REVIEWS

Review article
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The role of plasma serine leukocyte proteinase inhibitor in the body's defense against COVID-19

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Abstract

The COVID-19 pandemic continues, causing colossal damage to the population and the global economy. As COVID-19 is studied, new data are emerging regarding the risk of severe coronavirus infection in patients with α_1-antitrypsin deficiency. α_1-Antitrypsin is the main inhibitor and key endogenous regulator of the serine leukocyte proteinase activitry released from the granules of activated neutrophils to the cell surface and into the extracellular space. It has been established that the number of cases of severe course and death of COVID-19 in the territories of 68 countries of the world correlates with the frequency of the spread of mutations in the proteinase inhibitor gene among the population of these countries, at which the concentration of α_1-antitrypsin in the human blood plasma is 10 times lower than normal. All this contributes to the revision of a number of provisions of the pathogenesis and therapy of a new coronavirus infection.

The review presents an analysis of the literature on the role of an inhibitor of serine leukocyte proteinases in protecting the body from COVID-19. The participation of α_1-antitrypsin in the inhibition of SARS-CoV-2 penetration into the respiratory tract epithelial cells, in the protection of the vascular endothelium, blood plasma proteins and elastin of the lung tissue from the damaging effect of leukocyte elastase released during neutrophil degranulation and the formation of neutrophil extracellular traps (NETs) is considered. The role of α_1-antitrypsin in suppressing inflammation by limiting the secretion of proinflammatory cytokines and neutrophil extracellular traps into the blood has been shown. The individual links in the pathogenesis of the new coronavirus infection have been detailed, which will allow revising the strategy for reducing the risks of severe course of COVID-19.

Keywords: *COVID-19, SARS-CoV-2, α_1-antitrypsin, neutrophil extracellular traps, leukocyte elastase*

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Роль плазменного ингибитора сериновых лейкоцитарных протеиназ в защите организма от COVID-19

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Аннотация

Пандемия COVID-19 продолжается, нанося колоссальный ущерб населению и мировой экономике. По мере изучения COVID-19 появляются новые данные относительно риска тяжёлого течения коронави-

русной инфекции у пациентов с дефицитом α_1-антитрипсина (ААТ). ААТ — основной ингибитор и ключевой эндогенный регулятор активности сериновых лейкоцитарных протеиназ, высвобождаемых из гранул активированных нейтрофилов на поверхность клеток и во внеклеточное пространство. Установлено, что число случаев тяжёлого течения и летального исхода COVID-19 на территориях 68 стран мира коррелирует с частотой распространения среди населения этих стран мутации в гене протеиназного ингибитора, при которой концентрация ААТ в плазме крови человека в 10 раз ниже нормы. Всё это способствует пересмотру ряда положений патогенеза и терапии COVID-19.

В обзоре представлен анализ литературы о роли ингибитора сериновых лейкоцитарных протеиназ в защите организма от COVID-19. Рассмотрено участие ААТ в ингибировании процесса проникновения SARS-CoV-2 в эпителиальные клетки дыхательных путей, в защите эндотелия сосудов, белков плазмы крови и эластина лёгочной ткани от повреждающего действия лейкоцитарной эластазы, высвобождаемой при дегрануляции нейтрофилов и формировании нейтрофильных внеклеточных ловушек. Показана роль ААТ в супрессии воспаления посредством ограничения секреции в кровь провоспалительных цитокинов и нейтрофильных внеклеточных ловушек. Детализированы отдельные звенья патогенеза новой коронавирусной инфекции, что позволит пересмотреть стратегию снижения рисков тяжёлого течения COVID-19.

Ключевые слова: *COVID-19, SARS-CoV-2, α_1-антитрипсин, нейтрофильные внеклеточные ловушки, лейкоцитарная эластаза*

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Introduction

COVID-19 (Coronavirus disease-2019) is a potentially severe acute respiratory infection caused by the novel coronavirus (SARS-CoV-2) belonging to the group of betacoronaviruses. Rapidly spreading within different countries, this virus can cause a serious disease in some patients who may develop severe acute respiratory syndrome, disseminated intravascular coagulation (DIC) and multiple organ dysfunction [1, 2].

The development of acute respiratory distress syndrome (ARDS) results in life-threatening impairment of the respiratory function [3]. The mortality among ARDS patients who need mechanical ventilation is 20–50% [1, 4].

