/moderate and severe conditions [82].

Impaired immune tolerance

Serum study of severe patients demonstrates that they produce antibodies with multiple types of specificity against interferon-stimulated cells, and these antibodies block functionally the formation of ISG-expressing cells associated with mild disease. Excessive humoral response and the production of autoantibodies (auto-Ab) incite the immune system against itself in many COVID-19 patients, and this sets targets for immunotherapy to allow the immune system to ensure antiviral protection. Phenotypic differences between the two groups of patients with varying degrees of COVID-19 severity may reflect or depend on systemic factors affecting all cell populations. It turned out that serum of 10% of patients contains antibodies against IFN-α [82], which is consistent with results of the study by P. Bastard et al. [74], where they were also revealed in 10.2% of COVID-19 patients. People who lack certain interferons are more susceptible to infectious diseases. The autoantibody system suppresses the IFN response to prevent damage from inflammation caused by pathogens. In 10.2% (101 out of 987) of patients with life-threatening COVID-19, at the beginning of the severe course of the disease, there were high titers of neutralizing auto-IgG against IFN-ω (13 patients), 30 times higher than in the general population, against 13 types of IFN-α (IFN-α1, α2, α4, α5, α6, α7, α8, α10, α13, α14, α16, α17, α21; 36 patients) and against both inter-

ferons (IFN-α and IFN-ω; 52 patients), 19 of them had anti-IFN-β auto-Abs. These auto-Abs neutralize high concentrations of the corresponding type I interferons and their ability to block SARS-CoV-2 infection *in vitro*. Before the pandemic, they were present in only 4 out of 1227 (0.33%) healthy people. These auto-Abs were not detected in patients with asymptomatic or mild SARS-CoV-2 infection ($p < 10^{-16}$); 1.5% of patients with severe COVID-19 and autoantibodies against type I IFN also had auto-Abs against other cytokines (IFN-γ, GM-CSF, IL-6, IL-10, IL-12p70, IL-22, IL-17A, IL-17F, and/or TNFβ), while 95 (94%) patients with life-threatening pneumonia out of 101 patients with auto-Abs were men. All patients tested had low or undetectable serum IFN-α levels during the acute phase of the disease. B-cell autoimmune phenocopy of type I IFN congenital defects is the cause of life-threatening COVID-19-associated pneumonia in at least 2.6% of women and 12.5% of men; 49.5% of patients with auto-Abs were over 65 years old compared with 38% of patients without auto-Abs, i.e. the frequency of circulating auto-Abs against type I IFN increases with age. The unique age profile is one of the features of COVID-19, in which mortality doubles every 10 years after the age of 50 years [5]. These findings explain partly the male predominance of patients with life-threatening COVID-19 and the increased risk with age, and pave the way for the prevention and treatment of severe COVID-19, including plasmapheresis, plasmablast depletion, and administration of recombinant type I IFNs that do not bind to autoantibodies (e.g. IFN-β) [74].

The auto-Ab response is evident in all severe patients and targets ISG+ cell populations and their generation. Antibodies in many of these patients have direct specificity for determinants on the surface of ISG-expressing monocytes. How and why ISG tolerance is impaired during SARS-CoV-2 infection remains to be determined. One of the candidates for the role of a B-cell response modulator is direct infection of monocytes with the SARS-CoV-2 virus. If during an early immune response ISG proteins are presented together with the proteins of the pathogen, the tolerance of the immune system to ISG proteins can be impaired. Conversely, infection of monocytes with a virus can cause excessive B cell responses to many antigens, not just those generated during infection. Targeting of over-abundant and autoreactive B cells with drugs such as rituximab can probably overcome the global suppression of protective ISG-mediated antiviral immunity. The presence of circulating neutralizing anti-IFN-α auto-Abs is closely associated with low serum IFN-α levels. Plasma of patients with neutralizing auto-Abs against type I IFN neutralizes the ability of IFN-α2 to block infection of cells with SARS-CoV-2 *in vitro* and *in vivo*, in contrast to plasma of healthy people and patients infected with SARS-CoV-2, without auto-Abs [74]. A similar block of generation of the ISG signature in response to IFN-α was noted in different cell populations, including lymphocytes [82]. These data indicate that patients' blood contains a sufficient amount of auto-Abs to neutralize the corresponding type I IFNs and block their antiviral activity against SARS-CoV-2. For some patients with neutralizing auto-Abs against type I IFN, these antibodies are known to be present in them before the pandemic, therefore, they are the cause and not the result of severe infection with SARS-CoV-2. These results are of direct clinical relevance. First, patients infected with SARS-CoV-2 can be screened for auto-Abs to identify individuals at risk of developing life-threatening pneumonia. Second, such patients recovering from severe COVID-19 should be excluded from convalescent plasma donation [74]. Removal of these auto-Abs restores the induction of IFITM3 expression and the overall yield of interferon-induced monocytes [82].

