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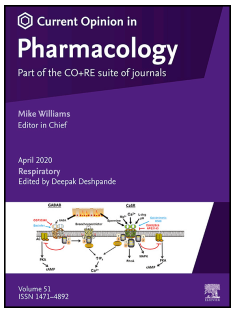

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PURINERGIC SIGNALING PATHWAY IN SEVERE COVID-19

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ABSTRACT

Substantial efforts have been made to understand the immune response during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, in order to identify and characterize risk factors, immune mechanisms responsible for the induction of tissue injury and potential therapeutic targets. Purinergic signaling pathway has shown to modulate the inflammatory processes in the course of several infectious diseases, but its role in the Coronavirus disease 19 (COVID-19) has not been clearly defined. Inflammation is usually associated to the release of ATP from different cell types, starting a cascade of events through the activation of a set of different purinergic receptors. This Review summarizes the evidence showing the involvement of the purinergic system in the inflammatory condition that characterizes severe COVID-19.

INTRODUCTION

Epidemiological data have confirmed over 600 million Coronavirus disease 19 (COVID-19) cases and almost 6.5 million deaths worldwide. However, the COVID-19 pandemic is far from over as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has mutated over time giving rise to the appearance of new variants of concern (https://coronavirus.jhu.edu/map.html). A real-time progress has been made in controlling the ongoing pandemic, especially through vaccination having been administered so far more than 12 billion vaccine doses. The clinical manifestations of acute COVID-19 are highly variable, ranging from asymptomatic infection and mild symptoms in most cases [1-4] to life-threatening severe disease [5-8]. Even though the number of pediatric patients with COVID-19 increased after the spread of variants with greater transmissibility, children show a lower severity and mortality compared to adults [9]. Moreover, up to ~20% of both adults [10] and children [11] will develop long COVID-19. It is well established that severe COVID-19 is associated with a dysregulated inflammatory response. Because the purinergic signaling has shown to be involved in the regulation of inflammatory responses in the course of several infectious diseases [12-16], we will try here to summarize the evidence suggesting its participation in the pathogenesis of severe COVID-19.

PURINERGIC SIGNALING PATHWAY

ATP is released from activated, stressed, apoptotic or necrotic cells being tissue damage induced by neutrophils one of the most relevant sources of extracellular ATP. Controlled ATP released by viable cells can progress through different mechanisms such as vesicular transport, anion channels, and connexin and pannexin hemichannels [17,18]. In the extracellular space, ATP is hydrolyzed to ADP, AMP, and adenosine by enzymes

belonging to four ectonucleotidase families: the ectonucleotide pyrophosphatase/phosphodiesterase family, the ectonucleoside triphosphate diphosphohydrolase family including CD39, the alkaline phosphatase family, and the ecto-5'-nucleotidase known as CD73. These ectonucleotidases are widely expressed by immune and non-immune cells, and display different abilities to hydrolyze ATP, ADP, and AMP. Thus, the relative tissue expression of these enzymes determines the local concentration of different purinergic ligands, and the consequent activation of purinergic receptors, which comprise nineteen different receptors able to recognize ATP, related nucleotides, and/or adenosine. Purinergic receptors include ionotropic P2X (P2XR) and metabotropic P2Y receptors (P2YR) families that promote inflammasome activation in monocytes, neutrophils, macrophages and dendritic cells, and modulate antigen receptor signaling in T cells [18,19]. Moreover, ATP promotes neutrophil recruitment and activation perpetuating tissue injury [20]. Of the four adenosine receptor subtypes, the G protein-coupled A2A and A2B receptors are commonly upregulated in response to the activation of immune cells and play an important role in the regulation of the inflammatory responses [21].

A DYSREGULATED IMMUNE RESPONSE CHARACTERIZES SEVERE COVID-19

The damage mediated by SARS-CoV-2 might explain some of the pathological findings in COVID-19, however, there is compelling evidence suggesting that the host immune response also plays a key role. Autopsies of deceased COVID-19 patients have revealed very little active viral infection and large accumulation of activated immune cells, suggesting that organ failure is mediated, at least partially, by infiltrating immune cells [22]. In fact, severe COVID-19 in adults is associated with an overactive inflammatory

response characterized by high levels of IL-1β, IL-6, TNF-α, IFN-γ, MIP-1α and 1β, and VEGF [23,24]. In addition, an increased neutrophil count and a high neutrophil-to-lymphocyte ratio have shown to predict a worse outcome [25,26]. High number of neutrophils have been found in the bronchoalveolar lavage (BAL) fluid of severe COVID-19 patients while lung autopsies revealed variable levels of neutrophil infiltration [27,28]. Moreover, increased blood concentrations of a variety of neutrophil products such as NETs, myeloperoxidase, and calprotectin have been described in patients with severe COVID-19 [29,30]. Thrombosis and coagulopathy are also common findings in severe disease [31]. Although the underlying mechanisms have been not clearly defined and characterized, it is clear that the damage of the endothelium, the activation of platelets, the release of NETs, and an increased expression of tissue factor in target tissues, can certainly play an active role [32,33].

