

# Apoptosis and P53 Activation are involved in COVID-19 Pathogenesis

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## Abstract

The problem of preserving epithelial stem cells in the lungs and other tissues as well as providing the possibility of their reparation seems to be very important in SARS-CoV-2 infection. A delayed or insufficient type I IFN response and the associated early virus replication result in massive cell death due to apoptosis and necroptosis induced by the virus through caspase 1 and 8 activation as well as increased p53 activity. Increased p53 activity reduces the possibility of cell proliferation and differentiation, which hinders lung epithelial reparation (and apparently in other injured tissues as well) and this, in turn, increases inflammation, leads to dysfunction of the corresponding organs and predetermines further development of fibroses. Increased p53 activity also enhances apoptosis (not only the virus-infected cells, but also the adjacent, accidentally “captured” cells may be its “victims”), which additionally increases tissue injury. Perhaps besides the antiviral and anti-inflammatory medications used currently for COVID-19 treatment, other drugs and methods should be developed and used to maintain tissue stem cell populations as well as to reduce excessive p53 activity and apoptosis.

SARS-CoV-2 is the coronavirus responsible for the pandemic viral pneumonia known as COVID-19. COVID-19 has a wide spectrum of clinical manifestations ranging from asymptomatic or mild disease to severe viral pneumonia which may lead to Acute Respiratory Distress Syndrome (ARDS), a cytokine storm and multiple organ failure [1]. SARS-CoV-2 enters the human host cells by means of binding its S (spike) protein with angiotensin converting enzyme-2 (ACE2) with the participation of serine protease TMPRSS2 [2]. ACE2 receptors are widely distributed in various tissues and organs of the human body including the cells of the heart, kidneys, mucosae of the upper respiratory tract, and endothelial cells of all blood vessels (including capillaries) [3]. That is why as soon as SARS-CoV-2 enters the circulation, it spreads widely, which explains the ability of this virus to generate systemic diseases. Actually COVID-19 patients suffer

not only from ARDS, but also from other complications such as myocardial injury, arrhythmia, acute renal injury, neurological complications [4]. However the lungs are the main target organ for SARS-CoV-2. In fact 80% ACE2 expressing human cells have been shown to be type 2 alveolar epithelial cells (AT2) – self-sustaining progenitors for AT1 (differentiated cells, which are a component of the blood-air barrier. Besides, TMPRSS2 is highly expressed on AT2 [2,3].

In the host cells RNA viruses are recognized by the host immune system through three main classes of pattern recognition receptors: Toll-, RIG-I- and NOD-like receptors. Signal effectors NF-κB and IRF3/7 are activated as a result of recognition. NF-κB promotes the transcription of proinflammatory cytokines such as TNF-α, IL-1β and IL-6, further causing inflammatory responses Th1 and Th17 with subsequent IFN-γ and IL-

17 secretion. IRF3/7 promotes the production of type I IFNs, which, in turn, initiate the transcription of IFN-stimulated genes (ISGs) [5].

SARS-CoV-2 penetration into the epithelial and endothelial cells after binding to ACE2 initiates cell death pathways (apoptosis, necroptosis) through caspase 1 and 8 activation [6] as well as IL-1 $\beta$  and IL-18 transition to their bioactive form and their release via necroptosis. IL-1 $\beta$  additionally induces the expression of other proinflammatory cytokines such as TNF- $\alpha$  and IL-6 [7]. So cell death and inflammatory responses are closely associated during SARS-CoV-2 infection [8].

The dual mode of cell death pathways induced by SARS-CoV-2 in the epithelial lung cells may lead to successful antiviral responses or immune pathogenesis depending on the degree of cell death activation. Many factors (including the initial dose of the viral infection, the success of the host immune response, comorbidities) may influence its level. The inflammatory responses of the infected cells in cases of massive infection or rapid early replication of the virus can additionally cause immune cell infiltration into the lungs with subsequent overproduction of proinflammatory cytokines, which may lead to a severe course of the disease [8].

So hypercytokinemia is a distinctive feature of COVID-19. Serum levels of some of these cytokines (mainly IL-1 $\beta$ , IL-1Ra, IL-6, IL-7, IL-10, IP-10 and TNF- $\alpha$ ) allow to differentiate mild, moderate and severe cases of the disease [9]. In severe cases hypercytokinemia may increase and go into a cytokine storm leading to such critical conditions as ARDS, disseminated intravascular coagulation or multiple organ dysfunction.