As it turned out, autoantibodies are present in 60–80% of hospitalized COVID-19 patients, and anticytokine auto-Abs are especially common, often to several cytokines at once, with a large number of different specificities generated in individual patients. In rare patients, IgG antibodies to ACE2 were detected. Some types of autoantibodies appeared *de novo* after SARS-CoV-2 infection. The authors concluded that SARS-CoV-2 induces the development of new IgG autoantibodies in a significant proportion of hospitalized COVID-19 patients and that severe COVID-19 may impair self-tolerance. One of the most important unanswered questions is that it is not known yet what leads to the loss of tolerance and the development of autoimmunity in COVID-19 patients and why certain molecules are targeted. The study of these mechanisms continues [84].

Clinical characteristics of SARS-CoV-2 infection

SARS-CoV-2 infection causes a wide range of clinical manifestations, from asymptomatic cases to rapid lethal outcome. The severity of SARS-CoV-2 infection is associated with dysregulated immune responses [18]. At the beginning of the epidemic, the most common symptoms were shortness of breath (in more than 50% of all COVID-19 patients [85, 86]), fever and cough, but now new features are emerging, caused by

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the viral infection itself or its consequences [87]. The inflammation is initially triggered in the lungs by SARS-CoV-2-induced damage to the alveolar epithelial cells, causing extensive infiltration of immune cells. This local inflammatory response causes a cytokine release syndrome known as cytokine storm, which leads to hyperinflammation of many organs with subsequent tissue damage and death of patients [88]. Other pathophysiological mechanisms, such as endotheliosis, dysregulation of the RAAS, thrombosis, lymphocytopenia, and T-cell anergy, can also contribute to morbidity [89, 90]. Up to 94% of all COVID-19 patients have one or more chronic (concomitant) diseases, and the most common of them are hypertension, chronic obstructive pulmonary disease, diabetes, cardiovascular and cerebrovascular pathologies [91].

Hypohemoglobinemia, damage to the endothelial glycocalyx

In COVID-19, SARS-CoV-2 infection decreases the concentration of hemoglobin in the blood and disrupts the process of oxygen transport by hemoglobin, causing oxygen deficiency in patients [92]. According to W. Liu and H. Li [93], the coronavirus attacks the 1-beta chain of hemoglobin, releasing iron ions from it to form porphyrin, so that less and less hemoglobin will be available for gas exchange. In addition, iron ions (Fe2+ or Fe3+), which are part of the structure of oxyhemoglobin, are toxic in a free state and increase the oxidative stress of the blood. When SARS-CoV-2 destroys hemoglobin, these ions enter the blood and tissues, where the main effects of the virus emerge, oxygenation decreases, and hypoxia increases, which may explain shortness of breath and fatigue in some patients after recovery, although their lungs have been completely cleared of the virus [94, 95]. The destruction of hemoglobin is evidenced by an increase in serum levels of ferritin, C-reactive protein, lactate dehydrogenase and erythrocyte sedimentation rate [93]. Low oxygen saturation even with APV is additional evidence. These patients may require regular blood or serum transfusions supported by hyperbaric oxygen [96]. The destruction of hemoglobin interferes with the delivery of oxygen to vital organs. Given that anemia is relatively common in people over 60 years of age, a further decrease in organ oxygenation may explain partly the increased mortality in older patients. Binding of the virus to hemoglobin leads to the release of free radicals and further hypoxia and can cause a heart attack or cardiac arrest [93]. All this is based on the damage to the endothelial glycocalyx due to the binding of serum albumin by the SARS-CoV-2 virion. Low albumin levels were revealed in 80% of deceased patients with COVID-19 [5].

Sepsis and septic shock

Some COVID-19 patients recover without additional support other than oxygen. The condition of the others deteriorates suddenly with the development of ARDS which occurs when fluid accumulates in the alveoli and is associated with sepsis [96]. The oxygen level in their blood drops sharply, and dyspnea occurs [97]. Computed tomography images show ground glass opacity in the lungs of such patients. They usually require APV, and mortality is high among them. Autopsy revealed that the alveoli were filled with fluid, eukaryotic material, and dead lung tissue [98]. Along with this pathology, organ failure of the renal, hepatic and cardiovascular systems is accelerated by sepsis of the capillary network. Thrombosis, multiple organ failure, and sepsis symptoms enable to diagnose systemic septic shock in almost all cases of lethal outcome. The mechanism of sepsis development is systemic and manifests itself as a syndrome of increased capillary permeability surrounding the alveoli of the lungs and capillaries of all other organs. The epithelium is lined with a gel-like layer of interconnected proteins, endothelial glycocalyx, which is required to maintain capillary microcirculation, adsorption and reabsorption through capillary membranes and the distribution of fluid through tissues. The destruction of this layer leads to sepsis and septic shock, which can be limited by maintaining the physiological concentration of plasma protein, especially albumin. Serum albumin transports hormones and free fatty acids and maintains oncotic pressure, but SARS-CoV-2 virions competitively bind to albumin, which disrupts its transport function and the formation of endothelial glycocalyx [5].

