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COVID-19: FEATURES OF THE PATHOGENESIS OF THE DISEASE AND TARGETS FOR IMMUNOTHERAPEUTIC EFFECTS

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An analysis of current scientific literature on the pathogenesis of the coronavirus infection that caused the 2019 pandemic, COVID-19, was carried out. The structure, genome, introduction into the cell and the life cycle of the SARS-CoV-2 virus that caused the pandemic, the mechanisms of protection of the virus from the host's immune system, features of the clinical picture of coronavirus infection, the pathogenesis of viral pneumonia, in particular, disruption of the renin-angiotensin system, cytokine storm, participation of the complement system in the pathogenesis of COVID-19 are reviewed. The models of infections caused by SARS-CoV and SARS-CoV-2 in laboratory mice are also considered.

Keywords: coronavirus; COVID-19; pathogenesis; cytokine storm; complement; antibody-dependent enhancement; immunotherapy.

COVID-19: ОСОБЕННОСТИ ПАТОГЕНЕЗА ЗАБОЛЕВАНИЯ И МИШЕНИ ДЛЯ ИММУНОТЕРАПЕВТИЧЕСКОГО ВОЗДЕЙСТВИЯ

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Произведен анализ современной научной литературы в области патогенеза коронавирусной инфекции, ставшего причиной пандемии 2019 г., — COVID-19. Рассмотрены строение, геном, внедрение в клетку и жизненный цикл вируса SARS-CoV-2, вызвавшего пандемию, механизмы защиты вируса от иммунной системы хозяина, особенности клинической картины коронавирусной инфекции, патогенез вирусной пневмонии, в частности нарушение работы ренин-ангиотензиновой системы, цитокиновый шторм, участие системы комплемента в патогенезе COVID-19. Рассмотрены также модели инфекций, вызываемых SARS-CoV и SARS-CoV-2, на лабораторных мышах и перспективы иммунотерапевтического воздействия на инфекции, вызываемые SARS-коронавирусами.

Ключевые слова: коронавирус; COVID-19; патогенез; цитокиновый шторм; комплемент; антителозависимое усиление инфекции; иммунотерапия.

Virion structure and the genome of COVID-19

Coronaviruses are represented by spherical particles of 100–120-nm diameter, and their genomic RNA(+) is of 27–42-kb length bound to nucleocapsid proteins that form a spiral ribonucleocapsid located inside the icosahedral core formed by matrix (M) proteins. The outer lipid membrane is formed when the virus nucleus exits from the intracellular membranes. Coronaviruses contain spines of 20–40 nm length with bulges at the ends, because of which the viral particles take the shape of a corona [3].

The genome of SARS-CoV-2 is represented by a single-stranded (+) RNA of length 29.9 kb, and it is 79.5% identical to the genome of the SARS-CoV virus; the location of the reading frames in the genomes of these viruses is identical. The identity of the SARS-CoV-2 genome to the MERS-CoV genome is approximately 50% [4]. At least 10 open reading frames are known, and two of them, which occupy two-thirds of the entire genome, encode for two polyproteins, which are degraded into 16 non-structural proteins (nsp) that implement replication and transcription [5]. The remaining one-third of the genome encodes structural proteins, namely viral envelope protein (E), nucleocapsid protein (N),

List of abbreviations

ACE2 — angiotensin-converting enzyme 2; IFN — interferon; IL — interleukin; TNF — tumor necrosis factor.

membrane protein (M), viral spike protein (S), and several other proteins with unknown functions [6].

Cell penetration and life cycle of the virus

The receptor for the SARS-CoV-2 and SARS-CoV viruses is the angiotensin-converting enzyme 2 (ACE2), which is expressed in most organs and tissues, particularly in the lungs, heart, kidneys, and intestines, as well as in vascular endothelial cells [7, 8].

Thus, the receptor protein is expressed both in the organs that act as the main targets for viral infection and in the organs and tissues, the role of which remains to be established in the pathogenesis of viral infection.

The spike protein S of the SARS-CoV-2 virus binds to ACE2 with a high affinity ($Kd = 1.2 \cdot 10^{-9}$ M); and the affinity of the SARS-CoV protein S is 4-times lower ($Kd = 5.0 \cdot 10^{-9}$ M) [9].

It is possible that the higher affinity for ACE2 contributes to the higher contagiousness of SARS-CoV-2 when compared to SARS-CoV. Furthermore, the protein S is cleaved by the cell membrane proteinase TMPRSS2 into proteins S1 and S2, while S2 is involved in the penetration of the virus into the cells [10], after which the viral RNA enters the cytoplasm, where the transcription of viral proteins and RNA replication occur [11]. Coronaviruses multiply rapidly; therefore, in the cytoplasm of human Vero E6 cells, 8 after infection with SARS-CoV and SARS-CoV-2 with a multiplicity of infection of 3, two-layer vesicles were noted, in which the viruses were assembled, after which the vesicles merge into larger ones, and the viruses leave the vesicles and exist from the cell. Titers of SARS-CoV and SARS-CoV-2 viruses in the culture medium begin to increase from 6 h after infection; by 14 h after infection, they range from $1 \cdot 10^7$/mL to $1 \cdot 10^8$/mL with a flattening of the curve [12].

