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Novel therapeutic targets for SARS-CoV-2-induced acute lung injury: Targeting a potential IL-1β/neutrophil extracellular traps feedback loop

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ABSTRACT

Most COVID-19 infected individuals present with mild flu-like symptoms; however, 5–10% of cases suffer from life-threatening pneumonia and respiratory failure. The pathogenesis of SARS-CoV-2 and its pathology of associated acute lung injury (ALI), acute respiratory distress syndrome (ARDS), sepsis, coagulopathy and multi-organ failure is not known. SARS-CoV-2 is an envelope virus with S (spike), M (membrane), N (nucleocapsid) and E (envelop) proteins. In a closely related coronavirus (SARS-CoV), the transmembrane E protein exerts an important role in membrane-ionic transport through viroporins, deletion of which reduced levels of IL-1β and a remarkably reduced lung edema compared to wild type. IL-1β is generated by macrophages upon activation of intracellular NLRP3 (NOD-like, leucine rich repeat domains, and pyrin domain-containing protein 3), part of the functional NLRP3 inflammasome complex that detects pathogenic microorganisms and stressors, while neutrophils are enhanced by increasing levels of IL-1β. Expiring neutrophils undergo "NETosis", producing thread-like extracellular structures termed neutrophil extracellular traps (NETs), which protect against mild infections and microbes. However, uncontrolled NET production can cause acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), coagulopathy, multiple organ failure, and autoimmune disease. Herein, we present arguments underlying our hypothesis that IL-1β and NETs, mediated via NLRP3 inflammasomes, form a feed-forward loop leading to the excessive alveolar and endothelial damage observed in severe cases of COVID-19. Considering such assertions, we propose potential drug candidates that could be used to alleviate such pathologies. Considering that recent efforts to ascertain effective treatments of COVID-19 in severe patients has been less than successful, investigating novel avenues of treating this virus are essential.

Introduction

Coronaviruses (CoVs) are characterised by surface spike proteins and an unsegmented positive RNA genome [1]. While most CoVs infect domestic animals including pigs and chickens, 6 have been confirmed as zoogenic, also infecting humans [1]. These include severe acute respiratory syndrome coronavirus (SARS-CoV), middle east respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. SARS-CoV-2 is the causative factor behind the ongoing coronavirus disease 2019 (COVID-19) pandemic.

SARS-CoV-2 is an envelope virus with S (spike), M (membrane), N (nucleocapsid) and E (envelop) proteins [2,3]. SARS-CoV (~76% homology with SARS-CoV-2) possesses a 76-amino acid long transmembrane E protein [4], deletion of which decreases the viral titre 20-fold [5], and decreased inflammation via the NF-κB pathway within infected mice [6]. The ion channel activity of SARS-CoV was also mapped to the transmembrane domain of the E-protein [7]. A recombinant virus lacking E-protein decreased apoptosis and inflammation [8]. Virulence in mouse mutants lacking the SARS-CoV E-protein ion channel activity (EIC^-) was severely reduced, although viral replication remained unaffected [9]. Strikingly, EIC^- mutants produced low levels of proinflammatory cytokines including IL-6, IL-1β and TNF-α as compared to wild type mice, including remarkably reduced lung edema [9].

IL-1β is generated by resident macrophages upon activation of membrane-bound or cytosolic pattern recognition receptors (PRRs) which detect pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The most relevant PRR in macrophages is the intracellular NLRP3 (NOD-like, leucine rich repeat domains, and pyrin domain-containing protein 3) [10–13]. NLRP3 exists as a latent monomer in quiescent cells. Upon stimulation, NLRP3

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recruits ASC (an adapter protein) and pro-caspase-1, forming a functional NLRP3 inflammasome complex by oligomerization [10–13]. A feed-forward mechanism for IL-1β generation exists, i.e., IL-1β can activate the NLRP3 inflammasome (an intracellular multi-protein complex that detects pathogenic microorganisms and stressors) and vice versa [10–13], representing a potential mechanism of generating the exaggerated cytokine response in sepsis.

A diverse array of intracellular mediators have also been implicated in activating the NLRP3 inflammasome [10], and current consensus posits that these mediators induce a common cellular signal [10,13]. The major intracellular events proposed include activation of ion channels, reactive oxygen species, and lysosomal damage. Acute lung injury associated with SARS-CoV infection can be attenuated by mutating the viral E-protein which codes for viroporins [9], transmembrane proteins with ion-exchange properties that can activate NLRP3 inflammasomes [14].

Neutrophils also play a key role as the first line of defense in most infections including viral infections [15]. Indeed, COVID-19 patients present with higher neutrophil counts in blood [16]. Neutrophils undergo a unique type of cell death termed "NETosis" [17], whereby expiring neutrophils produce thread-like extracellular structures called neutrophil extracellular traps (NETs). The main constituents of NETs include DNA, modified histones, and cytotoxic proteins including neutrophil elastase (NE), myeloperoxidase (MPO) and cathepsin [17]. NETs mainly perform a protective function, forming mesh-like structures to trap microbes. However, uncontrolled NET production has been heavily implicated as a causative factor of ALI, ARDS, coagulopathy, multiple organ failure and autoimmune disease [15]. Interestingly, IL-1β produced by the NLRP3 inflammasome is a key inducer of NETs [18]).