High levels of chemokines and their receptors are also observed in COVID-19 patients. Accordingly, many increased regulatory transcripts of chemokines are detected in BALF samples of COVID-19 patients, including neutrophil recruitment mediators [9]. These results agree with data of lung infiltration by monocytes, macrophages and neutrophils, in contrast to lower lymphocyte counts [10]. It should be noted that cytokine storms occur in COVID-19 patients in spite of a decreased viral load, and this fact suggests that not only the virulence of the virus but also an excessive immune response of the host may be responsible for such an outcome. In such situations IL-

1 $\beta$ , IL-18, IFN- $\gamma$  and IL-6 are the key mediators of hyperinflammation [11].

It should be noted that conditions of both hyperinflammation (which may lead to a cytokine storm) and immunodeficiency (namely lymphopenia and suppression of early interferon response) are combined in COVID-19.

Lymphopenia is one of the most prominent COVID-19 markers; it is observed in over 80% patients [9]. Tests have shown that all lymphocyte subsets are decreased including CD4+ and CD8+ cytotoxic T-cells [12], natural killer (NK) cells, memory cells and regulatory T cells as well as B cells [13]. Decreased lymphocyte counts are closely associated with a severe course of the disease [13]. In addition to a quantitative decrease, T cells demonstrate an increased level of depletion and a reduced functional diversity [12]. Lymphopenia seems to be due to the fact that in spite of the very low ACE2 concentration on the lymphocytes the virus can directly infect T cells, though it is unable to replicate in them [14]. So T cell infection may lead to cell death via apoptosis, necroptosis or pyro ptosis [14]. In such situations infected epithelial cells release a number of cytokines including TNF- $\alpha$  which enhances T cell apoptosis [15]. Thus, lymphocytes are another category of cells which, along with the epithelium and endothelium, suffers as a result of uncontrolled cell death in situations of massive apoptosis.

Type I IFNs are of great importance to protect the host against viral infections because they promote intracellular RNA degradation and virus clearance and trigger a long-term adaptive immune response [16]. However coronaviruses have been shown to be able to suppress the type I IFN response. In fact the Nsp1 protein of SARS-CoV-2 can almost totally prevent the translation of not only interferons and other proinflammatory cytokines, but also of the antiviral IFN-Stimulated Genes (ISGs) [17,18]. Nsp1 effectively interferes in the mechanism of cell translation, which leads to a stop in host protein production and hinders the normal functions of the defense systems of the cell. In such situations the virus can use its resources up to their exhaustion and cell death [18]. So the main links of the innate immune system which depend on the translation of such antiviral protection factors may be ineffective [19].

The kinetics and intensity of the antiviral response are the decisive determinants of COVID-19 outcome. In mild and moderate COVID-19 cases the early antiviral response (mostly of type I IFNs) allows to reduce the viral load quickly and prevents T cell depletion and hypercytokinemia [11,20].

In COVID-19 cases with a severe course of the disease a delayed or low antiviral response results in increased lung cytokine / chemokine levels, impaired virus-specific T cell responses and acute clinical deterioration [21]. Early viral replication leads to massive epithelial, endothelial and T cell apoptosis and perhaps may directly enhance coagulopathy. Actually it has been shown that vascular thrombosis may be caused by a direct effect of SARS-CoV-2 on platelets, as both ACE2 receptors and serine protease TMPRSS2 have been found on them [22]. The danger of a high viral load may lie in the fact that – according to some data – the S part of the SARS-CoV-2 protein is a superantigen that causes T cell activation, and this – according to the authors – may be one of the main causes of the cytokine storm [23]. Besides, it has been shown that in cases with a delayed immune response to coronaviruses, type I IFNs can themselves induce hyperinflammation and intensify the cytokine storm [17,21].

It should be taken into account that besides the antiviral and pro-inflammatory activity, type 1 IFNs have antiproliferative and proapoptotic functions [24]. IFN signalling has been shown to interfere with lung repair during recovery from the flu, with IFN- $\lambda$  most effectively controlling these processes. In such situations the p53 protein induced by type 1 IFNs directly reduces epithelial proliferation and differentiation [25]. Active proliferation of AT2 is essential for lung repair. Indeed, in normal conditions AT2 showed a low turnover rate, but after a flu-induced lung injury they rapidly proliferated starting from Day 5–7 after infection, which correlated with recovery from infection [25]. Thus, delayed long-term production of type 1 IFNs makes the viral infection worse: it disrupts lung epithelial regeneration through p53 activation, which leads to epithelial loss and increases the severity of the disease. Increased p53 activity also enhances apoptosis (not only the virus-infected cells, but also the adjacent, accidentally “captured” cells may be its “victims” [25], which additionally increases tissue injury.