The continued increase in deaths, along with the steadily increasing number of SARS-CoV-2-infected people, urges scientists, healthcare and pharmaceutical professionals to ramp up the research aimed at studying molecular mechanisms of pathogenesis of this dangerous infection, at search for effective therapeutic and preventive agents for COVID-19, which would decrease significantly the mortality rates [5, 6].

Do alpha-1 antitrypsin (AAT) levels in human plasma affect the severity of the infection caused by SARS-CoV-2?

COVID-19 is divided into 3 stages of disease progression [4], each of them corresponding to the stages of SARS-CoV-2 travel along the respiratory tract:

1) the asymptomatic condition (from 1 to 2 days), when the virus binds to the receptors of epithelial cells in the nasal cavity, enters them and starts replicating;

2) the disease manifestation stage, when acute protective inflammatory response develops in the upper respiratory tract; within a few days, this response may or may not prevent the further virus replication and migration into airways and lungs;

3) the alveolar phase featuring apparent clinical manifestations, when the virus affects type 2 alveocytes and endotheliocytes, and induces their massive apoptosis accompanied by hyper-secretion from activated neutrophils into the bloodstream and the interalveolar space of neutrophil extracellular traps (NETs).

Note that prior to the alveolar phase, the disease progresses only in 19–20% of the people infected with SARS-CoV-2 [1, 4]. Even then, in 14% of patients, the disease is characterized by relatively mild endothelial dysfunction and moderate impairment of the respiratory function (moderate disease) [4]. Only in 5% of patient, this phase may end in alveolar collapse with intensive endothelial injuries, pulmonary edema and extensive lung tissue destruction typical of ARDS (severe disease) [2–4]. Severe hemostatic disorders are recorded in 100% of patients with severe COVID-19. SARS-CoV-2 enters the bloodstream through damaged regions of the endothelium, causing viremia and septic shock resulting in death in 20–50% of cases, even when properly treated [1].

The comparison between these data and the relative number of residents of the European continent and the United States, who have mutations in the proteinase inhibitor gene governing the AAT production in the body showed an interesting pattern. The proportion of severe and moderate COVID-19 cases associated with the alveolar phase of the disease (19–20%) was almost

similar to the proportion of people having decreased AAT levels in their plasma, resulting from 5 most commonly encountered genetic mutations (15–17%) [7]. The mutation of the proteinase inhibitor gene in the Z allele was seen as most unfavorable, being characterized by the highest (up to 10–15% of the normal level) decrease in AAT levels in peripheral blood. This congenital pathology is generally implied when researchers use the term "AAT deficiency" in their publications [7, 8]. Another interesting pattern comes to light: Carriers of this mutation account for 4–5% in Europe and the United States, while, according to statistics, the frequency of critical COVID-19 cases in these countries is approximately 5%.

AAT is a protein produced by the liver; it participates in inactivation of enzymes. Its primary function is to protect the lungs from the elastase released from neutrophil granules in response to the invasion of an infectious agent and serving as a biochemical marker of an inflammatory process. If the activity of elastase is not inhibited by AAT, it will cause damage to the lung tissue.

AAT deficiency inevitably leads to the development of emphysema both as the primary cause and associated with chronic bronchitis, chronic obstructive pulmonary disease or any other chronic nonspecific lung disease [9, 10]. Other AAT deficiency-related diseases include systemic vasculitis, Type 1 diabetes, rheumatoid arthritis and other autoimmune inflammatory diseases [7]. Patients with chronic obstructive pulmonary disease, emphysema and autoimmune diseases have a high risk of developing severe COVID-19 [2].

The epidemiological studies by H. Yoshikura showed that among 68 countries in the world, the severity of the COVID-19 epidemic strongly correlated with the prevalence of AAT deficiency: both in the number of infectious complications ($r = 0.8584$; $p < 0.05$) and in the number of fatal outcomes ($r = 0.8713$; $p < 0.05$) [11]. It has been found that in countries with a high prevalence of AAT deficiency (Europe and the United States), high COVID-19 death rates were observed for more than 6 months, regardless of changing incidence, while in the countries with a low prevalence of AAT deficiency (Japan, China and other countries of Asia), after the first wave of the epidemic, the number of deaths was steadily decreasing, though the total number of patients remained unchanged or even increased [11].

