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Inflammasomes and Pyroptosis as Therapeutic Targets for COVID-19

Jeremy K. Y. Yap,* Miyu Moriyama, ‡ and Akiko Iwasaki†‡‴§

The inflammatory response to severe acute respiratory syndrome–related coronavirus 2 infection has a direct impact on the clinical outcomes of coronavirus disease 2019 patients. Of the many innate immune pathways that are engaged by severe acute respiratory syndrome–related coronavirus 2, we highlight the importance of the inflammasome pathway. We discuss available pharmaceutical agents that target a critical component of inflammasome activation, signaling leading to cellular pyroptosis, and the downstream cytokines as a promising target for the treatment of severe coronavirus disease 2019–associated diseases. *The Journal of Immunology, 2020, 205: 000–000.

While the race for a vaccine against the severe acute respiratory syndrome–related coronavirus 2 (SARS-CoV-2) is in full swing, current treatments available for coronavirus disease 2019 (COVID-19) are limited to supportive care. One of the greatest enigmas surrounding COVID-19 is the diverse disease trajectories of COVID-19 patients. Some displayed little to no symptoms, whereas others develop severe fever and pneumonia, leading to acute respiratory distress syndrome and ultimately to death. It is becoming increasingly clear that the innate immune system is a major player in patients’ response to the virus infection. Serum levels of both pro- and anti-inflammatory cytokines are markedly higher in severe cases than in moderate cases of COVID-19, suggesting that a cytokine storm, also known as cytokine release syndrome, is associated with increasing disease severity (1). Additionally, leukocytosis and lymphocytopenia are hallmark clinical features of severe cases of COVID-19 (1). These observations allude to an overdrive in inflammation as a mismanaged antiviral response against SARS-CoV-2 that lead to poor clinical outcomes.

SARS-CoV-2 is a positive-sense RNA virus. As such, its pathogen-associated molecular patterns will be recognized by RNA-sensing pattern recognition receptors, including TLR3, TLR7, and TLR8 in the endosome as well as retinoic acid–inducible gene 1 (RIG-I)–like receptors in the cytosol (2). Suggestion of SARS-CoV-2 activating the inflammasomes and pyroptosis being at the core of pathogenesis comes from the fact that lactate dehydrogenase (LDH) levels are highly elevated in patients that go on to develop severe disease (Y. Han, H. Zhang, S. Mu, W. Wei, C. Jin, Y. Xue, C. Tong, Y. Zha, Z. Song, and G. Gu, manuscript posted on medRxiv). LDH is a cytosolic enzyme that is released to the extracellular environment upon membrane rupture. In fact, LDH release is used to monitor pyroptosis (3). Additionally, cytokine released as a result of inflammasome activation, IL-1β, as well as its response gene product, IL-1RA, are found to be elevated in the sera of COVID-19 patients (4).

The key to overcoming excessive inflammatory activity is to target a crucial regulator of cellular inflammation while leaving the antiviral pathways intact. Pathogen- or alarmin-induced activation of nucleotide-binding oligomerization domain (NOD)–like receptors leads to inflammasome assembly into a colossal molecular scaffold that generates a platform for the mass recruitment and activation of caspase-1 with the help of a “bridge” filament protein, the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) (Fig. 1). Proteolytic activation of caspase-1 subsequently catalyzes the maturation and secretion of proinflammatory cytokines, specifically IL-1β and IL-18 (5). The most well-characterized of the inflammasomes is the NOD-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome, which has been implicated in a plethora of diseases ranging from autoinflammatory diseases to neurologic disorders. Importantly, the NLRP3 inflammasome is also involved in antiviral responses and virus-associated illnesses.

