A rationale for targeting the P2X7 receptor in Coronavirus disease 19 (Covid-19)

Francesco Di Virgilio[1], Yong Tang[2], Alba Sarti[1], and Marco Rossato[3]

[1]Univ. of Ferrara
[2]Chengdu University of Traditional Chinese Medicine
[3]University of Padova

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Abstract

Acute respiratory distress syndrome (ARDS) is the main cause of morbidity and mortality in Coronavirus disease 19 (Covid-19) for which as of now there is no effective treatment. ARDS is caused and sustained by an uncontrolled inflammatory activation characterized by a massive release of cytokines (cytokine storm), diffuse lung edema, inflammatory cell infiltraton and disseminated coagulation. Macrophage and T lymphocyte dysfunction plays a central role in this syndrome. In several experimental in vitro and in vivo models, many of these pathophysiological changes are triggered by stimulation of the P2X7 receptor. We hypothesize that this receptor might be an ideal candidate to target in Covid-19-associated ARDS.

An effective treatment for Covid-19 is still lacking, although anecdotal evidence suggests that IL-6 blockade is beneficial (Mehta *et al* ., 2020), and blockers of other pro-inflammatory cytokines such as IL-1β are being considered in Covid-19 patients showing hyperinflammation (Monteagudo *et al* ., 2020). In such a dramatic situation, it might be necessary to explore unconventional, but rationale, therapeutic options. There is evidence that severely ill Covid-19 patients develop a cytokine storm syndrome causing acute respiratory distress syndrome (ARDS), the main cause of morbility and mortality (Mehta *et al*2020). This is thought to be due to uncontrolled, virus-mediated, direct activation of lung macrophages in a process reminiscent of the macrophage activation syndrome (MAS) observed in some rheumatologic diseases such as systemic juvenile idiopathic arthritis (sJIA), adult onset Still's disease and systemic lupus erithematosus (LES), although it is debated wheter hyperinflammation in Covid-19 should be considered MAS sensu strictu (Crayne *et al* , 2019; Henderson *et al* ., 2020). Covid-19 patients show massive infiltration by inflammatory cells (neutrophils, monocyte/macrophages) in their lungs, and increased blood levels of IL-1β, IL-6, IL-2, IL-7, TNF-α and several other pro-inflammatory cytokines and chemokines (Guo *et al* ., 2019). While main target of coronaviruses are lung epithelial cells, they also infect macrophages and dendritic cells, where they cause abortive infection and sustained activation, that in turn drives hyperinflammation (Fehr and Perlman, 2015). Thus, macrophage inhibition is likely to be a crucial step to prevent the extensive lung injury caused by Sars Cov-2. A promising target receptor to down-modulate macrophage responses might be the P2X7 receptor (P2X7R).

The P2X7R is a plasma membrane receptor gated by extracellular ATP, the earliest and most ubiquitous damage-associated molecular pattern (DAMP) released at all inflammatory sites, the lungs included (Tolle and Standiford, 2013; Di Virgilio *et al* ., 2020). Very interestingly, increased ATP levels are also found in bronchoalveolar lavage fluid (BALF) from patients with ARDS, or from mice with lipopolysaccharide (LPS)-induced acute lung injury (ALI) (Cicko *et al* , 2018). Increased extracellular ATP levels have also been direclty demonstrated*in vivo* in the lungs of mice inhaled with LPS thanks to the bioluminescent