Inherent antiviral immunity upon SarS-Cov-2 infection

In response to infection with SARS-CoV-2, protective reactions develop due to the activation, first of all, of innate, and then acquired of immunity and directed against the virus, but the immunopathogenesis of severe clinical forms of COVID-19 is associated with the formation of an unbalanced immune response, leading especially severe cases to respiratory distress syndrome and impaired lung functions. The imbalance of immunological reactions depends primarily on the development of the initial stages of the innate antiviral immune response.

After infection of the cells, the virus becomes uncoated and the viral nucleic acids appear in the cytoplasm. The cells of our body can resist infection by several viruses as a result of primary recognition by the innate immunity receptors of viral pathogen-associated molecular structures or patterns, the main molecules for the SARS-CoV-2 coronavirus are single-stranded RNA molecules and some viral proteins. The most important cytoplasmic sensory molecules for the recognition of viral nucleic acids are the members of the RLR family, namely retinoic acid-inducible gene I (RIG-I) and melanoma differentiation factor 5 (MDA5), which are located in the cytoplasm of most cells of the body and are represented by RNA-dependent ATPases related to the helicase family DExD/H-box. They consist of separate domains that recognize and bind to viral RNAs. All RLRs implement signaling using the adapter molecule mitochondrial antiviral signaling protein (MAVS) associated and dependent on the mitochondria and the TNF receptor activating factor 3 (TRAF3) molecule by activating the TANK/IKKγ/IKKε/TBK1 complex with the involvement of the intracellular factor TRAF-family binding kinase 1 (TBK1), followed by dimerization and phosphorylation of the regulatory factors interferon responsive factor 3 (IRF3) and IRF7, which move into the cell nucleus and interact with a region of DNA called as the IFN-stimulated response element that leads to sequential induction of gene expression, first IFNβ and then IFNα, which are necessary for the development of antiviral responses [13, 14].

The second pathway from the adapter molecule MAVS is associated with the involvement of intracellular signaling molecules tumor necrosis factor (TNF) receptor-associated factors (TRAF-2/6) acting on the IKK complex, which in turn activate the transcription factor NF-κB, promoting its translocation into the nucleus and inducing gene expression of pro-inflammatory cytokines. The emergence of foreign RNA and DNA in the cytoplasm of cells serves as a signal of the presence of infection, and the recognition of viral components by the cytoplasmic receptors

of innate immunity leads to the induction of the synthesis of not only interferons (IFNs) but also pro-inflammatory cytokines that stimulate the development of a typical inflammatory response as a component of antiviral defense reactions [15].

Interferons suppress viral infections through two main ways. First, by binding to specific cellular receptors, which causes an induction of the expression of ISG and the synthesis of several antiviral proteins. The functions of the molecules encoded by these genes include the suppression of the passage by viruses of the life cycle at almost all stages, including the penetration into the cell, translation of viral proteins, viral replication, viral assembly, and the final release into the environment. As a result, cells affected by IFN acquire the so-called "antiviral status" and cannot be infected by viruses. Second, all IFNs possess immunomodulatory properties, which enhance the work of innate and acquired antiviral immunity, activate the cytotoxicity of NK cells, promote the presentation of viral antigens to T lymphocytes, and stimulate the functions of several other cells involved in the protection mechanism against viruses [16, 17].

Mechanisms of the virus escape from the host's immune system

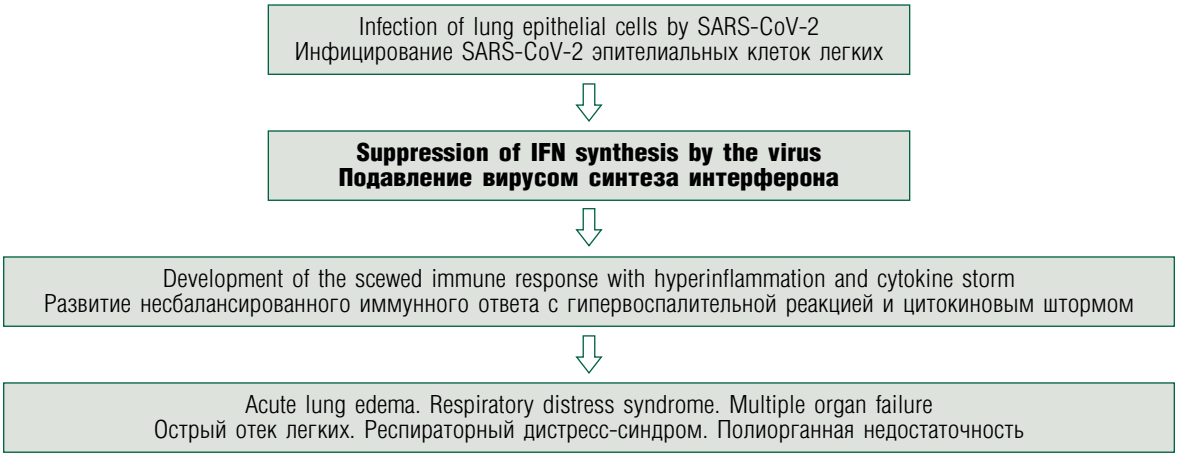
Apparently, the blockade of the IFN system is of particular importance in the pathogenesis of infectious diseases caused by highly pathogenic viruses, which include coronaviruses. IFN inhibits the replication of these viruses; therefore, the suppression of the synthesis and the action of IFN are especially important for their survival in the human body.