EVIDENCE OF PURINERGIC SIGNALING ACTIVATION DURING SEVERE COVID-19

Different studies have shown that ATP is released to the extracellular space in the course of severe COVID-19. Luu and coworkers [34] reported that the SARS-CoV-2 spike protein induces the transient opening of Pannexin-1 (Panx-1) channels in human lung epithelial cells, allowing the release of ATP and IL-1β. Consistent with these findings, the analysis of BAL from COVID-19 patients also showed high levels of these mediators. Single-cell RNA sequencing from nasal epithelia obtained from COVID-19 patients demonstrated a higher expression of *PANX1* mRNA compared with healthy individuals, while the analysis of lung tissues from lethal COVID-19 cases showed a high expression of the Panx-1 protein. Interestingly, Panx-1 blockers significantly prevented SARS-CoV-2 replication in human lung epithelial cells. Together, these observations suggest that

targeting Panx-1 channels might result in both, the inhibition of SARS-CoV-2 replication and the modulation of inflammatory mechanisms triggered by extracellular ATP in the course of COVID-19. On the other hand, by performing a single-cell profiling of BAL from COVID-19 patients and non-infected controls, Wauters and coworkers [35] found an upregulation of the purinergic receptor *P2RX7*, *NLRP3* and *IL-1β* genes in monocyte/macrophage cells from critical COVID-19 patients, but not in cells from uninfected or mild COVID-19 patients. In addition, the authors also reported higher levels of ATP in BAL samples from critical COVID-19 patients compared with patients suffering mild disease.

ATP plays a central role in the induction of inflammatory response via the stimulation of P2X7 receptor, which triggers the activation of the NLRP3 inflammasome [19], while ADP appears to play a major role in thrombotic events by inducing the activation of platelets through P2Y12 receptor [36]. CD39, which converts ATP to AMP, and CD73, that produces adenosine from AMP, are particularly relevant for the balance between the pro- and anti-inflammatory effects mediated by extracellular ATP [18]. Several works [37-39], recently reported the presence of high levels of ATP and ADP but low levels of adenosine in plasma from adults with severe COVID-19, suggesting that purinergic signaling might promote not only an inflammatory status but also thrombotic events. Pietrobon and coworkers also reported an impaired adenosine receptor expression and a reduced ATP hydrolysis capacity during COVID-19, favoring systemic inflammation [38]. In agreement with these observations, Da Silva and coworkers [39] reported a decreased ability to hydrolyze ATP in PBMCs from patients with severe COVID-19 together with an increased hydrolysis of ATP mediated by platelets. High levels of soluble CD39 (sCD39) in plasma were also reported by Diaz Garcia and coworkers [37], and

consistent with other studies [40,41], a higher expression of cell-surface CD39 was detected in different leukocyte populations including CD4+ and CD8+ T cells, FOXP3+ regulatory T cells, NK cells and monocytes. In addition, Symsek and coworkers [42] reported that CD39+ Tregs frequency increases with disease severity in adult patients but decreased in juvenile patients in an age-dependent manner . Since the levels of sCD39 in plasma were shown to be related to length of hospital stay and intensive care unit admission, it has been suggested that sCD39 might represent a promising biomarker for COVID-19 severity. Interestingly, Diaz Garcia and coworkers reported that the reversible antagonist of P2Y12 receptor Ticagrelor, significantly inhibits platelet activation induced by plasma from patients with severe COVID-19. Indeed, Ticagrelor has been proposed to prevent coagulopathy development in COVID-19 patients [43]. Notably, Wang and coworkers [41], showed that ENTPD1/CD39, an ectoenzyme defining exhausted T-cells, is upregulated in the lung, liver, spleen, and PBMCs of severe COVID-19 patients where expression positively correlates with markers of vasculopathy. They also noted an aberrant regulation of this ectoenzyme, as indicated by heightened levels of STAT-3 and HIF-1a, which contribute to CD39 modulation at the transcriptional level. These changes can contribute to a purinergic pathway imbalance, resulting in metabolic changes and T cell dysfunction. In this sense, Hou and coworkers [44] reported that SARS-CoV-2-specific T cells are found in peripheral blood from convalescents patients up to 1 year post-infection, however, these cells show an increased expression of exhaustion markers such as PD-1, Tim-3, TIGIT, CTLA-4, and CD39, suggesting a dysfunctional phenotype. Garcia-Villalba and coworkers [45], described that the concentration of soluble P2X7 receptor is elevated in the plasma of COVID-19 patients and shows a positive correlation with disease severity, suggesting that plasma levels of the P2X7 receptor could be a novel biomarker of COVID-19 severity. Moreover, they observed that a soluble form of P2X7

