

Clinical and biological data on the use of hydroxychloroquine against SARS-CoV-2 could support the role of the NLRP3 inflammasome in the pathogenesis of respiratory disease

To the Editor,

The use of hydroxychloroquine (HCL) has been very common in countries with a rapid spread of coronavirus disease 2019 (COVID-19), although controversial and the subject of heated scientific discussions with implications for the whole society.

On this topic, we have read with particular interest the articles published as early view records of your Journal, and among these a timely and accurate systematic review and meta-analysis.¹ Authors conclude the manuscript hypothesizing potential benefits on the symptoms related to cytokine release and on the incidence of radiological progression. This is consistent with what they report in the introductory part related to the potential mechanisms of action of the drug, which we would like to further clarify here.

High levels of proinflammatory cytokines have been detected in autopsy tissue from patients with severe acute respiratory distress syndrome (ARDS) and they have been advocated by some Authors as potentially involved in the development of ARDS also in patients with coronavirus infections.² Previous studies on severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome CoV allowed to detect a sharp increase in interleukin- β (IL-1 β) plasma levels during the course of the disease.

Mature and biologically active IL-1 β is produced by a caspase-1 mediated conversion of the cytokine precursor pro-IL-1 β . The activation of caspase 1 is in turn regulated by the nucleotide-binding and oligomerization domain-like receptor family, pyrin domain-containing 3 (NLRP3): a multiprotein complex consisting of an NLRP3 scaffold, an adapter apoptosis speck-like protein, and the effector procaspase-1.³ A two-step mechanism seems needed for a complete activation of the NLRP3 inflammasome. The priming step, activated by infections through pathogen-associated molecular patterns and by mechanical injuries through damage associated molecular patterns, leads to the upregulation of pro-IL-1 β , pro-IL-18, and inflammasome components. The second step allows the interaction of the components into the inflammasome structure thus leading to the production of active proinflammatory interleukins,⁴ (Figure 1).

SARS-CoV ORF3a protein, a lineage-specific accessory protein, has been shown to activate the NLRP3 inflammasome in lipopolysaccharide-primed macrophages, and it seems also capable of activating the pro-IL-1 β gene transcription through nuclear factor κ B. Interestingly, ORF3 and another NLRP3 inducer (ORF8) in SARS-CoV-2 were found to be highly divergent from ORF3a and ORF8b in SARS-CoV. Therefore, there is an urgent need to experimentally confirm the activation of the NLRP3 even by the novel coronavirus.⁵

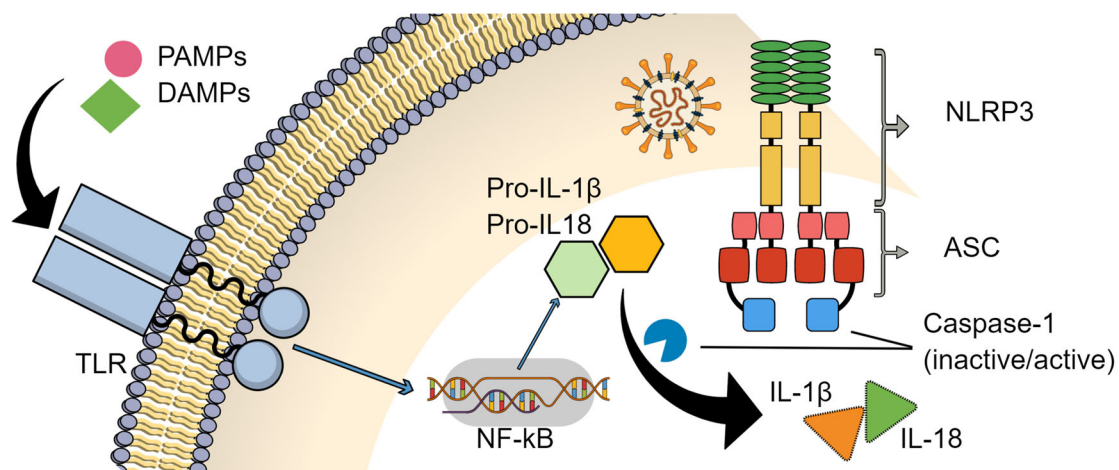


FIGURE 1 Activation of the NLRP3 inflammasome by a two-step mechanism. First, pathogen-associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) interact with the toll-like receptor (TLR) of alveolar macrophages leading to the induction of interleukin genes via nuclear factor κ B (NF- κ B). Secondly, the mature and active forms of interleukin-8 (IL-18) and IL-1 β are obtained from the action of caspase-1, part of the inflammasome protein scaffold [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Available evidence on the mechanisms of action of hydroxychloroquine and pirfenidone on NLRP3 inflammasome