The fight of the virus against a host's immune system begins immediately after its permeation into the cell. The nsp proteins organize the endoplasmic reticulum into vesicles surrounded by a two-layer membrane, in which the virus multiplies [18]. The vesicle membrane protects against receptors that recognize pathogen-associated molecular patterns that constitute the innate immune system [19]. These include toll-like receptors (TLRs), RIG-I-like receptors, NOD-1-like receptors, C-lectin-like receptors, and several others [20].

Several proteins of SARS-CoV and MERS-CoV counteract the innate immune responses. Viral proteins nsp1, nsp3 protein macrodomain, nsp-deubiquitinase, ORF3b, ORF6, and ORF9 suppress the action of IFN and IFN-stimulated proteins. In particular, the nsp1 suppress the action of IFN through 3 mechanisms, namely by the inactivation of the translation of host cell proteins by causing degradation of the host mRNA and by inhibiting the phosphorylation of STAT1. Nsp3 is a proteinase that disrupts IRF3 phosphorylation and NF-κB signaling. In addition to the abovementioned proteins, in whom the mechanisms of action have been established, IFN antagonists include the nsp7 and nsp15 proteins with unknown suppression mechanisms [21–23].

The SARS-Cov-2 coronavirus blocks the IFN system in the following ways. First, SARS-Cov-2 possesses viral proteins, particularly Nsp16, that suppresses the recognition by cellular pattern-recognizing receptors. Second, SARS-CoV-2 inhibits the synthesis of type I and type III IFNs by interfering with the signaling from pattern-recognition receptors. Third, it blocks signal transduction from IFN receptors [24, 25].

The blockade of the antiviral effect of IFN is extremely important for the COVID-19 pathogenesis, since the degree of suppression of the IFN system in coronavirus infection is associated with the severity of the clinical manifestations of the disease. In deceased patients with MERS-CoV-induced infection, the level of endogenous IFN synthesis was significantly lower than in the survivors [26, 27]. Clinical cases have revealed that, in COVID-19, insufficient IFN synthesis in the early stages of infection is decisive in the imbalance of innate immune responses, as was confirmed in a mouse model of SARS-CoV respiratory infection, in which unbalanced IFN synthesis and leukocyte release into the lung tissues were noted. The imbalance was attributable to the low synthesis of IFN at the initial stage of infection, which was accompanied by the lack of proper control over the development of coronavirus infection. In response to the intense replication of the virus, the synthesis of pro-inflammatory cytokines, followed by the hyperproduction of IFN itself, was triggered, although the untimely late synthesis of IFN only causes an increase in the inflammatory reaction with a massive release of leukocytes into the lung tissues [28, 29]. In patients with a lethal variant of severe acute respiratory syndrome, in the peripheral blood plasma, not only the levels of pro-inflammatory



COVID-19 immunopathogenesis
Иммунопатогенез COVID-19

cytokines and chemokines but also the levels of IFNα and IFNγ as well as several ISG products were increased [30, 31]. These data suggest that, when infected with SARS-CoV or MERS-CoV coronaviruses, a delay in IFN synthesis disrupts the proper control of viral replication, leading to lung infiltration with activated neutrophils and monocytes as well as intensive synthesis of pro-inflammatory cytokines with all consequences that it entails in the form of an acute hyperinflammatory reaction and the development of respiratory distress syndrome. However, it has also been revealed that the timing of IFN administration, namely its use only in the early phase of infection, can induce a protective therapeutic effect [27].

In clinical studies, in severe patients with COVID-19, not only decreased but also delayed IFN synthesis was registered, which was accompanied by an early onset of intensive synthesis of pro-inflammatory cytokines and the development of a cytokine storm [32]. Thus, if IFN is not synthesized immediately, but with some delay and in small quantities, this mechanism allows the coronavirus to replicate actively and induce an increased synthesis of pro-inflammatory cytokines. Figure 1 presents the general scheme of the COVID-19 immunopathogenesis.

Characteristics of the clinical presentation of coronavirus infection

Approximately 80% of all patients infected with SARS coronaviruses demonstrate the asymptomatic course of the infection or possess a mild to moderate respiratory illness, but approximately 20% of all infected patients develop a more severe illness that require hospitalization, and 5% of them patients even require forced ventilation of the lungs and increased monitoring in the intensive care unit.

After infection with COVID-19, the latency period can last from 2 to 14 days. The typical symptoms include cough, fever, shortness of breath, headache, ailment, and muscle pain [33, 34].

In patients with an unfavorable course of the disease, severe pneumonia with acute respiratory distress syndrome develops, which is characterized by pulmonary edema, accumulation of inflammatory cells in the lungs (such as neutrophils, macrophages, and lymphocytes), and severe hypoxia [35]. Histopathological examination of the pulmonary lesions in SARS infections, in addition to edema and infiltration of inflammatory cells, reveals stratification of the alveolar epithelial cells and the expansion and damage of alveolar septa. In the foci of inflammation, tissue necrosis, and hyperplasia have been noted. Damage to the walls of the interstitial arterioles of the lungs indicates that, in addition to the cytopathogenic effect of viruses, the inflammatory response is significant in the disease development [20].