receptor is released from human peripheral blood mononuclear cells upon inflammasome activation induced by LPS plus ATP. This observation is consistent with previous reports showing that the stimulation of the P2X7 receptor leads to the shedding of this receptor associated to extracellular vesicles [46]. The role of this secreted form of the P2X7 receptor remains to be defined. However, it should be mentioned that circulating exosomes from COVID-19 patients have shown to activate the NLRP3 inflammasome (36).

Contrasting with some of the results described above, Dorneles and coworkers [47] found lower levels of ATP in plasma from mild and severe COVID-19 patients, compared with healthy donors. The reasons for this contrasting observation are not clear. In addition, the authors reported lower adenosine plasma levels in the blood of severe COVID-19 patients compared with healthy donors, suggesting that this condition might contribute to the development of the dysregulated inflammatory response that characterize severe COVID-19. Moreover, an increased frequency of CD4+CD39+ T cells together with low frequencies of CD4+CD73+ and CD8+CD73+ T cells was also observed. Ahmadi and coworkers [48], on the other hand, also reported a reduced expression of CD73 in different lymphocyte populations including CD8+ T cells, NK cells and NKT cells in COVID-19 patients. Interestingly, the decreased expression of CD73 in CD8+ T cells and NKT cells was shown to be associated to an inflammatory signature characterized by an enhanced secretion of granzyme B, perforin, TNF-α and IFN-γ.

Shultz et al [49] performed an extensive analysis using public datasets of raw proteomics data acquired by mass spectrometry and raw genomics data obtained by microarray from COVID-19 patient samples. They found that plasmatic inosine levels are increased in

patients with severe COVID-19. They also observed an upregulation of *PANX1, CD39, CD38, P2RX7,* and *ADORA2B* mRNA genes. Consistent with these findings, by performing untargeted metabolomics analyses of serum samples, Dogan and coworkers reported an increased plasma concentration of inosine in COVID-19 patients compared with healthy donors [50]. Interestingly, it has been reported that inosine exerts a broad range of anti-inflammatory effects in experimental models of acute lung injury [51,52]. Wu and coworkers [53] also reported plasma metabolomic alterations during SARS-CoV-2 infection, including changes in GMP levels, a metabolite partially generated by CD39 and CD73, showing higher levels in fatal cases compared with mild COVID-19 patients. Finally, by studying children with COVID-19 we recently reported [54] that plasma levels of ATP were higher in infected children compared with healthy ones. Interestingly, plasma levels of ATP showed a negative correlation with the frequency of regulatory T cells but a positive correlation with the frequency of Th17 cells, suggesting a possible role for the extracellular ATP in the acquisition of an inflammatory signature by the T cell compartment. Interestingly, Symsek and coworkers [42], found that CD39-expressing Tregs increased with disease severity in adult patients while decreased in young patients in an age-dependent manner.

PERSPECTIVES

The purinergic system is a complex jigsaw puzzle of nucleotides, nucleosides, ectonucleotidases, receptors and transporters, able to modulate the course of inflammation associated to infectious, autoimmune, and neoplastic diseases. This complexity explains why it has long been difficult to define therapeutic targets among purinergic receptor and ectonucleotidases, useful for the treatment of inflammatory conditions. Severe COVID-19 is clearly associated to the development of an exacerbated inflammatory response and hence the use of anti-inflammatory agents such as

corticosteroids, Tozilizumab (a monoclonal antibody directed to the IL-6 receptor), or inhibitors of the JAK/STAT pathways have been incorporated as therapeutic tools. In spite that the purinergic signaling shows to be affected in severe COVID-19, further studies are needed to clearly define potential targets for the successful treatment of inflammatory and thrombotic processes underlying critical illness.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Writing original draft and reviewing, L.A., I.S., V.S., C.R. J.G. Conceptualization and editing, L.A. and J.G.

ABBREVIATIONS

Coronavirus disease 19, COVID-19; severe acute respiratory syndrome coronavirus 2, SARS-CoV-2; bronchoalveolar lavage, BAL; Pannexin-1, Panx-1.