Bai Let al. <i>Biochem Pharmacol.</i> 2019;169:113619.	Renoprotective effects of artemisinin and hydroxychloroquine combination therapy on IgA nephropathy via suppressing NF- κ B signaling and NLRP3 inflammasome activation by exosomes in rats.
Tang TT et al. <i>Cell Death Dis.</i> 2018;9(3):351.	Hydroxychloroquine attenuates renal ischemia/reperfusion injury by inhibiting cathepsin mediated NLRP3 inflammasome activation.
Eugenia Schroeder M et al. <i>Sci Rep.</i> 2017;7(1):1892.	Proinflammatory Ca ⁺ -activated K ⁺ channels are inhibited by hydroxychloroquine.
Frujita Y et al. <i>Arthritis Res Ther.</i> 2019;21(1):250.	Hydroxychloroquine inhibits IL-1 β production from amyloid-stimulated human neutrophils.
Burmeister R et al. <i>Inhal Toxicol.</i> 2019;31(7):274-284.	Prevention of crystalline silica-induced inflammation by the antimalarial hydroxychloroquine.
Schwarzbach CJ et al. <i>Neurology.</i> 2016;86(3):241-4.	Chorea in a patient with cryopyrin-associated periodic syndrome.
Lübow C et al. <i>Biochem Pharmacol.</i> 2020;175:113864.	Lysosomotropic drugs enhance pro-inflammatory responses to IL-1 β in macrophages by inhibiting internalization of the IL-1 receptor.
Roche P et al. <i>Cardiology.</i> 2013;126(1):59-61.	Pirfenidone and the inflammasome: getting to the heart of cardiac remodeling.
Wang Y et al. <i>Cardiology.</i> 2013;126(1):1-11.	Pirfenidone attenuates cardiac fibrosis in a mouse model of TAC-induced left ventricular remodeling by suppressing NLRP3 inflammasome formation.
Li Y et al. <i>Mol Immunol.</i> 2018;99:134-144.	Pirfenidone ameliorates lipopolysaccharide-induced pulmonary inflammation and fibrosis by blocking NLRP3 inflammasome activation.
Li X et al. <i>Exp Cell Res.</i> 2018;362(2):489-497.	NLRP3 inflammasome inhibition attenuates silica-induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells.

Abbreviations: IgA, immunoglobulin A; IL, interleukin; NF- κ B, nuclear factor κ B; NLRP3, pyrin domain-containing 3.

During infections, while the activation of the NLRP3 inflammasome tends to eliminate the viral load, most viruses have developed evasion strategies that involve different mechanisms. In summary, viral proteins can facilitate the ubiquitination and degradation of those of the inflammasome, or prevent their assembly. Some viruses are even capable of modulating the effector capacity of NLRP3, interacting with key elements, such as caspase-1.⁶ It is currently unknown whether SARS-CoV-2 is capable or not of resorting to the above reported strategies. However, it is clear that the inflammatory response is rapid and unbalanced, so as to quickly generate significant organ damage in the absence of signs of mitigation of the cytokine storm.

There is other noteworthy evidence indirectly supporting the contribution of an unbalanced NLRP3 inflammasome response to the development of acute pulmonary syndromes in the reported cases of severe COVID-19: in relatively recent times, some authors have highlighted how the activation of the NLRP3 inflammasome is crucial for a complete development of acute lung injury, ARDS, and refractory hypoxemia, the cardinal clinical features of severe COVID-19.⁷ IL-1 β and IL-18 (the latter also activated by caspase-1) are quick inducers of lung fibrosis. Moreover, the acute release of IL-1 β into the peripheral blood upregulates the expression of other proinflammatory cytokines, such as IL-6.


We would like here to highlight that HCL is a known NLRP3 inhibitor, and that a relevant part of its potential clinical effectiveness is certainly based on the downregulation of IL-1 β expression (Table 1). Furthermore, among the molecules capable of inhibiting NLRP3, we have found interesting information on the mechanism of action of pirfenidone. This drug has been approved for the treatment of idiopathic pulmonary

fibrosis. The therapeutic effects of pirfenidone, mainly measurable with the reduction of fibrosis, extend to other target organs, such as the heart, liver, and kidney. Together with the suppression of various pulmonary cytokines, pirfenidone reduces the formation of NLRP3 inflammasomes with reference to ideal models of pneumonia-induced ARDS. A couple of case reports support the hypothesis that the drug may accelerate the recovery of patients with fibrotic-phase ARDS.

In conclusion, available evidence suggests that a regulation of NLRP3 inflammasome activation may offer a promising therapeutic approach by inhibiting or slowing down the process of acute respiratory distress syndrome. In our opinion, further translational experiments are necessary to enrich the therapeutic possibilities against severe COVID-19.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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