The α_1-proteinase inhibitor made from human plasma [12] has been used for many years as replacement therapy of AAT deficiency for prevention of emphysema and other chronic inflammatory diseases associated with connective tissue destruction [7]. The intravenous therapy with α_1-proteinase inhibitor stabilizes hemodynamics and coagulation parameters in patients with septic shock and DIC-syndrome [13]. Currently, the inhibitor is being considered as a promising candidate for post-exposure prevention and treatment of COVID-19 [6, 12, 14]; therefore, urgent attention should be given to accumulation and analysis of the available information about the physiological function performed by the plasma AAT in the human body. Further on, based on the diagram presented in the **Figure**, we will look at the role of AAT from the perspective of engaging potential pathophysiological mechanisms of inactivation of the damaging effect of SARS-CoV-2 on the cells of microorganisms.

The role of α_1-antitrypsin in protection of cells and tissues against the destructive effects of leukocyte elastase

Plasma contains 7 protein inhibitors of serine proteinases (serpins) accounting for approximately 10% of all its proteins [10].

They include:

- SERPINA1 (α_1-antitrypsin, also known as α_1-proteinase inhibitor) protects lung tissue from leukocyte elastase (LE);
- SERPINA5 (protein C inhibitor);
- SERPINC1 (antithrombin) inhibiting blood coagulation proteases;
- SERPIND (heparin cofactor II);
- SERPINE1 (inhibitor of plasminogen activator);
- SERPING1 (C1 inhibitor) regulating the complement, kallikrein and contact system activation;
- SERPINF2 (α_2-antiplasmin) inhibiting plasmin and regulating fibrinolysis.

In patients with COVID-19, the balance between plasma serine proteases and their serpins, which is crucial for normal functioning of biological systems in the healthy body, is severely disturbed in 4 main proteolytic cascades (coagulation, complement, fibrinolysis and kallikrein) being affected by LE profoundly released from granules of activated neutrophils during their massive degranulation and NETosis of these cells in the lung tissue, liver and other organs. As a result, in patients with COVID-19, a "proteolytic storm" and the subsequent cytokine storm start developing along with the hyperactivity of the coagulation cascade, endotheliopathy and hypoxia followed by intensive degradation of elastin in the lung tissue and by thrombotic complications affecting vessels of different organs [4]. Based on the data of the recent clinical studies, the imbalance in the elastase-AAT system is observed in all patients with severe COVID-19, especially during the period preceding their death from the novel coronavirus infection [15].

The level of LE activity in nasopharyngeal swabs from patients diagnosed with COVID-19 is 3 times as high as the normal level [16], demonstrating a tenfold increase in blood and bronchoalveolar lavage fluid from patients with sepsis and ARDS [17, 18]. Having the broadest substrate specificity, during the imbalance in the enzyme-inhibitors system (AAT-elastase),