In addition to the lungs, which are the main focus of the disease, SARS infects several human tissues and organs, including the spleen, intestines, kidneys, liver, adrenal glands, parathyroid gland, pituitary gland, brain, pancreas, neurons of the central nervous system, and endotheliocytes of the vessels of several organs [36]. The most frequent abnormalities in the blood of patients with COVID-19 (as diagnosed by reverse transcription-polymerase chain reaction

[RT-PCR]) include an increase in the concentration of C-reactive protein, a decrease in the concentration of albumin, and an increase in the level of interleukin-6 (IL-6) and lactate dehydrogenase activity.

Cytological indicators include lymphopenia, decreased level of lymphocytes and eosinophils, and increased erythrocyte sedimentation rate [37–39].

In young and middle-aged patients with mild to moderate infection, the number of copies of the SARS viral genome, as determined by RT-PCR during the period from day 2 to day 4 after the onset of the first symptoms, can range from 10^3 to 10^9 copies in a throat swab and from 10^3 to 10^9 copies in a sputum sample; subsequently (usually starting from the day 7), these indicators decrease, but in some patients, even on the day 20, from 10^2 to 10^4 copies of viral RNA can be detected in the smears collected from the throat and sputum samples. The titers of viral RNA in the blood of different patients differ significantly, and this indicator is short-term [39].

Pathogenesis of viral pneumonia

1. Impairment of the renin-angiotensin system (RAS).

The SARS receptor of coronaviruses the ACE2 protein is a significant participant in the RAS that regulates the blood pressure and electrolyte balance and consists of two oppositely directed signaling pathways. In the classical pathway (ACE/AngII/AT1R), renin converts angiotensinogen synthesized in the liver to the peptide angiotensin I (Ang 1–10), which the angiotensin-converting enzyme (ACE) hydrolyzes in the endothelial cells of the lung capillaries to form angiotensin II (Ang 1–8). Angiotensin II, which interact with the AT1R and AT2R receptors, ultimately causes vasoconstriction, thereby increasing the blood pressure.

Angiotensin II also activates (mainly through the AT1R receptor) several more signaling pathways that in turn stimulate the inflammation of the walls of blood vessels as well as fibrosis, fibrinogenesis, and myocardial hypertrophy [40].

The oppositely directed signaling pathway (ACE2/Ang 1–7/Mas1R) is directed by the Zn-metallopeptidase of ACE2, which removes one amino acid from the angiotensin I molecule to form Ang1–9 and also one amino acid from the angiotensin II molecule to form the vasodilatory peptide Ang (1–7), which implements signaling through the Mas1R receptor. Thus, the vasodilating effect of ACE2 gets implemented in two ways, when the vasoconstrictor peptide angiotensin II is converted into vasodilatory Ang-(1–7), the receptor of which is Mas1R, and angiotensin I, which is a substrate for ACE, is converted into inactive Ang-(1–9), which cannot be a substrate for ACE. The activation of this pathway leads to the suppression of leukocyte migration, the expression of pro-inflammatory cytokines, and the stimulation of fibrinogenesis, while the inactivation of *ACE2* in mice leads to an increase in the pro-inflammatory action of angiotensin I [35, 40, 41].

Angiotensin II is mainly produced in the lungs. The inactivation of *ACE2* in mice caused an increase in the blood concentration of angiotensin II, increased inflammation in the lungs, increased vascular permeability, increased pulmonary edema, infiltration of the lungs with neutrophils and, as a result, deterioration of lung function. Conversely, the administration of recombinant ACE2 to mice reduced the above symptoms and improved the lung functions in models of acute lung injury [42, 43].

In mice infected with SARS-CoV, the expression of ACE2 in the lungs decreased. The administration of recombinant protein S (SARS) interacting with ACE2 to mice was also found to decrease the ACE2 expression in the lungs [43].

In patients with COVID-19, blood concentrations of angiotensin II were significantly higher than in healthy individuals, and it was correlated with the disease severity [44]. Thus, the impairment of the RAS is significant in the pathogenesis of pulmonary pneumonia and in acute respiratory distress syndrome in COVID and COVID-19.

2. Cytokine storm.

In COVID and COVID-19, alveolar epithelial cells are primarily affected, especially type II pneumocytes, which produce alveolar surfactant and are the precursors of type I pneumocytes, as well as epithelial cells of the upper respiratory tract. Protection against the innate immune system enables the virus to replicate rapidly in these cells, which is accompanied by a significant cytopathogenic effect and apoptotic death of some cells [45]. Inflammation develops in the lungs, in which alveolar epithelial cells synthesize pro-inflammatory cytokines and chemokines.

Massive infiltration of the lungs with monocytes, macrophages activated by the M-1 type, and neutrophils attracted by these cytokines and chemokines, in turn, provides with additional production of pro-inflammatory cytokines by these cells. In a cytokine storm, high blood levels of pro-inflammatory cytokines and chemokines correlate with the high levels of neutrophils and monocytes in the blood and lungs.

As a result, high concentrations of inflammatory mediators (e.g., IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1A, and TNF) were detected in the peripheral blood of COVID-19 patients [46]. Meanwhile, high concentrations of IL-2, IL-2, IL-6, IL-7, IP-10, IL-2R, IL-10, TNF, MIP1-α, MCP-1, and GCSF correlated positively with the disease severity [47].

Interleukin-6

The blood concentration of IL-6 is an indicator of COVID-19 severity [48]. In the initial period of the disease, high concentrations of IL-6 and C-reactive protein in the blood indicated the need to admit the patient to the intensive care unit [49].

