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Abstract

Following entry and replication of Severe Acute Respiratory Syndrome-coronavirus-2 (SARS-CoV-2) into ACE2 expressing cells, the LQIHFWHG FHOOV XQGHUJR O\VLV UHOHDVLQJ PRUH YLUXVHV EXW DOVR FHOO FRQWHQ WRJHWKHU ZLWK RWKHU FHOO FRQWHQWV $ FDVFDGH RI LQÀDPPDWRU\ F\WRNLQHV HQ DV ZHOO DV V\VWHPLF LQÀDPPDWLRQ 7KLV FDVFDGH RI LQÀDPPDWRU\ F\WRNLQHV LQ 6\QGURPH· &56 DQG LV DVVRFLDWHG ZLWK SRRU RXWFRPHV DQG GHDWK 0DQ\ VWX SDWLHQWV UHGXFHV GLVHDVH VHYHULW\ DQG GHDWKV +HUH ZH UHSRUW KLJKO\ VLJQL 71). ,/ DQG VROXEOH XURNLQDVH SODVPLQRJHQ DFWLYDWRU UHFHSWRU LQ &29,' S FLUFXODWLQJ P\HORLG FHOOV IURP WKH VDPH SDWLHQWV WKHUH LV LQFUHDVHG H[SU HDUO\ LQ WKH LQIHFWLRQ :H REVHUYHG LQFUHDVHG 1/53 JHQH H[SUHVVLRQ LQ P\HO ZLWK HOHYDWHG FLUFXODWLQJ /, OHYHOV :H FRQFOXGH WKDW HDUO\ 6$56 &R9 &56 7KXV 1/53 LV D WDUJHW WR UHGXFH WKH RUJDQ GDPDJH RI LQÀDPPDWRU\ F\WRNL

multi-organ failure. CRS contributes to and can be causal in COVID-19 however; the mechanism(s) for initiation of CRS in COVID-19 remains unknown. Numerous trials comparing standard of care in control patients as well as case reports have administered the IL-1 Receptor antagonist (IL-1Ra) anakinra in modest to severe COVID-19 patient, although there are at present no randomized trials. Emerging from these reports is the concept that targeting of IL-1 result in improved outcomes, including deaths. For example, high doses of anakinra reduces deaths as well as number of days in the hospital [3-5]. Anakinra has also been administered in less severe hospitalized patients and resulted in similar reduction in disease [6]. Since anakinra blocks the IL-1 Receptor, the e cacy of anakinra may be due to reducing IL-1 or IL-1 . Other studies report that speci cally targeting IL-1 with the neutralizing monoclonal antibody canakinumab also reduces outcomes. e intracellular processing of IL-1 into its biologically active form is largely governed by cytosolic macromolecular complexes termed in ammasomes [7,8]. Notably, it has been observed that viral proteins of the SARS-CoV virus ORF3a, ORF8b and Viroporin 3a activate the NLRP3 in ammasome [9-11]. More recently, in vitro studies showed that also SARS-CoV-2 induces the NLRP3 in ammasome formation [12]. Presence of NLRP3 in ammasome aggregates has also been shown in the lungs of fatal COVID-19 pneumonia and in PBMCs and tissues of COVID-19 positive post-mortem patients upon autopsy [13,14]. Notably, Rodrigues et al. have shown that SARS-CoV-2 virus can infect monocytes leading to the NLRP3 in ammasome formation in these cells [13]. ese studies con rm activation of the NLRP3 in ammasome in COVID-19 in moderate to severe cases. e use of an early treatment for non-hospitalized COVID-19 subjects in order to reduce the burden of hospitalizations and intensive care units is clear [15]. Here we show increased NLRP3 in non-hospitalized SARS-CoV-2 positive subjects. ese data support the rationale for early inhibition of NLRP3 to prevent in ammasome formation and the release of IL-1 in SARS-CoV-2 infection.

Methods

PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from drawn blood by gradient centrifugation using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). PBMCs were suspended in Roswell Parl Memorial Institute 1640 medium supplemented with 50 g/mL gentamicin, 2 mM glutamine, and 1 mM pyruvate and cultured for 24 hours.

Cytokines measurements

Plas 10 31i-/P <<Tm [(P 0.022l[si)2 (f IL)9 (-1)],-1 ,-(-2)313 ET

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Recent studies have demonstrated that oral administration of colchicine in 4159 non-hospitalized COVID-19 PCR positive patients reduced a composite end-point of hospitalizations and death in 4.6% of treated patients compared to 6.0% of placebo-treated subjects ($p<0.04$) [20]. Colchicine treatment also reduces the risk of cardiovascular events [21]. e mechanism by which colchicine is e ective in coronary artery disease as well as in COVID-19 is likely due to reduce IL-1 -mediated in ammation. However, colchicine does not directly inhibit NLRP3 [22]. Unlike speci c NLRP3 inhibitors, colchicine a ects integrins, cell migration and microtubule assembly. A signi cant advantage of targeting the NLRP3 in ammasome is the ability to reduce IL-18 processing. erefore, speci c NLRP3 inhibitors could be used to treat the Macrophage Activation Syndrome (MAS)-like disease in COVID-19, where IL-18 plays a pathological role. Elevated circulating IL-18 correlated with disease severity and poor outcomes in COVID-19 patients [2,13]. IL-18 is characteristically elevated in non-COVID-19 MAS [23]. Several case series reports that a MAS-like disease develops in COVID-19 patients with markedly elevated levels of D-dimer, which is indicative of MAS in COVID-19 [24]. Speci c NLRP3 inhibition will reduce both IL-1 as well as IL-18 and thus targets two agonists of COVID-19 disease.

References

1. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, et al. $Q LQÀDPPDWRU\ F\WRNLQH VLJQDWXUH SUHGLFWV &29,' VHYHULW\ DQG VXUYLYDO 1DWXUH 0HGLFLQH
2. /XFDV & :RQJ 3 .OHLQ - &DVWUR 7% 6LOYD - HW DO /RQJLWXGLQDO DQDO\VHV UHYHDO LPPXQRORJLFDO PLV¿ULQJ LQ VHYHUH &29,' 1DWXUH r