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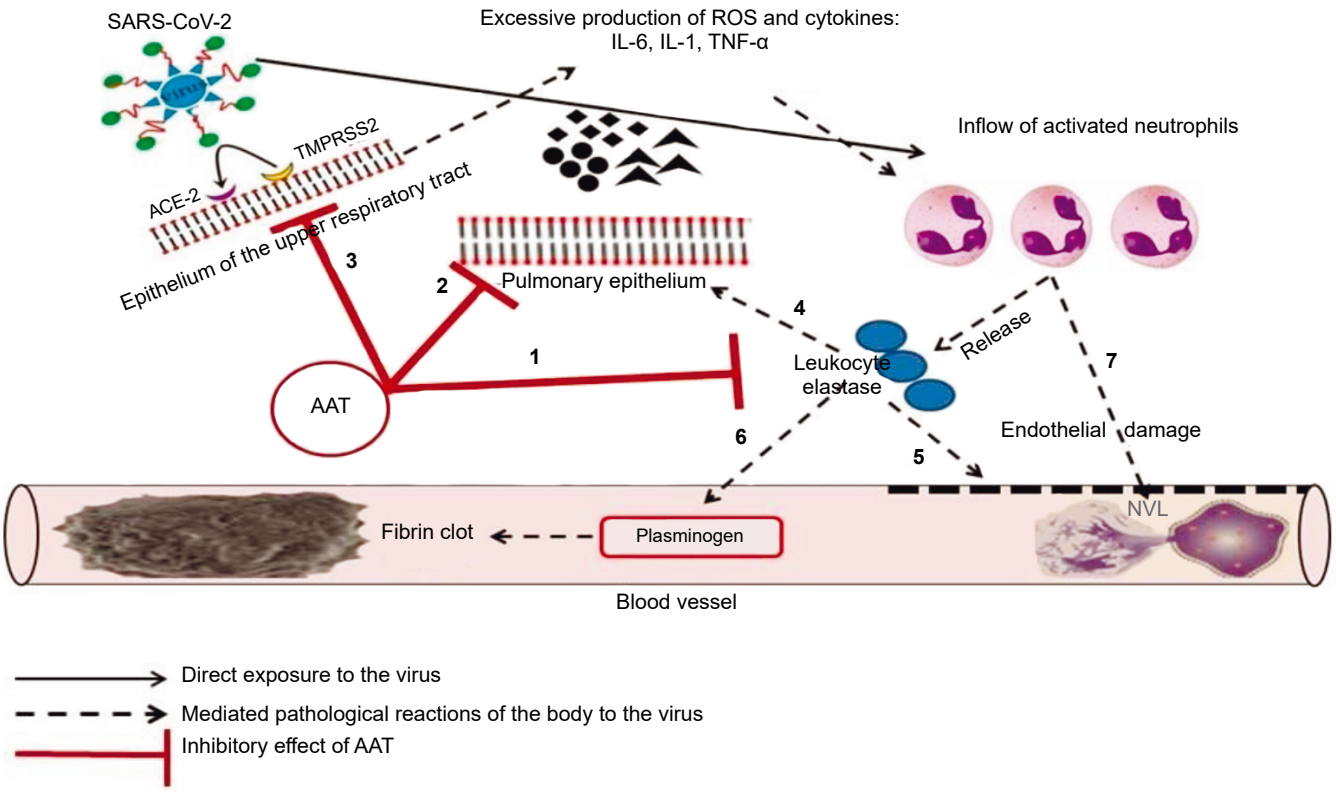

An exemplary scheme for countering SARS-CoV-2 by means of AAT.

1 — neutralization of the extracellular proteolytic activity of leukocyte elastase, preventing damage to the pulmonary epithelium (4), vascular endothelium (5), destruction of plasminogen (6) and excessive formation of NVL (7);
2 — endogenous suppression of overproduction of ROS and pro-inflammatory cytokines by cells, preventing the generalization of the inflammatory response;
3 — suppression of the process of penetration of coronavirus into epithelial cells due to inhibition of the activity of serine proteases (type 2 transmembrane protease serine 2 — TMPRSS-2, etc.), which cleave the SARS-CoV-2 S-protein into subunits.

this serine protease cleaves not only different structural proteins, including elastin, but also multiple plasma proteins: clotting factors, fibrinolytic factors, and those of the kallikrein-kinin system and the complement, including all the above serpins [9, 10]. The key role of LE in destruction of lung tissue in patients with chronic obstructive pulmonary disease, emphysema, other lung diseases and ARDS [19] and, consequently, the role of AAT in prevention of this damage has been confirmed [7]. The LE ability to inactivate nearly all regulatory components of proteolytic systems responsible for adaptation and protection underlies the critical role of LE in impaired regulation of blood coagulation processes in patients with tuberculosis [20], lung cancer [21] and, apparently, with COVID-19 [2, 4, 5, 15].

Severe hemostasis disorders in patients with severe COVID-19 present a sharp decrease in blood levels of antithrombin III and ADAMTS13 protease, which is associated with the proteolytic storm and cytokine storm [4], elastase secretion and resulting destruction of plasminogen without functionally active plasmin [2, 5], elevated levels of D-dimer and fibrinogen degradation products [1, 15]. Besides, NETs in peripheral blood of such patients account for more than 16% [22]. It is believed that in patients with acute septicemia, changes in the levels of clotting factors and an increased number of fibrinogen degradation products result from proteolytic cleavage of plasma proteins by serine leukocyte proteases (SLPs) [18, 20, 23].