It is presently unclear if SARS-CoV-2 activates the NLRP3 inflammasome. However, taking lessons from its predecessor, the severe acute respiratory syndrome–related coronavirus (SARS-CoV), which caused the severe acute respiratory syndrome global epidemic between 2002 and 2003, was shown...
to express at least three proteins that activate the NLRP3 inflammasome: envelope (E), ORF3a, and ORF8b. E protein localizes at the membrane enfolding the Golgi complex and the endoplasmic reticulum–Golgi intermediate compartment and function as an ion channel (viroporin) that facilitates Ca²⁺ leakage to the cytosol (6). In contrast, ORF3a localizes at the Golgi complex and plasma membrane, acting as a K⁺ channel (7). As NLRP3 is sensitive to high cytosolic Ca²⁺ but is instead inhibited by high K⁺ concentration, the viroporin activity of SARS-CoV presumably induces inflammasome activation via E protein–mediated Ca²⁺ leakage from intracellular storage and ORF3a-mediated cellular K⁺ efflux at the plasma membrane to the extracellular spaces (7, 8). The resultant disruption of intracellular ionic balance also promotes mitochondrial damage and generation of reactive oxygen species (ROS), which coactivates NLRP3 (7). SARS-CoV could also activate inflammasomes independent of its viroporin activities. E protein and ORF3a are able to stimulate NF-κB signaling to drive the transcription of inflammatory cytokines and chemokines, including IL-1β, IL-18, and IL-8, and prime NLRP3 expression to its functional level (9–12). ORF3a also activates NLRP3 inflammasome by promoting TNFR-associated factor 3 (TRAF3)–mediated ubiquitination of ASC (12). Finally, ORF8b activates NLRP3 through direct interaction of the leucine-rich repeat domain of NLRP3 (13). Given that SARS-CoV-2 shares ~79% overall genetic similarity with SARS-CoV and the amino acid sequences of SARS-CoV-2 and SARS-CoV E protein are 94.7% conserved, it is likely that SARS-CoV-2 could similarly activate the NLRP3 inflammasome (14, 15). Interestingly, however, a study on SARS-CoV-2 consensus sequence HKU-SZ-005b showed a remarkable distinction in ORF8 from that of SARS-CoV and a lack of the aggregation motif found in SARS-CoV ORF8b that triggers NLRP3 activation (14). ORF3a share 72% amino acid sequence identity between the two viruses, and of note, ORF3b is another region with distant sequences with only 32% identity (14). It would be interesting to determine whether SARS-CoV-2 ORF3a and ORF8 can likewise interact with NLRP3 or at least function as ion channels that would indirectly induce inflammasome activation. Although it is unknown for SARS-CoV2 infection, various innate immune receptor signals are proposed as the upstream contributors of RNA virus-induced NLRP3 inflammasome activation. These include Z-DNA binding protein 1 (ZBP1)/receptor interacting protein kinase 1 (RIPK1)/RIPK3 signaling (16, 17), and 2’ 5’-oligoadenylate synthetase/RNaseL pathway (18). Moreover, RIG-I is proposed to interact with ASC and induce IL-1β secretion after vesicular stomatitis virus infection independently of NLRP3 (19). In parallel with the viral protein-mediated inflammasome activation, it is possible that the RNA-sensing pathways trigger inflammasome activation upon SARS-CoV-2 infection.
There are various other NLRs beyond NLRP3 and the inflammasomes that may be just as consequential in host immune response against viral infections like SARS-CoV-2. These include NLRs that intensify inflammatory processes, such as NOD1 and NOD2 that similarly form multiprotein complexes known as NODosomes. NOD1 and NOD2 are expressed in leukocytes and epithelial cells, and the assembled NODosomes drive NF-κB signaling and type I IFN production (20). Conversely, there exists a unique subgroup of NLRs that function as negative regulators of inflammation, including NLRX1, NLRP12, and NLR family CARD domain containing 3 (NLRC3). These NLRs attenuate inflammation by modulating NF-κB signaling, type I IFN response, and ROS production, among other processes (20). Interestingly, it was reported that SARS-CoV-2 ORF9c protein can activate negative regulators of host inflammatory responses, including NLRX1, to block mitochondrial antiviral-signaling protein (MAVS) to hinder NF-κB–mediated cytokine production (21).

As a mechanism to drastically intensify disease pathogenesis, inflammasome activation can trigger cellular pyroptosis, a type of programmed cell death characterized by gasdermin D–mediated membrane rupture, and spontaneous release of cytosolic contents into the extracellular spaces. Upon inflammasome activation, caspase-1 and other noncanonical inflammasome caspases such as caspase-4, caspase-5 and caspase-11 activate gasdermin-D, which subsequently form pores on the cell membrane (22). These gasdermin-D pores facilitate the secretion of IL-1β and IL-18, and importantly, they also enable simultaneous influx of Na+ and water molecules, causing excessive cell swelling to the point of membrane rupture (22, 23). Pyroptosis of macrophages that have phagocytosed viruses rapidly releases a myriad of alarmins, including viral particles, cytokines, chemokines, LDH, ATP, and ROS, prompting an immediate reaction from surrounding immune cells and thus inducing a pyroptotic chain reaction. Moreover, pyroptosis would allow viral Ags and RNA to be disseminated in the circulation and possibly generate immune complex and deposition in target organs, such as kidney, to initiate severe inflammatory cascade.

