Pyroptosis targeting via mitochondria: an educated guess to fast-track COVID-19 therapies

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Abstract

Pyroptosis, is a specialized form of inflammatory cell death which aids the defensive response against invading pathogens. Its tight regulation is lost during infection by the severe acute respiratory coronavirus 2 (SARS-CoV-2) and thus uncontrolled pyroptosis disrupts the immune system and the integrity of organs defining the critical conditions in patients with high viral load. Molecular pathways engaged downstream to the formation and stabilization of the inflammasome - required to execute the process - have been uncovered and drugs are available for their regulation. On the contrary, pharmacological inferring of the upstream events - which are critical to sense and interpret the initial damage by the pathogen - is far from entirely elucidated. This limits our capacity to identify early markers and targets to ameliorate SARS-CoV-2 linked pyroptosis. Here we aim to raise attention to mitochondria and pathways leading to their dysfunction with the goal to inform early steps of inflammasome and devise tools to predict and counteract diseases by the SARS-CoV-2.

Key words: SARS-CoV-2, Pyroptosis, Mitochondria and Pharmacology.

Text

The 2019 coronavirus disease (COVID-19) has changed our lifestyles causing an unimaginable and unprecedented health crisis with impact at multiple levels.

The causative etiological agent behind what is the fastest spreading disease of the 21st century is the severe acute respiratory coronavirus 2 (SARS-CoV-2). Structurally this is an enveloped, positive-sense, single-stranded RNA virus that enters its host cell by binding to the angiotensin-converting enzyme 2 (ACE2) receptor through the interaction with the trimeric S spike glycoprotein (Kim et al, 2020; Hoffman et al, 2020). Similar to other coronaviruses [i.e. the highly pathogenic Severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle-East respiratory syndrome coronavirus (MERS-CoV)] SARS-CoV-2 infection is associated with overbearing and uncontrolled inflammatory response (He et al, 2006; Lau et al, 2013; Zhou et al, 2014). Although a great portion of patients infected by SARS-CoV-2 remains asymptomatic or develops very mild symptoms, others experience severe and acute respiratory syndrome leading to hospitalization and critical care treatment. Notably, patients with severe COVID-19, present elevated level of pro-inflammatory mediators (TNF-α and IL-6) in their peripheral blood which epitomizes poor prognosis linked with mortality (Hojyo et al, 2020; Santa Cruz et al, 2021). Thus, the uncontrolled elevation of cytokines delivers distress at various systemic levels irreparably damaging organs essential for life such as heart and kidney (Long et al, 2020).

Since the very beginning of the outbreak, it was clear that in SARS-CoV-2 patients the so-called cytokine storm played a crucial role in the pathogenesis of the disease and its most severe manifestations. Cytokine storm encompasses disorders of immune dysregulation characterized by systemic inflammation and multiorgan dysfunction. This is recapitulated in the severe COVID-19 cases in which the exacerbation of inflammation is a consequence of the unrestrained pathogen-associated molecular patterns. Clinical manifestations of the COVID-19 include acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS) and cardiac failure (Patel, Saxena and Mehta, 2021). Specifically, in COVID-19 cases the uncontrolled inflammatory response associates with leukopenia reflecting an high degree of cell lysis which follows the uncontrolled cellular demise consequence of the sustained pyroptosis (Ferreira et al, 2021). In addition, poor prognosis associates with coagulopathy (Klok et al, 2020) which is also linked with the inflammasome-mediated

pyroptosis in macrophages which release tissue factor (TF): an essential mediator of blood coagulation cascades (Wu et al, 2019).

Pyroptosis was firstly described in myeloid cells infected by pathogens (Cookson and Brennan, 2001) and originates etymologically from the Greek words *pyro* (fire) and *ptosis* (falling). It is a programmed execution of the cell which follows the stabilization of the supramolecular protein complexes called inflammasome. Pyroptosis (schematically summarised in **Figure 1**) is characterized by cellular swelling and rupture (lysis) to release the pro-inflammatory molecules such as pro-Interleukin 1β and pro-Interleukin 18 (IL-1β and IL-18) whose maturation follows activation of the caspase-1 (Yang et al, 2019). Pyroptosis is therefore a caspase-dependent process in which gasdermin D (GSDMD) is proteolytically cleaved to form pores on the plasma membrane instrumental for the release of cytokines (Shi et al, 2015).

Infection by pathogen drives pyroptosis by activating the nucleotide-binding oligomerization domain (NOD)–like receptors required for the assembly of the inflammasome. The latter establishes a platform for the mass recruitment and activation of caspase-1 with the help of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) acting as a bridging molecule. The NOD-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome is the most well-characterised among the NOD-like receptors. It is implicated in a plethora of diseases ranging from autoinflammatory to neurological disorders as well as virus-associated illnesses and antiviral responses. Activation of the NLRP3 inflammasome is now confirmed in COVID-19 patients in which acts as an indicator of the disease severity (Toldo et al, 2020; Courjon et al, 2021).