Among the serpins present in blood, AAT has the highest concentration (the normal range from 1.2 to 2 mg/ml) and provides about 80% of the total anti-proteolytic activity of the plasma [10]. This protein is mainly produced by hepatocytes and, to a lesser extent, by lung epithelial cells [8]; it is an inhibitor of three SLPs: elastase, cathepsin G and protease 3, though it is considered an LE inhibitor, as only elastase is present in very high concentrations (5.33 mM or around 67,000 molecules per granule) in mature human neutrophils [24] and is the most extensively studied SLP, which accounts for not less than 90% of the total proteolytic activity in primary granules of the cells [9]. In addition to AAT, LE can only bind to α_2-macroglobulin, which suppresses its activity less effectively. Other plasma serpins are not active towards LE [10].

When neutrophils are stimulated by chemoattractants, the specific quantity of SLPs are released from granules to the cell surface where they function, displaying their catalytic activity, in the presence of AAT, by suppressing the process of forming the bond be-

tween the enzyme and inhibitor of reactive oxygen species (ROS) generated by cells during the "respiratory burst" [25, 26]. Compared to the low surface expression of SLPs in non-activated cells, chemoattractants (fMLP and C5a) increase the expression 3-fold' however, with the preliminary priming of neutrophils with lipopolysaccharides (LPS), the SLP expression through the simulating effect of chemoattractants increases 10-fold. The level of protease surface expression increases 30-fold by the phorbol 12-myristate 13-acetate, an activator of neutrophils' functions [25] and conventional and most commonly used NETosis inducer [27, 28].

At the point of contact between the activated neutrophil and the endothelium, AAT and ROS regulate the catalytic activity of the surface LE in space and time, limiting the proteolytic effect of the enzyme to the distance not exceeding 1.33 μm from the cell and to the duration not exceeding 12.4 msec [24]. Normal regulation, when there is no AAT deficiency and/or no excessive neutrophil degranulation, results in moderate and fairly fast elastase-induced cleavage of cadherin responsible for tight junctions between epithelial cells, forming a gap, which is sufficiently wide for migration of neutrophils from the vascular bed [29]. The AAT deficiency leads to disruption of the process regulation, causing endothelial injury due to a 2.5-fold increase in the distance covered by the effect of the surface LE, and, more importantly, due to a 6-fold increase in the timespan, during which LE exerts its damaging effect on the endothelium [24].

Cleaving the core proteins of heparan sulfate proteoglycan, which is a component of the extracellular matrix made up by endothelial cells, LE profusely releases soluble heparan sulfate, which is an endogenous ligand of the Toll-like receptor 4 (TLR4) in various cells, and, consequently, activates the endogenous pathway to systemic inflammatory response syndrome [30]. The surface protease 3, acting jointly with LE, intensifies neutrophilic inflammation through degradation of granulin-epithelin precursor (progranulin) acting as a suppressor of inflammation [31]; on the cell surface of activated neutrophils, cathepsin G converts angiotensin I into angiotensin II, functioning as an angiotensin-converting enzyme, thus providing a significant additional factor for increased vascular permeability and disrupted regulation of the blood pressure [32].

Currently, severe complications in COVID-19 are explained by the excessive inflammatory response to SARS-CoV-2 [1, 3, 5, 22], highly intensive secretory azurophilic degranulation of neutrophils [16], which is induced by the virus, alongside quantitative and functional AAT deficiency [2, 11]. AAT can partially lose its functional properties, being affected by proteolytic enzymes [9], tobacco smoke [33], intensive oxidation during oxidative stress [2], and by adsorption of a large amount of LE on strands of the DNA molecule, which form the backbone of DNA networks of NETs [23]. The significantly increased production of ROS is observed in all viral respiratory infections, including COVID-19 [34]. The oxidative stress involves oxidation of a large number of iron ions present in lysosomes, thus leading to disrupted stability of lysosomal membranes [35]. Excessive neutrophil degranulation leads to massive death of neutrophils and to inevitable neutrophil autolysis (NETosis or secondary necrosis) accompanied by release of a large number of SLP molecules into extracellular space [27, 36]. In this context, AAT acquires an increasingly important role in protection of cells and tissues against the disruptive effect of LE.