SARS-CoV-2–induced inflammasome activation and pyroptosis in alveolar macrophages and recruited monocyte-derived macrophages could drastically aggravate symptoms of pneumonia, including acute respiratory distress syndrome and fever. It was established that the route of SARS-CoV-2 entry into cells is through the angiotensin-converting enzyme 2 (ACE2) receptor, and these are indeed expressed by cells in the lungs, including alveolar type 2 cells, respiratory epithelial cells, and macrophages, making them suitable targets for viral infection and potential inflammasome induction leading to pyroptosis (24, 25). The epithelial cells lining the airways are particularly vulnerable to pathogenic insults, owing to its large area of exposure to external environment. Following influenza A virus infection, the RIG-I receptor is essential in regulating NLRP3 inflammasome activation in response to elevated type I IFN production to induce pyroptosis of lung epithelial cells (26, 27). Pyroptosis of lung epithelial cells may confer protection against pathogens, as demonstrated in mice models of melioidosis (28). However, inflammasome signaling in lung epithelial cells is significantly enhanced in asthmatic patients, which aggravates tissue inflammation and worsens viral pathogenesis (29). It is predicted that pyroptosis in lung epithelial cells is likewise detrimental given the severe pneumonia experienced by COVID-19 patients. In contrast, pyroptosis in alveolar macrophage induces acute lung injury and exacerbates lung inflammation by promoting neutrophil infiltration into the lungs and augmented alveolar concentrations of cytokines IL-6, TNF-α, and IL-1β (30). The combination effects between leukocytosis and pyroptosis may be a major contributor to cytokine storms observed in COVID-19 patients. Another unsettling observation that is especially relevant to severe COVID-19 patients is that mechanical stretch of the lungs further amplifies lung inflammation via NLRP3 activation in alveolar macrophages and MAPK kinase 6–mediated high-mobility group box 1 (HMGB1) protein expression in alveolar epithelial cells (31, 32). Therefore, the use of NLRP3 suppressors in patients requiring the use of ventilators might be helpful in mitigating excessive lung tissue damage.

Widespread pyroptosis could lead to excessive tissue inflammation, organ failure, and death within minutes (33). Uncontrolled pyroptosis is especially detrimental in the elderly who are already experiencing an age-related chronic inflammatory condition known as “inflammaging” (34). Moreover, ageing individuals have impaired capacity to produce type I and type III IFNs because of TRAF3 degradation (35). These IFNs are not only key antiviral resistance factors but are also potent regulators of the inflammasomes (36). When tested in a mouse model of influenza A virus disease, the absence of innate resistance (due to deficiencies in TLR7 and RIG-I–like receptor signaling) led to a lethal disease only in the presence of caspase-1/caspase-11 activation (37). In this setting, recruitment of neutrophils to the lung and activation of NETosis led to the pathological and lethal disease. Treatment with DNase (to break up the DNA released by the NETs) as well as IL-1R antagonist (Anakinra) was able to reduce the severity of the disease. Thus, the impairment in antiviral resistance and unregulated inflammasome activation may underlie the perfect storm for the severe disease we observe in the COVID-19 patients.

Although there is indeed ample evidence advocating for inflammasome inhibition as a viable solution to treatment of hyperinflammatory responses in virus infections, there have been conflicting results in the roles of immune receptors in host immune defense against virus infections. For example, it was shown that mice lacking NLRP3 and caspase-1 exhibit much greater mortality to influenza A virus infection due to compromised immune response, including a reduction in neutrophil and monocyte migration, as well as decreased secretion of cytokines and chemokines (38, 39). This implies the significant temporal, cell type, and disease-specific functions that would alter their therapeutic potential in a particular context, and it is imperative that these factors should be taken into consideration in the design and use of inflammasome inhibitors.

Inflammasome activation and pyroptosis could be underappreciated events that are central to COVID-19 pathogenesis. Abnormalities in blood coagulation leading to thrombotic complications, including pulmonary embolism, are associated with poor prognosis in COVID-19 patients (40, 41). The suppression of inflammasome-mediated pyroptosis in macrophages might mitigate anomalous blood clotting by preventing
the release of tissue factor, which is an initiator of blood coagulation cascades (42). Recently, another complication of COVID-19 described among children takes the form of pediatric inflammatory multisystem syndrome, otherwise referred to as Kawasaki disease, characterized by acute inflammation of the medium-sized arteries, including the coronary arteries, resulting in artery aneurysms that, if left untreated, would cause myocardial ischaemia, infarction, and death (43, 44). Although the precise etiology of Kawasaki disease is yet obscure, it is suspected that pathogenic infections play a role in disease pathogenesis because of the seasonality of this disease, and SARS-CoV-2 is likely contributing to the present emergence of symptoms among pediatric COVID-19 patients (45). A comparison of 146 Kawasaki disease patients found an overwhelming signature for an acute disease, involving IL-1 signaling pathways (46), further substantiating the roles of aberrant inflammasome activity and justifying the use of inflammasome inhibitors in COVID-19 treatments. In another study, inhibition of complement-induced pyroptosis was able to reduce local inflammation at the lungs and spleen of mice infected with the Middle East respiratory syndrome coronavirus (47). Yet another potential benefit of NLRP3 inhibition is the possibility of ameliorating comorbidities associated with COVID-19, including hypertension, chronic obstructive pulmonary disease, type 2 diabetes, and cardiovascular disease, as NLRP3 inflammasome activation is implicated in these diseases, as well (48–51). These comorbidities strongly influence COVID-19 severity, and mitigating them might improve COVID-19 prognosis and significantly decrease the risk of death.

Several repurposed compounds with regulatory effects on inflammasome activity are currently being appraised in clinical trials as treatment for COVID-19. An example is tranilast, a tryptophan analogue that has a direct inhibitory action against NLRP3 (52), which is currently undergoing a randomized control trial in COVID-19 patients (registration number: ChiCTR2000030002 on the Chinese Clinical Trial Registry). Tranilast is initially approved for the treatment of allergic and inflammatory diseases such as bronchial asthma, atypical dermatitis, allergic conjunctivitis, keloids, and hypertrophic scars (53). Now, its effectiveness has also been recognized in the treatment of fibrosis, proliferative disorders, cancer, cardiovascular problems, autoimmune disorders, ocular diseases, diabetes, and renal diseases (53). Tranilast might be an attractive intervention for COVID-19 patients with comorbidities, given its wide array of therapeutic effects with minimal side effects. However, tranilast should not be used together with the anticoagulant drug warfarin, in the event that the latter is used clinically to control blood clotting in COVID-19 patients, as the two drugs are known to interact with each other synergistically to produce serious side effects (54). In an exciting recent development, a study shows that disulfiram, a US Food and Drug Administration–approved drug used to treat alcohol addiction, is a potent inhibitor of pyroptosis and gasdermin D–dependent cytokine release (55) and holds promise for COVID-19 therapy. Preventing gasdermin-D pore formation without disrupting inflammasome activation represents a promising approach, as one can restrict viral replication within cells by eliciting inflammasome-mediated apoptotic cell death instead of pyroptosis and cytokine release, thus limiting widespread tissue inflammation (56). Efforts are also on the way to block the cytokines downstream of inflammasomes, including IL-1β, using Anakinra, which are currently being tested in phase 3 clinical trials for COVID-19 (ClinicalTrials.gov identifier: NCT04330638 and NCT04324021). Treatment with Anakinra was previously shown to be effective in alleviating vasculitis, coronary lesions, and myocarditis in a mouse model of Kawasaki disease (57) and was followed by successful use as clinical intervention for cases of severe and resistant Kawasaki disease in newborn and very young children (58, 59).

Various dedicated inhibitors of NLRP3 have existed mostly in the form of clinically unproven experimental compounds, both of natural and synthetic origins (60). These compounds could either inhibit NLRP3 indirectly or directly target the NLRP3 core protein or its constituents, such as ASC and caspase-1. Pharmacological compounds that disrupt the signaling pathways upstream of inflammasome activation also hold promise. For example, the natural polyphenolic flavonoid silymarin (61), the plant sesquiterpene lactone parthenolide, as well as the synthetic IκB kinase-β inhibitor Bay 11-7082, inhibits the NF-κB pathway, thereby preventing the priming step of NLRP3 activation and the transcription of inflammatory cytokines (62). Inhibition of NF-κB–mediated inflammation was shown to improve survivability of SARS-CoV–infected mice (9). Furthermore, quercetin, a natural antioxidative flavonoid found in many plants, exhibited potent suppressive ability against inflammasomes, including NLRP3, by diminishing ASC speck formation and oligomerization (63). The same study demonstrated that quercetin likewise relieves vasculitis in a mouse model of Kawasaki disease, thus offering a plausible alternative to the use of Anakinra, although its clinical relevance remained to be determined. Another plant-derived dehydrocostus lactone found in traditional Chinese medicinal herbs, such as the roots of Sarsosuria lappa, similarly suppressed NLRP3 inflammasome activity by blocking ASC oligomerization, in addition to reducing neutrophil recruitment (64). These promising results illustrated a couple of noteworthy ideas: first, the effectiveness in a multipronged approach in inflammation inhibition, and second, the prospective benefits in screening for additional naturally occurring substances with anti-inflammatory properties and assessing their clinical potential in the treatment of COVID-19.

Clinically approved drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) can also be repurposed to selectively inhibit NLRP3. NSAIDs of the fenamate type such as flufenamic acid and mefenamic acid were shown to inhibit NLRP3 inflammasome by reversibly blocking volume-regulated anion channels, which regulate Cl– transport across plasma membrane (65). Additionally, it was suggested that NSAIDs also contribute to limiting the secretion of proinflammatory cytokines through their cyclooxygenase-1 (COX-1)–independent activity. At present, there is no evidence for or against the use of NSAIDs as COVID-19 treatment. Nevertheless, it is recommended that NSAIDs should be prescribed cautiously to COVID-19 patients, including when used as analgesic (66).

Finally, as discussed above, type I and type III IFNs can be used to suppress transcription of both IL-1β as well as inflammasome components. Type I IFNs have demonstrated efficacy against SARS-CoV infection in in vitro experiments but have generally failed in human trials (67). However, the
use of type I IFN in COVID-19 might still be effective for COVID-19 as SARS-CoV-2 is unusually sensitive to type I IFN pretreatment compared with SARS-CoV (K.G. Lokugamage, A. Hage, C. Schindewolf, R. Rajsbaum, and V. D. Menachery, manuscript posted on bioRxiv). In contrast, an advantage that type III IFNs have in COVID-19 treatment over type I IFNs is that the former do not induce proinflammatory effects in the lungs (68). Tests in preclinical models have also supported the effectiveness of type III IFNs in reducing the disease severity (69). Nonetheless, a careful clinical trial is warranted for the use of IFN treatments as, to date, the precise pathogenesis of COVID-19 is still unclear at this stage.

Conclusions

In this review, we propose that the benefits of inhibiting inflammasomes and/or pyroptosis are multifaceted and the search for inhibitor drugs in COVID-19 therapy would prove to be a worthwhile effort. However, although NLRP3 inhibition may be a promising intervention, a functional and balanced immune activity is still paramount for infection control and pathogen clearance. The importance of NLRP3 in repressing SARS-CoV-2 virulence is emphasized in a study that demonstrated that significantly dampened NLRP3-mediated inflammation in bats conferred disease tolerance in these hosts, providing an ideal reservoir for a range of zoonotic viruses, including SARS-CoV, Middle East respiratory syndrome coronavirus, and likely SARS-CoV-2 (70). In fact, some viruses such as the influenza virus, measles virus, Sendai virus, and Nipah virus have evolved mechanisms to suppress the NLRP3 inflammasome (71). Although shown to interact with anti-inflammatory immune receptors, whether SARS-CoV-2 also suppresses inflammasomes in infected cells is unknown. Considering the gravity of the present situation, it is worth expanding the screen for available NLRP3 inhibitors to evaluate their effectiveness in mitigating aberrant inflammatory responses in COVID-19 patients. It would also be important to determine the safety and the most suitable dosage of such inhibitor drugs through clinical trials as early as possible.

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Disclosures

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References

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