Interleukin-6 is produced by a wide range of activated immune cells, namely macrophages, dendritic cells, neutrophils, and lymphocytes [50]. In addition, the mechanisms of inflammation induction that are unique for coronaviruses were also discovered, and the S type protein of the SARS-CoV virus induced the production of IL-6 and TNF by macrophages [51]; in addition, the SARS-CoV N protein activated the expression of IL-6 by epithelial cells of the lung alveoli through intracellular transfer of the transcription factor NF-κB [52].

Thus, in coronavirus infections, IL-6 production is already induced in infected cells.

Interferon-γ

In COVID-19, the blood concentration of IFNγ increases with an increase in the viral load [53]. IFNγ appears to be produced by CD4 helper T lymphocytes. These cells also produce GM-CSF, which induces monocyte differentiation, which however increases the blood concentration of IFNγ. Previously, the association of high concentrations of IFNγ with the severity of pneumonia was demonstrated for infections caused by SARS-CoV-1 and MERS-CoV.

A high concentration of IFNγ in the blood of a COVID-19 patient is a reliable indicator for referring a patient to the intensive care unit [46, 54].

Tumor necrosis factor

TNF is also one of the key pro-inflammatory cytokines that are significant in the development of several inflammatory diseases and septic shock [50]. The blood serum concentration of TNF increases with SARS-CoV and, especially, extensively with SARS-CoV-2, which correlate positively with the disease severity [54, 55].

Interleukin-1

Interleukin-1 is also an important pro-inflammatory cytokine that plays a dominant role in the cytokine storm [41, 50, 55].

Involvement of the complement system in the pathogenesis of COVID-19

Inflammation, a characteristic of coronavirus infections, also extends to vascular endothelial cells, and electron microscopic examinations of the vessel walls of deceased COVID-19 patients indicate direct infection of endothelial cells with SARS-CoV-2 viruses; the accumulations of inflammatory cells and apoptotic bodies have been reported in the vascular intima [56].

In the blood vessels of the lungs of COVID-19 patients, the deposition of the terminal components of complement C5b-9, Cd4, and a proteinase associated with mannose-binding lectin MASP2 has been reported. Colocalization of viral protein S, C5b-9, and Cd4 in the interalveolar septa and microvessels was noted in 2 out of 5 patients examined [57].

In COVID-19 patients, the deposition of C5b-9, MASP2, and C4d was also noted in the endothelium of the skin microvessels [58].

A past study revealed that the nucleocapsid protein (N) of the SARS-CoV, SARS-CoV-2, and MERS-CoV viruses activates MASP2, which indicates the involvement of the lectin pathway of complement activation in endothelial damage and the development of angiopathy [59]. Narsoplimab, a human monoclonal antibody (IgG4) that binds with MASP2 and blocks the lectin pathway of complement activation, was used as a part of a combination therapy to treat 6 COVID-19 patients with acute respiratory distress syndrome. After the prescription of narsoplimab, the number of circulating endothelial cells, as well as the pro-inflammatory cytokines, decreased rapidly, and the activity of lactate dehydrogenase decreased, and the blood serum level of C-reactive protein also decreased.

In contrast to the control group, all test patients survived and recovered [59].

According to a study on SARS-CoV infection in $C3^{-/-}$mice knocked out on the C3 gene (C3 is the main component of the complement system and is involved in all 3 pathways of its activation); in such mice, viral pneumonia was less severe and lesser numbers of neutrophils and monocytes accumulated in their lungs, while the concentration of pro-inflammatory cytokines in the blood serum was reduced when compared to that in the control mice [60].

The use of the therapeutic antibody eculizumab (which binds with C5 and blocks its cleavage into C5a and C5b and the subsequent formation of the terminal complement complex C5b-9) in the complex therapy of patients with severe COVID-19 has shown encouraging results, as, in some patients, complete remission has been observed and partial remission in others [58, 61].

For instance, AMY-101, a peptide C3 blocker, was successfully used to treat one moderately severe COVID-19 patient [62].

Thus, complement activation contributes to the pathogenesis of COVID and COVID-19. Although the lectin pathway of complement activation is mainly involved in endothelial damage and microangiopathy, the blockade of the classical or alternative pathway of activation can also be considered as a part of the complex therapy of the disease.

3. Seroconversion and cellular immune response.

Seroconversion occurs starting from the day 5 after the onset of symptoms; it is registered in 50% of all patients on the day 7 and in 100% of all patients on the day 14 [25, 39]. In this case, the part of the antibodies referred to virus-neutralizing antibodies. These are antibodies to the receptor-binding domain of protein S1 (amk 318–510 of protein S) and to the HP2 domain (amk 1029–1192 of protein S), which block the binding of the virus to the ACE2 receptor and the virus fusion with the cell membrane, respectively [63]. Thus, neutralizing antibodies, binding to coronaviruses, can act independently, but under *in vivo* conditions, they can bind to other components of the immune system, for example, to complements and phagocytic cells, resulting in viral elimination [64, 65]. The titers of antibodies to the virus, the titers of neutralizing antibodies, as well as their dynamics in the blood serum can differ significantly in different patients [39].

Cellular immune response, as determined in the peripheral blood mononuclear cells of convalescent patients based on the number of cells producing IFNγ, upon stimulation with SARS-CoV-2 proteins (recombinant proteins were used to stimulate cells, namely N, S fragment containing a receptor-binding site, and SARS-protease CoV-2), was characterized by significant individual differences in this indicator [65].