Hypoalbuminemia is often registered in patients with conditions such as diabetes, hypertension, and chronic heart failure, i.e. in those most vulnerable to SARS-CoV-2 infection. Hypoalbuminemia, coagulopathy, and vascular diseases are associated in case of COVID-19 and enable to predict outcome regardless of age. Hypoalbuminemia is also known as a factor of sepsis and ARDS, when fluid accumulates in the alveoli. As the viral particles spread, the ratio between bound and unbound albumin increases. Decreased nutrient levels provoke cellular stress and apoptosis. Thus, the tolerance to the virus depends on the transport of nutrients and the amount of free albumin. Albumin therapy to replace bound albumin and increase its total plasma and

interstitial fluid concentrations may relieve systemic sepsis and prevent death. In septic shock, damage to the endothelial glycocalyx can cause inflammation of the vascular endothelium and uneven distribution of microvascular blood flow, as well as the release of nitric oxide, which is normally involved in the regulation of vascular homeostasis, but its uncontrolled release leads to damage to erythrocytes and reduces the ability of blood to carry oxygen [92].

Coinfectious complications

A decrease in the effector function and the count of cells involved in the clearance of pathogens increases the risk of coinfection with other viruses, bacteria and fungi, especially in the case of prolonged hospitalization of COVID-19 patients. Procalcitonin level, which is considered a marker of severe bacterial infections, does not increase in patients with uncomplicated COVID-19 upon admission, but its progressive increase is associated with an almost 5-fold increased risk of severe disease [99]. The incidence of secondary bacterial or fungal infections is significantly increased in patients with severe COVID-19; the overall incidence of coinfection in COVID-19 patients is estimated at 8% [100]. Bacteremia was detected in 12% of patients receiving invasive APV, and in less than 2% of other patients [101]. These studies probably underestimate the actual frequency of coinfection in this population. It was established that 21% of patients infected with SARS-CoV-2 have another additional respiratory pathogen, including rhinovirus/enterovirus and respiratory syncytial virus [102].

Another aspect of the severe course of COVID-19 is associated with circulating *mitochondrial DNA* (MT-DNA) secreted by damaged cells of solid organs and causing inflammation. Quantification of circulating MT-DNA level in prospectively collected acellular plasma samples from COVID-19 patients at the time of admission to the hospital revealed its sharp increase in patients admitted to intensive care units, who eventually died. Multivariate regression analysis showed that high levels of circulating MT-DNA are an independent risk factor and an early indicator of lethal outcome after adjusting for age, gender, and comorbidities, as reliable as clinically established indicators of systemic inflammation [103].

CONCLUSION

Most patients infected with SARS-CoV-2 have no symptoms or develop mild to moderate symptoms and recover in 1–2 weeks. However, 5% of patients have pneumonia, and many of these patients require intubation and are admitted to intensive care units.

Until now, there is no specific therapy for COVID-19. One of the factors determining the lack of effective methods of treatment and drugs is the lack of knowledge of the pathophysiology of COVID-19. The study of the complex relationship between various proteolytic defense systems functioning in the human vasculature during COVID-19, and the role of the mediators involved in it, will provide the possibilities of their pharmacological modulation.

SARS-CoV-2 affects the lungs and other organs, including the heart, kidneys, brain, spleen, eyes, and digestive tract. Therefore, further research is required to expand our understanding of organ damage and to define diagnostic, prognostic, and therapeutic strategies in the clinic.

In this review, we aimed to elucidate the mechanisms of immunoinflammatory, thrombohemostatic, and other manifestations of COVID-19. The levels of inflammatory biomarkers and coagulation biomarkers differ significantly in COVID-19 patients, indicating the existence of different biochemical/clinical phenotypes in which different cellular systems predominate. The development of new drugs to treat this disease requires awareness of its molecular pathways and critical target molecules. Blocking virus penetration pathways, including receptors and enzymes, and controlling immune responses are promising strategies for reducing multiple organ dysfunction.

ADDITIONAL INFORMATION

Author contribution. Golota A.S., Shcherbak S.G. — writing of the article; Shneider O.V., Vologzhanin D.A. — revision and writing of the article; Kamilova T.A. — search and analytical work, revision of the article. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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