Characteristically, COVID-19 patients had increased regulation of apoptosis, autophagy and p53 pathways in PBMC [26] and a strong induction of IFN and p53 signalling in collected BALF samples [27] as compared with healthy control groups. Epithelial loss contributes to the development of ARDS, pneumonia, and increased susceptibility to bacterial superinfections. Thus, repair of the damaged epithelial tissue is of paramount importance to maintain lung function and barrier defense. It should be noted that a significant epithelial injury unaccompanied by its timely repair also leads to lung fibrosis, which is a common complication of COVID-19. Indeed, insufficient or impossible repair of functionally important cell categories specific for a given tissue is the basic cause of fibrosis development [28]. So there is the question of the consequences of injury of the stem tissue cells and the corresponding untimely and limited renewal of differentiated cells (or even its total failure) in cases with a severe course of infection caused by insufficient immunity and the inability of the host organism to limit the reproduction of viruses [29]. This apparently also applies to cardiomyocytes, hepatocytes, renal epithelium, since ACE2 receptors are widely distributed in the human body and were found in 72 types of human tissues [3]. It should be noted that COVID-19 is accompanied by coagulopathy and vascular thrombosis, which additionally injures tissues due to insufficient oxygen supply. Indeed, the bulk of the complications in patients after COVID-19 are in the lungs, heart, kidneys and brain, but in severe cases the disease may affect any organ. Even a mild course of the COVID-19 disease does not guarantee that there will be no complications; and they may not appear immediately – serious problems with the lungs or heart are found in some patients only over time [4,9].

Perhaps besides the antiviral and anti-inflammatory medications used currently for COVID-19 treatment, other drugs and methods should be developed and used to maintain tissue stem cell populations, as well as to reduce excessive p53 activity and apoptosis.

In this regard, data on the successful treatment of severe COVID-19 forms using multipotent Mesenchymal Stem Cells (MSCs) are of great interest [30,31]. The anti-inflammatory and immunomodulatory properties of MSCs are well known and have been used in many clinical studies [32]. Besides, MSCs behave as tissue

protective agents which inhibit apoptosis, limit oxidative damage, and enhance regeneration [32]. For example, Keratinocyte Growth Factor (KGF) and Hepatocyte Growth Factor (HGF) released by MSCs protect alveolar epithelial cells from apoptosis with increased Bcl-2 expression and inhibition of the HIF1 protein [33]. During apoptosis-related hypoxia MSCs induce the expression of a number of factors such as vascular endothelial growth factor (VEGF), HGF, and TGF- $\beta$ 1 which can reverse endothelial cell apoptosis [31].

Apoptosis was observed at different stages of viral infections in SARS-CoV-2 patients [31]. Therefore effective control of apoptosis in COVID-19 patients is very important. The best studied apoptosis-marker molecules are caspases which trigger and implement caspase-dependent apoptosis as well as the p53 protein which initiates the triggering of mitochondrial apoptosis. Initiation of apoptotic processes occurs in various pathological conditions and leads to the death of cells (the survival of which is undesirable for the organism) but it can be excessive. Apoptosis markers play an important role in the study of the development of pathological processes in the immune, bronchopulmonary, excretory, cardiovascular systems. The p21 and p16 proteins which are p53 targets and mediators in signal transmission during mitochondrial apoptosis are being actively studied with the aim of its possible regulation [34].

Interestingly, blocking necroptosis strongly inhibits SARS-CoV-2-induced inflammatory responses [8] which are the main determinant of disease progression in COVID-19 patients, as research has shown [11]. So the question of whether methods limiting necroptosis and pyroptosis can be included in the therapy of COVID-19 requires further evaluation [8].

Besides, attention should be paid to research that has shown increased viability of functionally important cells of a number of tissues when substances reducing excessive p53 activity are used, thus allowing to decrease apoptosis and remove or reduce the restrictions on the processes of proliferation and differentiation of cells necessary for repair processes [35,36]. Indeed, it has been shown that a decrease in p53 activity has a beneficial effect on the repair of injured tissues. Short-term inhibition of p53 obtained by the use of a pharmacological inhibitor PFT- $\beta$  in combination with KGF improves the recovery of

thymic epithelial cells and enhances the recovery of T cells in irradiated recipients after bone marrow transplantation [35]. At present active development of p53-targeted drugs that increase the radio resistance of normal tissues by regulating p53 is underway [36].

So during SARS-CoV-2 infection it is important to control the processes of cell apoptosis and necroptosis induced by activated caspases 1 and 8 with the aim to reduce the excessive proinflammatory response and prevent massive death of the alveolar epithelium, endothelium, and T lymphocytes. This is especially important in cases of a delayed or weak interferon response of the host, which results in early and rapid viral replication and associated massive apoptosis of these categories of cells. Perhaps besides the antiviral and anti-inflammatory medications used currently in severe cases of COVID-19 disease, with a delayed IFN response and increased p53 activation which prevents proliferation and differentiation of stem cells, other drugs and methods should be developed and used to regulate p53 activation.

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