Models of infections caused by SARS-Cov and SARS-Cov-2 in laboratory mice

The need for a model of coronavirus infection in small rodents, primarily in mice, is extremely high. Meanwhile, although SARS-CoV can infect several small animals, including rodents, the disease caused by the virus is asymptomatic or mild in them. For example, when BALB/c mice of 6–8 weeks of age are inoculated intranasally with SARS-CoV virus, viral replication in the respiratory tract reaches a maximum on the day 2 after infection, and the maximum virus production is registered on the day 5 after infection. However, the signs of mild pneumonia can be found only in some animals [66].

In adult BALB/c mice aged 4–6 months and in old mice aged 12-14 months, on days 3–6 after intranasal infection at a dose of 500 TCID 50%, weight loss, water depletion, and tousled fur are noted, along with the signs of interstitial pneumonia detected through histological examination [67].

To obtain SARS-CoV infection models that correspond to the human COVID to the greatest possible extent, attempts have been made to adapt the virus to multiply in the lungs of mice; as a result, viruses have been obtained that cause lethal diseases in models of intranasal infection in wild-type mice.

1. SARS-CoV v2163.

The Urbani SARS-CoV strain, which is not lethal to BALB/c mice, originally produced in Vero cells, was serially passaged 25 times in the lungs of BALB/c mice, which resulted in the preparation of a highly virulent strain designated as v2163. The v2163 virus induced signs of the disease in BALB/c mice aged 5-6 weeks, which included up to 20% weight loss, tousled fur, lethargy within 3–4 days, and death (average lifespan was 5.9 ± 1.4 days with infection

at a dose of 103.5 TCID 50/mouse). The v2163 virus increased the production of IL-1α, IL-6, MIP-1α, MCP-1, and RANTES in the blood of mice, and high IL-6 expression correlated with mortality. The infection largely mimicked a human disease, albeit there was no hyaline membrane formation in the lung pathology.

The v2163 virus was detected in the lungs and nasopharynx of inoculated mice on days 3 and 6 after inoculation, but it is entirely rarely revealed in the blood serum. Virus was not isolated from the kidney, brain, spleen, intestine, liver, or heart tissues of v2163-infected mice within the sensitivity of the virus detection method.

Nine mutations were detected in the v2163 genome, which affected 10 amino acid residues in the genes encoding viral proteins, such that 5 mutations were located in the nsp3, nsp9, nsp13, and 3b/m protein genes and 4 mutations were in the S protein gene [68].

2. SARS-CoV MA15.

SARS-CoV (Urbani strain) was adapted to propagate in the airways of BALB/c mice by successive passages. After 15 passages, a virus named MA15 was obtained, which is lethal for mice after intranasal administration [69].

The average lifespan of dead animals in this model was longer than with the infection with v2163, and it was 13.5 ± 6.9 days. Mortality is preceded by a rapid increase in the viral titers in the lungs and viremia, which is accompanied by lymphopenia, neutrophilia, and pathological changes in the lungs. Viral antigens were detected in large quantities in bronchial epithelial cells and alveolar pneumocytes, and necrotic cellular debris were revealed in the respiratory tract and alveoli, albeit the pneumonia was mild and focal. MA15-infected mice probably died from viral infection with extensive, virus-mediated destruction of pneumocytes and ciliated epithelial cells. In addition to the respiratory tract, the M-15 virus was detected in insignificant titers in the liver, spleen, and brain samples within 1–4 days after infection at a lethal dose.

Sequencing of the SARS-CoV MA15 genome showed that this virus has a different spectrum of mutations when compared to v2163. In total, 6 mutations were revealed (2 in the *nsp5*, 1 in *nsp9*, 1 in *nsp13*, 1 in S, and 1 in *M*), and only one mutation of them in the *S* gene coincides with a similar mutation in v2163 [68, 69].

3. Transgenic mice expressing the human ACE2 receptor.

In a past study [70], transgenic mice expressing the human ACE2 protein were obtained.

The human ACE2 protein is expressed in the lungs, heart, kidneys, and intestines. On days 3 and 7 after inoculation, SARS-CoV replicated in the lungs of transgenic mice more efficiently than in the lungs of wild-type mice. In addition, the transgenic mice showed more severe lung lesions, including interstitial hyperemia and hemorrhages, monocytic and lymphocytic infiltration, protein exudation, proliferation, and desquamation of alveolar epithelial cells. Other pathological changes, including vasculitis, degeneration, and necrosis, were revealed in the extrapulmonary organs of the transgenic mice, and the viral antigen was detected in the brain. Thus, transgenic mice were more susceptible to SARS-CoV than the wild-type mice, and the susceptibility was associated with severe pathological changes that resembled human SARS infection.

In another study [71], transgenic mice expressing the human ACE2 protein were infected with SARS-CoV-2. Animal weight loss and viral replication in the lungs were evidently registered. The typical histopathology was interstitial pneumonia with infiltration of a significant number of macrophages and lymphocytes into the alveolar interstitium as well as the accumulation of macrophages in the alveolar cavities. Viral antigens were noted in the bronchial epithelial cells, macrophages, and alveolar epithelium.