α_1-Antitrypsin as an endogenous suppressor of inflammation and regulator of formation of neutrophil extracellular traps

Currently, SLPs released during neutrophil degranulation are seen as signaling molecules, which, similar to hormones, control the functions of different cells involved in the inflammation and coagulation processes. The signal pathway is built through proteolytic modulation of expression and activity of receptors on cell surface as well as through regulation of the production and secretion of cytokines by cells [37, 38]. Platelets, lymphocytes, macrophages, endothelial and epithelial cells express special, proteolytically activated surface receptors (protease-activated receptors — PARs), which act as protease sensors during inflammation and immune response. Binding to the receptor of the PAR family, the protease cleaves off the N-terminal peptide, thus opening the attached ligand (peptide, protease agonist). The ligand activates the receptor, triggering a cascade of signal responses leading to rapid transcription of genes responsible for stimulation of cytokine production and controlling the cell death at the inflammation site [39, 40].

Through PAR1 and PAR2 on epithelial cells, surface SLPs of activated neutrophils regulate the secretory function of the epithelium and the leukocyte transepithelial migration [37]. Activating epithelial cells and cleaving the cadherin responsible for their adhesion, LE induces apoptosis of epitheliocytes [41]. The data from Suzuki et al. demonstrate that in this case, the apoptosis is induced via the pathway that is controlled by proteolytic activation of PAR1 on the surface of the lung epithelial cells - PAR-1–NF-κB–p53 [42]. Through PAR4, cathepsin G activates the secretory function of platelets, regulating their interaction with neutrophils and other cells at the inflammation site. Through PAR2, all the three SLPs regulate the secretion cytokines and chemokines by T-lymphocyte helpers and other leukocytes [37, 39]. The experimental data show that mouse lymphocytes, having lost PAR2 expression due to the genetic mutation, start producing much less interferon-gamma (IFN-γ) and interleukin-17 (IL-17), responding to the antigen stimulation [43]. The PAR expression on

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the surface of neutrophils leads to activation of these receptors by surface SLPs and, consequently, to increased production and release of proinflammatory cytokines by neutrophil granulocytes stimulated by LPS, phorbol 12-myristate 13-acetate or chemoattractants [39].

The AAT regulating the activity of SLPs in human plasma is a powerful endogenous inflammation suppressor limiting ROS production [44] and release of proinflammatory cytokines into bloodstream. During the whole-blood antigen stimulation of leukocytes in samples from patients with inherited AAT deficiency, cells produce IL-6 and IL-1 3.4 and 8.4 times as much as the leukocytes in the blood of donors without this pathology. The hypersecretion and release of cytokines into plasma of patients with AAT deficiency were inhibited by the AAT protein that had been isolated from the blood, if it was added in its normal physiological concentration to the blood before the antigen stimulation; it provided the effectiveness of suppression for IL-6, tumor necrosis factor α (TNFα) and IL-1 — 97, 91 and 47%, respectively. Spontaneous production of IL-1, IL-6 and TNFα by individual leukocytes could be activated by diluting blood with RPMI, as the dilution decreased the concentration of α_1-proteinase inhibitor (AAT protein) in the plasma [8].

The cytokine storm and proteolytic storm in patients with severe COVID-19 are accompanied by massive production of NETs by activated cells [1, 2, 4, 22]. NETosis is a ROS-dependent process, which can be described as genetically determined programmed death of neutrophils; it is associated with successive irreversible morphological alterations in cells: chromatin decondensation; fragmentation of nuclear and lysosomal membranes; mixing of nuclear content with cytoplasmic proteins; plasma membrane rupture; release of histone-decorated DNA webs (NETs) into extracellular space; large quantities of LE molecules as well as two other SLPs and different cationic bactericidal proteins from cytoplasmic granules [2, 27].

The fact that viruses can induce NETosis through their direct impact on neutrophil granulocytes has been recognized relatively recently, and the molecular mechanisms of NETs formation have been insufficiently studied in the context of viral infection. The above process can be induced by endosome TLR7 and TLR8 in HIV-1 or by β_2 integrins in hantavirus-caused infections [45], though there are no data confirming that SARS-CoV-2 penetrates neutrophils or can be adsorbed in large quantities on the cell surface to induce the process of active azurophilic degranulation. Nevertheless, the results of pathoanatomical studies confirm the presence of diffuse pulmonary infiltrates in the lung tissue of patients who died from COVID-19; infiltration was caused by NETs-forming neutrophils [2, 3]; high levels of NETs in peripheral blood smears stained using the Romanowsky-Giemsa technique (over 16%), could be used as a criterion for negative prognosis for COVID-19 and risk of its fatal outcome [22]. In the context of the novel coronavirus infection, hypersecretion of NETs promotes the development of alveolitis, endothelium damage, platelet activation and many other processes, which eventually induce intravascular blood coagulation [1]. The central role in development of severe fibrinolysis failure can be played by adsorption of large quantities of LE molecules in NETs [2], which strongly disrupts the SLP inhibition by its plasma AAT inhibitor [23].