SARS-CoV-2 (not dissimilarly to its predecessors) expresses at least three proteins capable to activate the NLRP3 inflammasome: ORF8b, envelope (E) and ORF3a, (Yap, Moriyama and Iwasaki, 2020).

ORF8b (i) holds the potential to directly activate the inflammasome via the leucine-rich repeat (LRR) domain of the NLRP3 protein and has been demonstrated to co-localize with both NLRP3 and ASC (Shi et al, 2019).

The E protein (ii) is a viroporin (oligomeric complexes that act as ion channels) found on the membrane encapsulating the ER-Golgi intermediate compartment (ERGIC) (Torres et al, 2007) capable to mobilize Ca^{2+} in the cytosol via which the NLRP3 inflammasome responds (Murakami et al, 2012; Nieto-Torres et al, 2015).

Independently from this the viroporin protein E promotes the transcription of inflammatory cytokines and chemokines, including IL-1β, and IL-18.

Finally, ORF3a (iii) establishes NLRP3 inflammasome assembly via its activity as a K^+ channel (Chen et al, 2019). Furthermore, the intracellular accumulation of Reactive Oxygen Species is *per se* an activator of NLRP3 inflammasome whose assembly can be also obtained through ASC ubiquitination mediated by binding to TNFR-associated factor 3 (TRAF3) (Siu et al, 2019).

Activation of the NLRP3 inflammasome therefore emerges as a molecular signature which predicts release of inflammatory cytokines prodromal to organ damage and immune system deregulation central to COVID-19 pathogenesis.

More recent research has indicated mitochondrial dysfunction as key event for NRLP3 activation, and with the increased understanding of the COVID-19 pathogenesis, we are keen to advocate further attention on the role mitochondria play in pyroptosis.

Mitochondria are pivotal to initiate assembly and activation of the inflammasome following engagement of pathogen associated molecular patterns (PAMPs). Their dysfunction associates with the increase in Ca^{2+}, metabolic modifications and lysosomal damage. More specifically mitochondrial ROS (mtROS) (i), oxidized mitochondrial DNA (mtDNA) in the cytosol (ii) and mitochondrial antiviral signalling protein (MAVS) (iii) aid formation of the inflammasome.

Even though the accumulation of mtROS is key to this (Zhou et al, 2011; Nakahira et al, 2011) ill-defined remains the hierarchy of molecular events which define, accompany or characterize the redox stress. On the other hand, established is that the oxidised mtDNA is consequence of the boost of ROS triggered by the accumulation of Ca^{2+} (Murakami et al, 2012) and the promoted synthesis of mtDNA is now linked to a specific signalling cascade subsequent to TLR binding (Zhong et al, 2018).

MAVS facilitates inflammasome assembly by directly interacting with NLRP3 alike Cardiolipin and Mitofusin-2 (Ichinohe et al, 2013; Iyer et al, 2013). However, MAVS ia also able to recruit the E3 ligase TRAF3 which mediates ASC ubiquitination amplifying inflammasome activation (Guan et al, 2015).

It is therefore evident that despite the attempts to uncover the precise upstream molecular events that culminate in inflammasome stabilization at mitochondrial level more is needed to exhaustively inform those.

Hitherto evident is that the promotion of mitochondrial quality control via selective autophagy (mitophagy) limits NLRP3 activation by eliminating damaged or stressed mitochondria (Zhong et al, 2016; Lin et al, 2019). Accordingly, de-ubiquitination of mitochondrial proteins by ROS drives the NLRP3 inflammasome complex assembly for the blockage of mitophagy (Zhang et al, 2019). Finally, NF-kB which is a bonafide read-out of mitochondrial distress signalling pathway (Desai, East et al, 2020) primes NRLP3 expression.

Deciphering the role of mitochondrial dysfunction in pathogen-induced NLRP3 activation will unveil novel opportunities for targeting the inflammasome and the mediated cell death which holds severe and systemic consequences.

The increased mechanistic awareness of the upstream processes of pyroptosis will therefore pave the way to an improved pharmacology to prevent feed forward mechanisms which amplify the inflammasome. Thus could also deliver better protocols for the management of COVID-19 patients.

Most of the efforts to this end are devoted to prevent intracellular access of the virus (of which vaccines are the archetype) but very few to repress and prevent pyroptotic cell death once the virus has entered the cell.

Considering this an aspect in need of greater attention, we are highlighting that mitochondrial-dependent upstream events of NLRP3 inflammasome activation may provide early molecular read-outs to predict and/or counteract severity of toxicity in SARS-CoV-2 infected cells. This will form the basis for innovative treatments against the uncontrolled inflammation in COVID-19 patients.

Conflict of interests

The authors declare that they have no competing interests.

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