Transgenic mice, in which the epithelial cells human ACE2 were expressed, were obtained in another past study [72]. After intranasal inoculation of SARS-CoV, the mice developed a lethal infection that began in the epithelium of the respiratory tract with the subsequent involvement of the alveoli and the detection of the virus in the brain. Infiltration of macrophages and lymphocytes to the lungs and the subsequent activation of pro-inflammatory cytokines and chemokines in both the lungs and the brain were noted. Transgenic mice expressing human ACE2 were also reported in later studies [73, 74].

Undoubtedly, the model using transgenic mice expressing human ACE2 corresponds to the clinical presentation of COVID in humans to a greater extent than any other models.

Immunotherapy for coronavirus infections

Currently, drugs for immunotherapy of coronavirus infections are under development, in preclinical or, at the best, under clinical trials. Immunotherapy can be represented by active (vaccination) and passive immunotherapy. Passive immunotherapy includes the transfusion of blood plasma of recovered patients and the administration of monoclonal antibodies or immunoadhesins.

1. Interferon.

Immunotherapy can also include the administration of type I or type III IFN preparations, since SARS-CoV-2 suppresses the induction of IFNα and IFNβ in the patient's body [75], which inhibit the innate immune defense system.

The advantage of IFN over other antiviral drugs, which often exert an antiviral effect against a narrow range of certain viruses, which is attributable to the fact that all IFNs of types I and III exhibit antiviral activity against almost all types of DNA and RNA of viruses, launching a program for the synthesis of antiviral proteins in the cells as well as also activate the antiviral immunity. As a result, IFNs ensure the involvement of all possible antiviral mechanisms in the development of a unified defense reaction of the body against the invading virus.

The use of genetically engineered IFN drugs enables surpassing the inhibitory effect of the virus on its synthesis and the manifestation of the effect of IFN in full and in the optimal time frame for blocking the spread of the virus any further. IFN preparations for topical intranasal administration can have a therapeutic effect at the initial stage of the disease and a preventive effect during an epidemic. The main approach to the therapy of COVID-19 with IFN drugs should be timely treatment in the early stages of infection, before the development of a complete symptom complex of life-threatening conditions.

Earlier in clinical practices, in case of infection with coronaviruses SARS-CoV and MERS-CoV, the protective effect of recombinant IFNα preparations was established only with early use, that is at the onset of the disease development but before the emergence of symptoms of severe pulmonary pathology. Later administration of IFN does not provide a positive trend when compared with that in the placebo group [76, 77]. Apparently, such differences in the efficiency of the therapeutic effect of IFN, which depends on the duration of the prescription of drugs, are explainable from the perspective of the immunopathogenesis of coronavirus infection. At the initial stage of infection, there is a lack of endogenous IFN and the introduction of a recombinant analog from the outside can compensate for this deficiency, which plays an important role in the further progression of the infectious process. On the contrary, at more advanced stages, a hyperinflammatory response with increased synthesis of pro-inflammatory cytokines may develop. The administration of IFN during this period is inappropriate, as it can lead to the aggravation of the cytokine storm and exacerbation of inflammation in the lung tissues [78, 79].

Tests of recombinant IFNα2b drugs conducted in China when infected with SARS-CoV-2 have demonstrated reduction in the duration of inoculation of coronavirus from the respiratory tract and the simultaneous reduction of the duration of detection of the elevated levels of IL-6 and C-reactive protein in the blood plasma of COVID-19 patients. Based on the experience of fighting the coronavirus epidemic in China, IFNα preparations are included in the national guidelines for the treatment of COVID-19 patients. Currently, the preparations of recombinant IFN are included in the recommendations of the Ministry of Health of the Russian Federation to combat coronavirus. In adults, IFN is recommended for local inhalation at a dose of $5 \cdot 10^6$ IU twice a day [79].

2. Vaccination.

Despite the fact that the first coronavirus epidemic caused by SARS-CoV occurred in 2002, until date, no coronavirus vaccine has been approved for use in any country. The information dispersed by the mass media about the production and distribution of millions of doses of a particular vaccine should be considered solely as an accelerated preparation for large-scale phase III clinical trials, which will take a long time (several months and years of follow-up of vaccinated and unvaccinated cohorts of the trials participants) to evaluate the results. Information on this issue can be tracked on the corresponding website of the World Health Organization [80]. Currently, a variety of vaccines are being deve-

loped and tested, namely inactivated whole-virion vaccines, live attenuated vaccines, virus-like particles, vaccines based on non-replicating viral vectors, protein subunit vaccines alone and in combination with adjuvants, and RNA and DNA vaccines, with more than 100 different vaccines being proposed in total [81–84]. Large-scale trials of different vaccines conducted in different countries are expected to enable comparison of the efficiency and duration of immunity with the use of different types of vaccines over a period of time.

Notably, there is a danger of side-effects (such as a cytokine storm and an antibody-dependent increase of infection) with vaccination [81, 82].