luciferase probe pmeLUC (Cicko *et al* . 2018). The P2X7R has a central role in inflammation as it is one of the most potent stimulator of the NLRP3 inflammasome, and therefore of caspase-1 activation and IL-1β and IL-18 release (Di Virgilio *et al* ., 2017) (Figure 1). P2X7R stimulation also promotes release of other cytokines and chemokines, e.g. IL-6, TNF-α, monocyte chemotactic protein-1 (MCP-1), IL-8, CC-chemokine ligand 3 (CCL3) and CXCL2, of pro-fibrotic factors such as TGF-β, and extracellular matrix remodelling factors such as metalloproteinase-9 and tissue inhibitor of metalloproteinase (TIMP)-1 (Di Virgilio *et al* ., 2017; Riteau *et al* , 2010). The P2X7R is a potent trigger of reactive oxygen species (ROS) production, therefore its overactivation (as it is the case at sites of heavy inflammation) may inhibit lymphocyte functions by impairing mitochondrial metabolism, as it has been shown in monocytes during sepsis (Martinez-Garcia *et al* 2019). Dysfunctional mitochondria are a feature of exhausted T lymphocytes (Desdín-Micó *et al* ., 2018). Although no specific data are available in Covid-19, reduced T cell function, as documented by the increased expression of T lymphocyte exhaustion markers, is a feature of coronavirus-induced lung infection (Diao *et al* ., 2019). It is known that inability of cytotoxic T lymphocyte and NK cells to lyse virus-infected cells occurs in MAS, and a is main cause of the sustained cytokine release driving the cytokine storm typical of this syndrome and of Covid-19 as well (Crayne *et al* ., 2019). In addition, a key feature of ARDS is the extensive pulmonary edema which is largely dependent on release of vascular endothelial growth factor (VEGF). Although the pathogenic role of VEGF in ARDS is controversial, blood VEGF levels are reported to be increased in Covid-19 patients, and this has prompted a clinical study aimed at testing the efficacy of bevacizumab administration (NCT04275414) (*https://clinicaltrials.gov/ct2/results?cond=&term=NCT04275414)* . While mostly known for its pro-inflammatory activity, it is also well documented that the P2X7R is a potent inducer of VEGF release and neo-angiogenesis *in vivo* , and accordingly it has been shown that its blockade inhibits VEGF-dependent increase in vascular permeability (Clapp *et al* ., 2019). Thus P2X7R targeting might prove beneficial to fight the early exudative phase in ARDS. Furthemore, thromboembolic complications are common among critically ill Covid-19 patients (Tang et al. 2020a), which has led to explore the protective effect of heparine administration Tang *et al* ., 2020b). Inhibition of the P2X7R might be beneficial also to treat thromboembolism since its stimulation promotes massive release of tissue factor (Baroni *et al* , 2007; Furlan-Freguia *et al* ., 2011).

A commonly used experimental model for ARDS, is the intratracheal application of LPS in mice. This causes ALI, an acute lung inflammation reminiscent of human ARDS (Cicko *et al* ., 2018). Early work showed that pharmacological blockade of the P2X7R, or its genetic ablation, substantially reduced inflammatory cell infiltration, cytokine levels and lung damage in acute inflammatory response (Wang *et al* ., 2015; Cicko *et al* ., 2018). P2X7R deficiency was also shown to reduce alveolar macrophage death and pro-IL-1α release in the lungs of LPS-treated mice (Dagvadori *et al* ., 2015). Monitoring the P2X7R as an inflammatory biomarker in Covid-19 by recently developed radiopharmaceuticals has been recently proposed (Juengling *et al.* , 2020)

On the basis of these convergent observations, we suggest that the P2X7R could be an ideal candidate receptor for pharmacological targeting in ARDS.

Small molecule drugs targeting the P2X7R have been developed by most Pharma Industries and have undergone extensive Phase I Clinical Trials revealing an excellent safety profile (Arulkumaran *et al* ., 2011). However, therapeutic efficacy in a number of chronic inflammatory diseases investigated in Phase II turned out to be limited, which led major Pharma Industries to drop or outsource P2X7R clinical research. A relevant exception is Johnson & Johnson that in late 2019 started a new Phase II clinical trial to test the efficacy of P2X7R blockade in depression (Cully, 2020). Therapeutic efficacy of P2X7R blockade has never been tested in a disease condition characterized by uncontrolled hyperinflammation, as in Covid-19-dependent ARDS. We propose that P2X7R antagonists should be given a chance at least for the compassionate treatment of Covid-19 patients with rapid evolutive ARDS.

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Legend to Figure 1

Central role of the P2X7R in Sars-Cov-2-dependent lung inflammation. Sars-Cov-2 infects lung mononuclear phagocytes, besides epithelial cells, triggering massive ATP release which feeds-back on the P2X7R expressed on macrophages and antigen-presenting cells (APC). P2X7R activation down-modulates MHC-I expression, and at the same time promotes IL-6 release, NLRP3-mediated IL-1β and IL-18 release, VEGF secretion and thus lung edema. Release of several other cytokines and chemokines is also promoted by P2X7R activation. Stimulation of P2X7R on APC-stimulated T lymphocytes causes exhaustion and T cell polarization. The combined effect of all these factors promotes the pathophysiological changes typical of ARDS.