Nuclear chromatin decondensation, which takes place when NETs are formed, is induced by LE. Excessive neutrophil degranulation drives LE into cell nuclei, where it cleaves nuclear histones responsible for keeping the chromatin of non-activated cells in tight condensed condition. In fact, NETosis is regulated by elastase as well as by myeloperoxidase intensifying the LE effect [27]. The key role played by LE in NETosis induction is proved *in vivo* by the ability of the specific low-molecular-weight LE inhibitor (sivelestat) to prevent formation of NETs and save animals suffering endotoxin shock induced by lethal doses of LPS of gram-negative bacteria. This inhibitor, which was approved in Japan and South Korea for treatment of patients with ARDS, was delivered into bodies of laboratory animals with special nanoparticles extending its protective effect [46].

NETs accumulation in blood and different organs is caused by imbalance between NETosis and DNase I-controlled NETs elimination from the body [3]. This imbalance can be associated with insufficient activity of the nuclease (for example, in patients with atherosclerosis) [32], while the decrease in the DNase I activity is explained by significant alterations of structural and adhesive properties of NETs formed during AAT deficiency [28]. The non-balanced formation of NETs is observed in all known autoimmune and chronic inflammatory diseases associated with uncontrolled destruction of connective tissue. As patients with the above diseases have a high risk of developing severe COVID-19 [2] and the diseases are accompanied by inherited (and/or acquired) AAT deficiency [7], the plasma SLP inhibitor (AAT protein), which controls LE activity, may play a critical role as a factor limiting the NETs formation and accumulation in lungs and peripheral blood of patients with COVID-19 [6].

α_1-Antitrypsin prevents SARS-CoV-2 entry into epithelial cells

The access to epithelial cells targeted by all coronaviruses that can cause a life-threatening disease (SARS-CoV, MERS-CoV and SARS-CoV-2) is provided by the viral S protein, which binds to the surface receptor of the angiotensin-converting enzyme 2 or to the collagen receptor — dipeptidyl peptidase 4 (CD26) when people are infected with MERS-CoV. To accelerate the process of coronavirus entry into target cells, the S protein must be cleaved proteolytically into two

subunits (S1 and S2) by TMPRSS2 expressed on the surface of epithelial cells of the respiratory and gastrointestinal tracts, the upper parts of which are sites of entry for infection [34, 47].

Using the combined 20-liter bronchoalveolar lavage fluids collected from SARS-CoV-2 seronegative healthy donors, L. Wettstein et al. isolated fractions of different proteins to assess their ability to inhibit SARS-CoV-2 entry into epithelial cells [12]. They identified the fraction that was most effectively inhibited the early intracellular stage of development of viral infection. In this fraction, they identified a specific protein (AAT serpin) responsible for inhibiting SARS-CoV-2 entry into epithelial cells of human lungs and into epithelial Vero E6 cells expressing surface TMPRSS2. The specificity of AAT in inhibition of SARS-CoV-2 entry was demonstrated by its inability to suppress viral pseudoparticles carrying G protein of the vesicular stomatitis virus.