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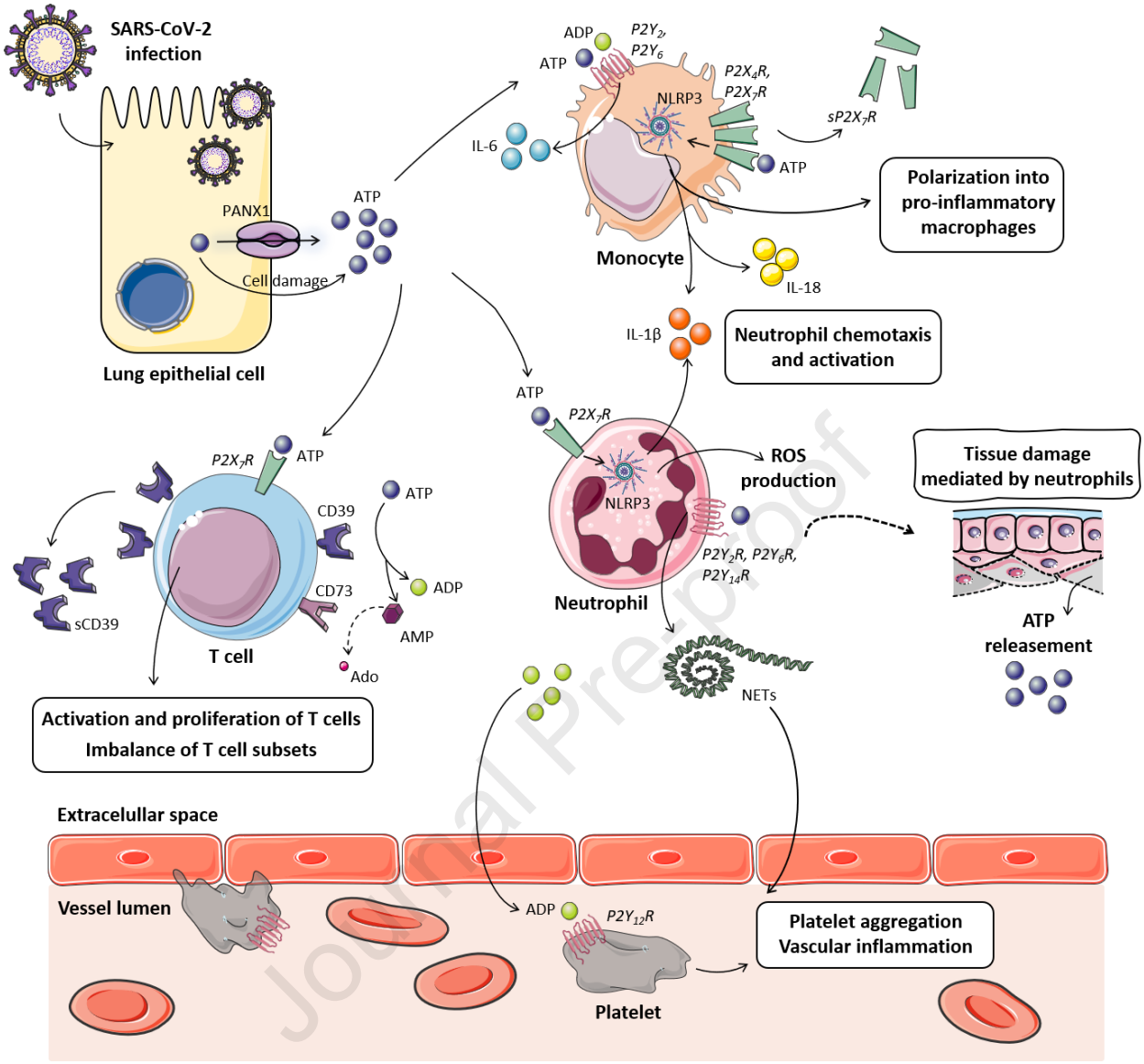
LEGEND TO FIGURE 1.

Pro-inflammatory pathways related with purinergic signaling during severe COVID-19. SARS-CoV-2 infection of respiratory epithelial cells promote cell stress and cell damage inducing ATP release to the extracellular space through different mechanisms, including the transient opening of PANX1. Injury of different cell types in the course of severe COVID-19, such as tissue damage mediated by neutrophils, also contributes to the enhancement in the extracellular concentration of ATP (not shown in the Figure). Severe COVID-19 is associated to an increased expression of CD39 and a decreased expression of CD73. This result in an increased ATP/ADP: adenosine ratio that promotes the activation of immune cells and platelets through the P2X7R and P2Y12R, respectively, and the development of inflammatory and thrombotic events. Abbreviations: PANX1, pannexin 1; Ado, adenosine; P2X7R, P2X7 receptor; NLPR3, NLR family pyrin domain containing 3; NETs, Neutrophil Extracellular Traps; ROS; Reactive Oxygen Species; P2Y12R; P2Y12 receptor. The figure was drawn by using pictures from Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: