SARS-CoV-2 and the NLRP3 Inflammasome

Julia Somers[1], Gökçe Yağmur Summak[2], Ebru Kocakaya[2], Ömür Beşbınar[2]

[1]Oregon Health and Science University, Department of Molecular and Medical Genetics
[2] Ankara University, Stem Cell Institute

Abstract

Inflammasomes are endogenous sensors of viral infection activated by damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs). Once activated by DAMPs or PAMPs, inflammasomes assemble and catalyze the autoactivation of Caspase-1 and subsequent maturation of proinflammatory cytokines. Although these multiprotein systems are key to innate immunity, the overactivity of inflammasomes can perpetuate a hyperactive immune response causing damaging inflammation. In COVID-19, devastating inflammation contributes substantially to morbidity and mortality. Patients with severe COVID-19 exhibit high levels of IL-1B and IL-18 indicating robust activity of the NLRP3 inflammasome. The mechanisms by which SARS-CoV-2 incites the NLRP3 inflammasome have not been extensively characterized, but may be inferred from those of its close relatives. Several proteins from other coronaviruses and other coronaviruses have been found to preferentially incite the NLRP3 inflammasome, both by direct association with inflammasome components and indirect disruption of ion gradients. Here we present our curation of these mechanisms and the possible targets in the NLRP3 inflammasome system for COVID-19 therapeutics.

Inflammasomes

Inflammasomes are multi-protein pro-inflammatory systems that are activated by viral infection or stress. By activating a class of caspases known as inflammatory caspases, which spur the maturation of proinflammatory cytokines such as IL-1β, inflammasomes engage in innate immune defenses. There are multiple inflammasome systems (NLRP1 / NALP1b, NLRC4 / IPAF , NLRP3 / NALP3 and AIM2) each containing a sensor protein, an adaptor protein, and the cellular protease caspase-1.

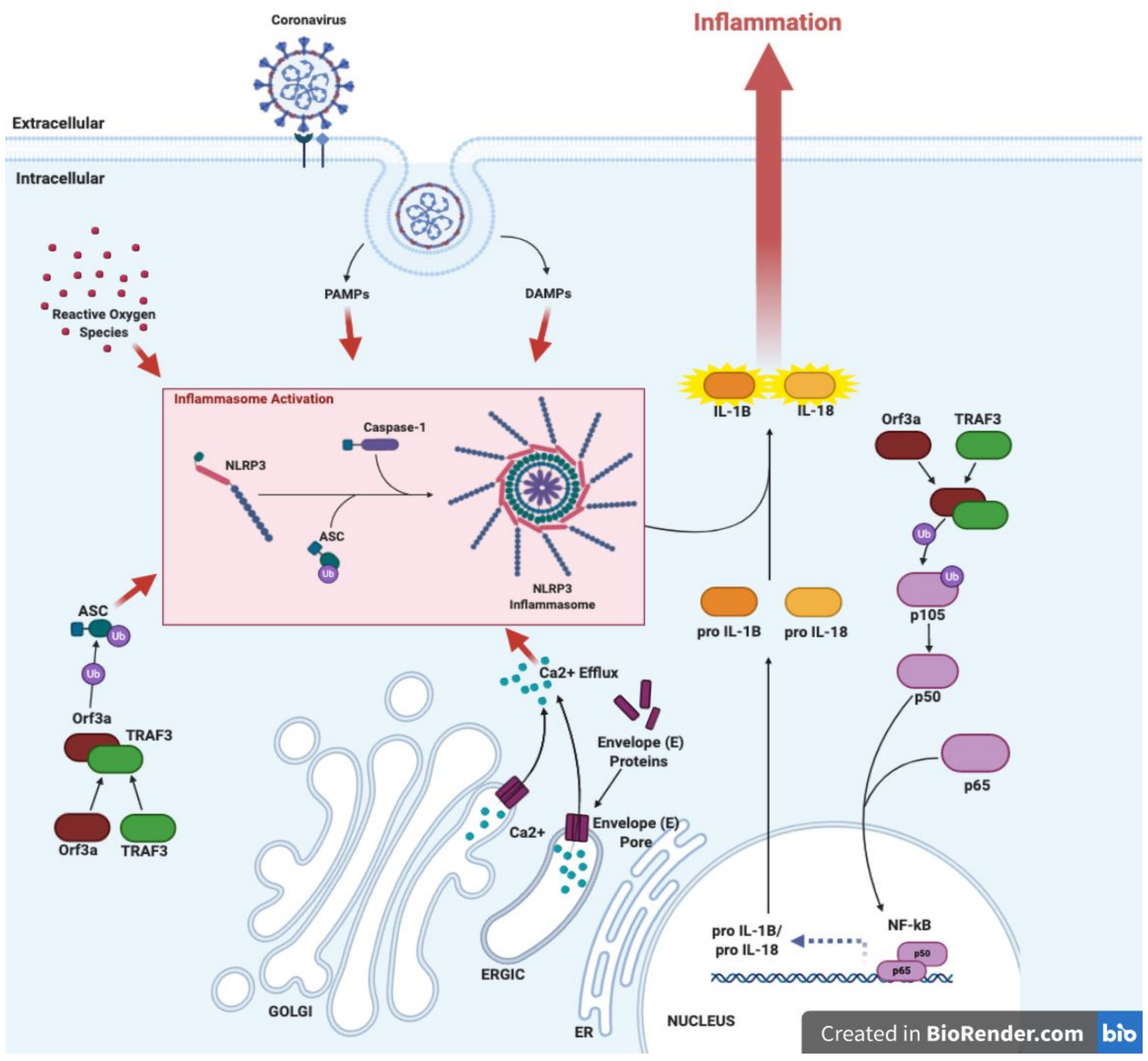
NLRP3 inflammasomes are particularly sensitive to RNA viruses. SARS, MERS, and SARS-CoV-2 inflammatory patterns show robust activity of the NLRP3 inflammasome. NLRP3 inflammasome activation is a two signal process. The first signal is the expression of inflammasome components and IL-1 family cytokines. Stimulated TLRs activate NF-Kβ signaling, resulting in increased synthesis of precursor proteins, including NLRP3, pro-IL-1β, and pro-IL-18. The second signal triggers is NLRP3 oligomerization and subsequent inflammasome assembly. A variety of signals can trigger NLRP3 oligomerization, including the RNA-sensing kinase PKR, reactive oxygen species, and ionic gradient disruption. NLRP3 oligomers then recruit the adaptor ASC, and caspase-1 to form the central structure of the NLRP3 inflammasome. The mature complex converts facilitates autoactivation of caspase-, which cleaves proIL-1β and proIL-18 into their mature forms.

IL-1β and IL-18 drive immune responses and contribute to the physiological characteristics of viral infection. IL-1B and IL-18 induce the proinflammatory cytokines TNF and IL-6, and IFN-g, IL-13, IL-4, and IL-8, respectively (Farag et. al. 2020)

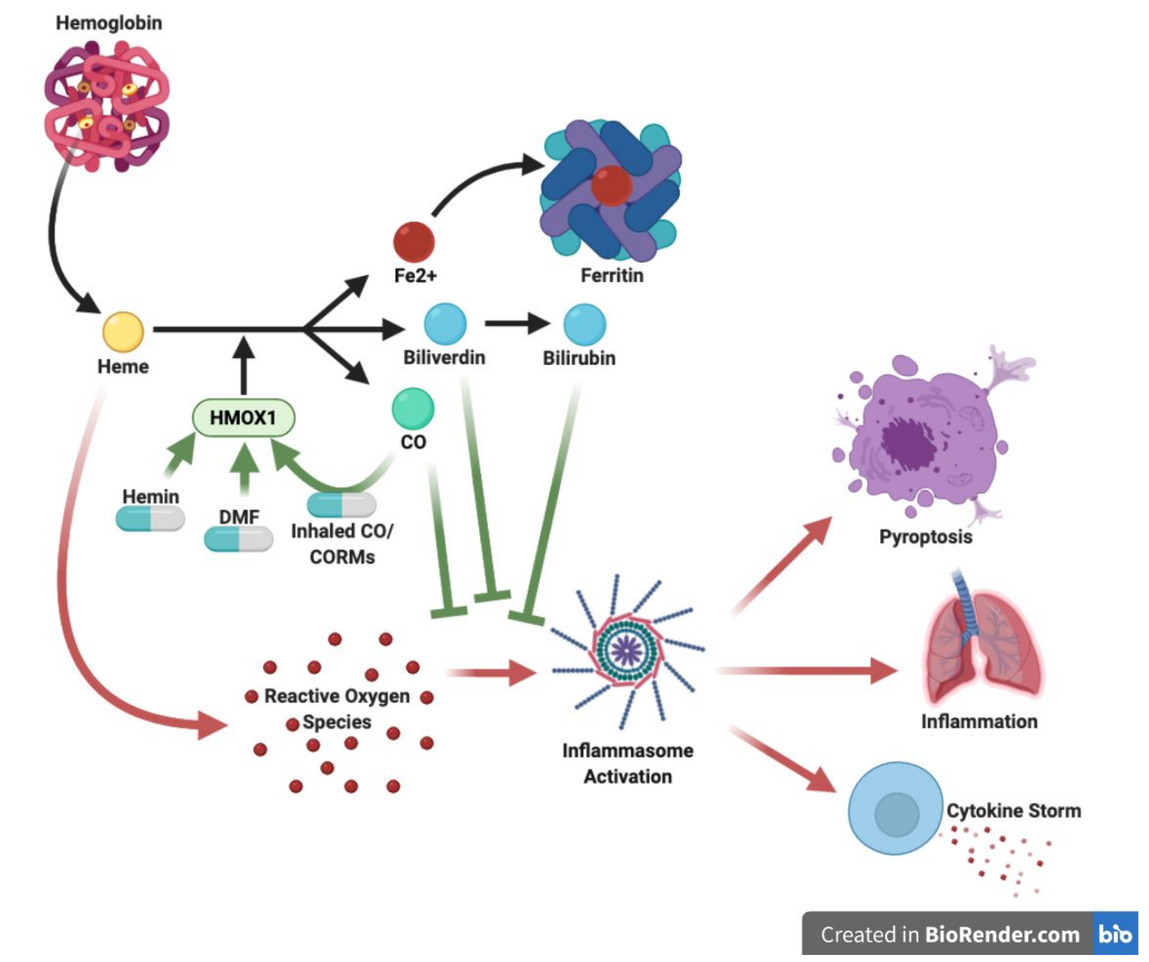
Coronaviruses incite the NLRP3 inflammasome

With regard to coronavirus infection (SARS, MERS and SARS-COV2), studies have documented higher levels of IL-18 and IL-1β not only in patients' blood, but also in the lungs and lymphoid tissues, suggesting increased activation of inflammasomes. Recent efforts have elucidated the mechanisms by which coronaviruses activate the NLRP3 inflammasome. Specific viral proteins, including the viroporins E and ORF3a trigger inflammasome assembly. Viroporins are capable of producing ion-permeable pores in various membranes, and their function is required for replication and virulence (Farag et. al. 2020). Siu et. al. demonstrated pro-IL-1β gene expression and IL-1β secretion were sufficiently activated by ORF3a. They discovered that by encouraging TNF receptor-associated factor 3 (TRAF3)-mediated ubiquitination of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), the SARS-CoV ORF3a accessory protein stimulates both signals for NLRP3 inflammasome activation (Siu et. al. 2019). Another group demonstrated that in lipopolysaccharide-primed macrophages, the SARS ORF3a activates the NLRP3 inflammasome by influencing K+ efflux and mitochondrial ROS (Chen et. al. 2019). In a similar fashion, coronavirus E protein localized to the golgi and the ER-golgi intermediate compartment (ERGIC) and created ion channels that released Ca2+ into the cytosol. The disruption of the Ca2+ gradient was sufficient to activate the NLRP3 inflammasome, representing another mechanism of coronavirus-induced inflammasome activity (Nieto-Torres et. al. 2015).

Thus far, mechanistic studies of coronavirus mediated NLRP3 inflammasome activation have been restricted to SARS and MERS. Even so, the NLRP3 inflammasome appears to be highly activated in COVID-19. Robust expression of IL-1 family cytokines, and consistencies with clinical manifestations of diseases known to be driven by NLRP3 inflammasome support that NLRP3 contributes substantially to COVID-19 pathophysiology (Ratajczak et. al. 2020, van den Berg et. al. 2020, Freeman et. al. 2020).



Activation of the NLRP3 inflammasome by coronaviruses.
Assembly of the NLRP3 inflammasome is triggered by signals of cell distress. NLRP3 oligomers associate with ASC to create a platform for caspase-1 auto-activation. Activated caspase-1 cleaves pro-IL-1B and pro-IL-18 to produce mature proinflammatory cytokines. Several coronavirus proteins have been documented to incite the NLRP3 inflammasome. Both E (dark purple)and the Orf3a (dark red) are known activators of the inflammasome system. These proteins, and possibly their function, are conserved across SARS, MERS, and SARS-CoV-2.



Suppression of the NLRP3 inflammasome by HMOX1
The NLRP3 inflammasome is a major contributor to the inflammation that is characteristic of COVID-19. NLRP3 inflammasome is suppressed by the products of the heme catabolism. Specifically, the antioxidants carbon monoxide (CO) and Bilirubin/Biliverdin, quell the formation of reactive oxygen species that activate the NLRP3 inflammasome. The heme catabolism pathway is driven by the enzyme heme oxygenase-1. Heme oxygenase-1 can be induced by several pharmacological agents, including the FDA approved drugs hemin and dimethyl fumarate. Low dose inhaled carbon monoxide and carbon monoxide releasing materials (CORMs) also activate the HMOX1 pathway and have demonstrated profound results against pulmonary inflammation.

Inhibition of the NLRP3 inflammasome by HMOX1

Elevated inflammasome activity can be attenuated by inducing HMOX1 with the FDA approved drug Panhematin (hemin). In a mouse model of sepsis, hemin administration inhibited the NLRP3 inflammasome by decreasing expression of ASC, NLRP3, and caspase-1; upregulating both gene and protein expression of HMOX1; elevating HMOX1 enzymatic activity; and reducing IL-1B and IL-18 levels, which significantly reduced inflammation and protected against acute lung injury (Luo et al., 2014). Hemin provided similar protection in a model of D-Galactosamine- and lipopolysaccharide-induced hepatic inflammation. Survival was markedly improved by pretreating mice with hemin. These mice maintained higher glutathione and lower lipid peroxidation levels, indicative of decreased oxidative stress. They also expressed lower levels of the inflammatory cytokines TNF-a and IL-1B and showed decreased interaction of NLRP3 with TXNIP. TXNIP is a member of the redoxisome system which activates NLRP3, possibly as a result of ROS accumulation (Yoshihara et al., 2013). Hemin also interfered with the assembly of the inflammasome by preventing association of NLRP3, ASC, and caspase-1 (Kim & Lee, 2013). Suppression of the NLRP3 inflammasome by HMOX1 stimulation could be an effective treatment in COVID-19.

Conclusions

COVID-19 inflammation is the cause of substantial morbidity and mortality. Inflammasomes control inflammatory programs in response to signals of viral infection or cell damage. Coronaviruses incite the NLRP3 inflammasome through various mechanisms, an effect which appears to be conserved in COVID-19. This makes the NLRP3 inflammasome a potential target for COVID-19 inflammation. HMOX1 targeted therapeutics are readily available and may be useful for suppressing the overactive NLRP3 inflammasome in COVID-19 patients with severe disease.

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