According to K.Y. Oguntuyo et al., the blood sera from individuals who were not exposed to SARS-CoV-2 effectively inhibited the entry of this virus into epithelial cells. The inhibition was provided by AAT that could induce neutralization of the proteolytic activity of TMPRSS2 and LE [14]. Although the modulating effect of LE on SARS-CoV-2 penetration into cells has not been confirmed experimentally *in vitro*, this effect was thoroughly studied in modeling of interaction between epithelial cells and SARS-CoV (the 2002 virus) [48] and can be important as the factor that can significantly accelerate the spread of the virus *in vivo*, when a lot of activated neutrophils migrate to the inflammation site and there is an imbalance in the elastase-AAT system. Active elastase, which has a damaging effect on epithelial cells and their receptors [37, 42], contributes to the 100–1,000-fold increase in the speed of coronavirus penetration into epithelial cells, and, consequently, to the dramatically increased intensity of its intracellular replication [49]. In addition to TMPRSS2 and elastase, the S protein that provides access for coronaviruses to target cells can be cleaved by other proteases, which change the permeability of cell membranes: trypsin, cathepsin and TMPRSS11a. The employment of additional proteases in cleavage of the S protein may broaden the range of coronavirus-susceptible target cells and can be an important factor of pathogenicity [47].

In laboratory animals, the combined intranasal injection of SARS-CoV and LPS of gram-negative bacteria caused acute viral infection and severe pneumonia, while the injection of the same dose of coronavirus alone into the upper respiratory tract did not cause any acute viral disease progression. In these tests, non-toxic doses of LPS were used; these doses did not induce any substantial pathologic changes in the body. The authors explain this phenomenon by the well-known ability of LPS to functionally activate neutrophils, which results in release of LE and other SLPs onto cell surface, and by the stimulating effect of LE on the virus entry into epithelial cells of the upper respiratory tract. Otherwise, it would be impossible to explain why the development of severe lung infection, when virus and LPS were jointly delivered into the body, was suppressed by specific LE inhibitors [50].

Discussion

Although coronaviruses have been studied for almost 20 years, the pathogenesis of the novel coronavirus infection caused by SARS-CoV-2 still needs investigation, especially regarding the mechanism responsible for a cascade of responses leading to multiple organ dysfunction. Effective methods of prevention and treatment of this potentially dangerous infection are still being looked for. When most of the global population has not been vaccinated against COVID-19, studies focusing on the existing and sufficiently safe antiviral and anti-inflammatory agents should be continued in the context of using them against SARS-CoV-2 to prevent any possible activation of pathophysiological mechanisms inducing severe COVID-19.

The present-day hypothesis about the pivotal role of NETosis in the COVID-19 pathogenesis and NETosis-associated disruption of AAT regulated LE activity [2] is closely connected with the new assumptions about the role of neutrophil granulocytes in the immunopathogenesis of a wide range of different diseases of infectious and non-infectious etiology [51]. NETosis as a mechanism of NETs formation was discovered in 2004, during the period between the outbreak of coronavirus infection in 2002 and the current SARS-CoV-2-caused pandemic. The discovery serves as a powerful motivator for further research underlying this assumption. Clinical trials for dornase alfa (recombinant human deoxyribonuclease I, DNase I) [3] as well as for specific TMPRSS2 inhibitors (camostat) [52] and LE inhibitors [5] have been launched recently. Specialists expect that the strategy of concurrent administration of nuclease and protease inhibitors during antioxidant therapy will be very efficient. The treatment will be targeted not only at clearance of NETs, but also at prevention of formation of new NETs *in vivo*.

New information about molecular mechanisms of COVID-19 pathogenesis, which is obtained promptly through joint efforts of scientists from different countries, is of exceptional interest to specialists exploring the pathogenesis of viral and bacterial, especially dangerous infectious diseases. For example, the pathogenesis of pneumonic plague and tularemia is still poorly studied, as the fact that phagocytosis-resistant cells of *Yersinia pestis* and *Francisella tularensis* are able to induce the lagged death of host neutrophils became known only in the new millennium [53, 54]. This delay results in massive neutrophil autolysis in peripheral blood on the 2nd–3rd day following the aerogenic infection and causes sweeping generalization of the inflammatory process leading to fast development of DIC

syndrome, which is explained by release of huge quantities of LE molecules into the plasma [36].

Conclusion

Thus, the analysis of the literature data highlights an important role of the quantitative and functional deficiency of the SLP plasma inhibitor, AAT, in the pathogenesis of the novel coronavirus infection and gives grounds for using drugs suppressing the activity of TMPRSS2 and LE for the purpose of prevention and treatment of COVID-19. Further large-scale clinical and experimental studies in this field will elucidate the mechanism of the novel coronavirus infection pathogenesis and will be instrumental for further development of efficient means and methods that will help win the fight against SARS-CoV-2.

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