3. Development of therapeutic monoclonal antibodies.

It was initially believed that neutralizing antibodies are antibodies to the domains of protein S, namely to the receptor-binding domain of the S1 subunit and to the HR1 and HR2 domains of the S2 subunit, which are important for the entry of a virus into the cell. It has been revealed that these domains contain a large number of immunogenic epitopes, which indicates the possibility of obtaining a large number of neutralizing antibodies that differ from each other [83]. Subsequently, it turned out that some antibodies to the proteins of the nucleoprotein (NP) and the viral envelope (E) also possess neutralizing properties. Moreover, some neutralizing antibodies have cross (SARS-CoV–SARSCoV2) activity [84]. Thus, there is a wide range of virus-neutralizing monoclonal antibodies that are promising for humanization and subsequent introduction into clinical practice. Some past studies [83, 84] share basic information, including the mechanism of action, about 30 different monoclonal antibodies against SARS-CoV-2. The clinical trials site in a past study [85] currently has 10 registered clinical trials of monoclonal antibodies against SARS coronaviruses.

4. Development of immunoadhesins.

Immunoadhesins are hybrid recombinant proteins that contain the extracellular domain of a specific receptor fused to the constant region of immunoglobulin.

The immunoglobulin constant region (Fc) confers stability to immunoadhesin *in vivo* and (if necessary) provides binding to complement and Fc receptors of immune cells. The first immunoadhesin to have introduced into public healthcare practice was etanercept, which consists of an extracellular fragment of the TNF receptor and human IgG1 Fc. Etanercept, which is known for >20 years, is effective in the treatment of several chronic inflammatory diseases.

A recombinant protein comprising of an active extracellular domain of the ACE2 protein fused to Fc was initially proposed for normalizing blood pressure and is currently tested in clinical trials [86].

However, since ACE2 binds to protein S with high affinity ($Kd = 1.2 \cdot 10^{-9}$ M for SARS-CoV-2 S protein and $Kd = 5.0 \cdot 10^{-9}$ M for SARS-CoV) [9], such a protein can bind effectively to SARS coronaviruses by blocking the viral receptor-binding domain and competing with ACE2 located on the cell surface. Indeed, ACE2-Fc immunoadhesins bind actively to SARS coronaviruses and neutralize them *in vitro*; particularly, the ACE2–NN–Fc variant was constructed, in which the peptidase activity of ACE2 was inactivated by replacing two amino acids in the active center of the enzyme. This immunoadhesin caused 50% neutralization of SARS-CoV at a concentration of 2 nM when tested in a culture of permissive cells [87]. An identical immunoadhesin obtained by other authors demonstrated a high neutralizing activity against SARS-CoV and SARS-CoV-2 *in vitro*, while the semicirculation time in the blood of mice *in vivo* ($T^1/_2$) was 5.2 days, while $T^1/_2$ of the ACE2 extracellular domain, not bound with Fc, was <2 h [88].

Using genetic engineering methods, it is possible to replace the Fc regions of both humanized monoclonal antibodies and immunoadhesins (for example, FcIgG1 can be replaced with FcIgG2 or FcIgG4) or individual amino acids in Fc using targeted mutagenesis in order to reduce binding to complement or to certain Fc receptors of immune cells, including those with receptors FcγRIIa and FcγRIIb, which is responsible for antibody-dependent intensification of infection [89]. These works are currently ongoing. For example, in the ACE2-Fc immunoadhesin molecule, binding to all Fcγ receptors was inhibited by mutagenesis, while the modified immunoadhesin was highly stable *in vivo*, it could penetrate well into lung tissues, and had a preventive effect upon infection in mice with SARS-CoV-2 [90].

5. Transfusion of plasma of the recovered patients.

It has been assumed that, since the blood plasma donor is a recovered patient, the plasma contains neutralizing antibodies in sufficiently high titers to achieve a therapeutic effect and, at the same time, does not cause any antibody-dependent increase in the state of infection. Blood for obtaining serum was collected from convalescent patients after 2–3 weeks of the first symptoms of disease emergence. One plasma dose was 200–600 mL. Since the determination of the titer of virus-neutralizing antibodies takes time, the blood plasma transfusion is often performed "blindly." This type of therapy has demonstrated its effectiveness (although it is ineffective in terminal patients), which is widely used empirically and analyzed in numerous clinical trials [92].

6. Use of known monoclonal antibodies.

In order to suppress the cytokine storm, monoclonal antibodies and immunoadhesins that are well-known and widely applied in the medical practice can be used against the most significant pro-inflammatory cytokines as well as antibodies that block the complement system [58, 59, 61, 84, 91].

Information on the numerous ongoing clinical trials of such drugs for the treatment of SARS-CoV-2 can be obtained from the clinical trials website given elsewhere [85]. In particular, the following drugs have been tested:

- antibodies against IL-6 and its receptor (i.e., tocilizumab, sarilumab, and siltuximab);
- anti-IL-17 antibody (secukinumab);
- anti-IL-1 beta antibody (canakinumab), soluble IL-1 beta receptor (anakinra);
- antibodies against TNF (i.e., infliximab and adalimumab) and TNF immunoadhesin (i.e., etanercept);
- complement activation blocking antibodies (i.e., narsoplimab, eculizumab, tocilizumab, and vilobelimab).

Conclusions

The treatment of coronavirus pneumonia involves serious difficulties, as has been demonstrated both in the treatment of patients and in animal models [93]. Currently, numerous antiviral and anti-inflammatory drugs are being developed and tested in different countries, including the immunotherapeutic drugs mentioned earlier in the review. The data accumulated until date on the structure of the SARS-CoV-2 virus and its genome and on the pathogenesis of COVID-19 suggest the development and implementation of vaccines, monoclonal antibodies, and immunoadhesins that have been found effective against coronavirus infection into healthcare practice.

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