Hypothesis: IL-1β and NETs form a feedforward loop, leading to the excessive alveolar and endothelial damage observed in severe cases of COVID-19

Although in most cases the recent COVID-19 pandemic involves mild flu-like symptoms such as a sore throat and fever, 5–10% of cases involve life-threatening pneumonia and respiratory failure, resulting in significant global COVID-19-induced morbidity and mortality [16]. The pathogenesis of SARS-CoV-2 and its ability to cause acute lung injury (ALI), acute respiratory distress syndrome (ARDS), sepsis, coagulopathy and multiorgan failure is not known. Considering the apparent close link and modulation between the NLRP3 inflammasome, IL-1β, and NETs, perhaps a causative factor underlying ALI in COVID-19 patients is attributable to NLRP3 inflammasome activation via viroporin-mediated ionic channel activity of SARS-CoV-2 during cellular invasion. Subsequently, activated NLRP3 would induce excessive NET production by neutrophils, resulting in ALI/ARDS.

Studies of patients with SARS-CoV infections implicated involvement of cytokines IL-1β, TNF-α, and IL-6 as causative factors [2]. IL-1β induces generation of other cytokines, including IL-6 and TNF-α, thereby contributing to the "cytokine storm" of inflammatory diseases [19]. IL-1β is generated by the NLRP3 inflammasome, primarily by resident macrophages upon activation of their pattern recognition receptors (PRRs) [10]. A feed-forward mechanism for IL-1β generation exists, i.e., IL-1β can activate the NLRP3 inflammasome and vice versa [10,11,20], potentially leading to an exaggerated cytokine response. The release of pro-inflammatory cytokines in SARS-CoV infection is attributed to E-protein-mediated increases in calcium permeability of ERGIC/Golgi membranes [7,21]. This cytosolic calcium influx activates the NLRP3 inflammasome and IL-6 production [7,21]. Importantly, cationic disturbances due to viroporins are the major cause of the lung edema caused by SARS-COV [18,20]. This lung edema and diffuse lung injury also occurs in COVID-19 patients and exhibits the characteristic ground-glass appearance on CT scans of the lungs.

Evaluation of the hypothesis

Generally, a two-step process results in optimal functioning of the NLRP3 inflammasome; priming and subsequent activation. A priming signal induced by extracellular PAMPs (e.g. LPS) will activate the TLR4/NFκB pathway to increase synthesis of NLRP3 and pro-IL-1β. Subsequently, a second extracellular signal by DAMPs (e.g., ATP) induce NLRP3 oligomerization with ASC and pro-caspase-1 [8,9,20,21]. Pro-caspase-1 of the assembled complex is converted to the active form (via autocatalysis) and cleaves latent pro-IL-1β to the mature IL-1β, which is subsequently released into the extracellular space [10–13]. Influx of calcium also results in stimulation of the inflammasome NLRP3 [21], while several studies have also implicated K^+ efflux during inflammasome activation [12,13].

IL-1β produced by activated inflammasomes can recruit and activate neutrophils, producing excessive NETs. In turn, NETs can further activate more inflammasomes [18]. In ALI/ARDS caused by infections, a massive migration of neutrophils into the alveoli is observed due to chemokines produced by epithelial cells and macrophages [15]. Migrated neutrophils are stimulated by stimulants such as IL-1β in the alveolar space to produce abnormal amounts of NETs, the enzymatic content of which causes potent lung injury [22]. Neutrophils elastase (NE) cleaves cadherins and endothelial cytoskeletal proteins resulting in increased vascular permeability [22] and apoptosis of epithelial cells, resulting in pro-inflammatory cytokine release.

MPO produced by NETs causes epithelial cell necrosis and apoptosis [22]. DNA, a major component of NETs with antigenic properties, causes an increased release of pro-inflammatory cytokines and generation of an abnormal immune response [22]. Endothelial damage during endothelial injury results in release of Von Williebrand factor (vWF), which activates platelets and neutrophils [23]. The activated platelets then stimulate neutrophils to produce NETs, which trap RBCs, platelets and proteins such as fibrin resulting in clot formation [24]. Several studies have implicated the role of NETs in sepsis, due to overactive and cytotoxic immune responses which culminate in multi-organ failure and death [25].

Consequences of hypothesis and discussion

To prevent/reduce ALI/ARDS in COVID-19 patients, it would thus be prudent to explore therapeutics that can block the 1) NLRP3 inflammasome pathway; 2) production of IL-1β from macrophages; or 3) excessive production of NETs (Fig. 1). Glyburide, an antidiabetic drug, can block NLRP3 inflammasome activation by inhibiting ATP-sensitive K+ channels [26]. However, the required dosage to inhibit NRLP3 *in vivo* is high, and would result in significant hypoglycaemia [27]. A potential alternative is *16673-34-0*, an intermediate substrate of glyburide with no hypoglycaemic activity [27]. A further option is Colchicine, which non-selectively inhibits NRLP3 inflammasomes at the P2X7 ATP receptor, and prevents assembly of the ASC complex [28,29]. Amantadine works as an antiviral agent against the influenza virus by blocking the ion channel protein M2 (viroporins) [30,31].

Ankinara can block IL-1β, potentially disrupting the IL-1β/NET feedback loop [18]. NET production has been blocked using recombinant DNase-1 (Dornase alfa), histone deacteylase inhibitors (HDACi) and IL-6 blockers [18,32]. The neutrophil elastase (NE) inhibitor, Sivelestat, has been approved to treat ARDS, although no change in survival rates has yet been observed [33]. While Dornase alfa, sivelestat, ankinara and colchicine are safe and FDA-approved drugs, specific clinical trials are required to evaluate the efficacy of these drugs against COVID-19 in order to prevent severe lung damage and ARDS in such patients.

Recent efforts to ascertain effective treatments of COVID-19 in severe patients has been less than successful. Indeed, Hydroxychloroquine treatment in COVID-19 patients may result in increased lethality [34,35]. To this degree, it is essential that novel avenues of treating this

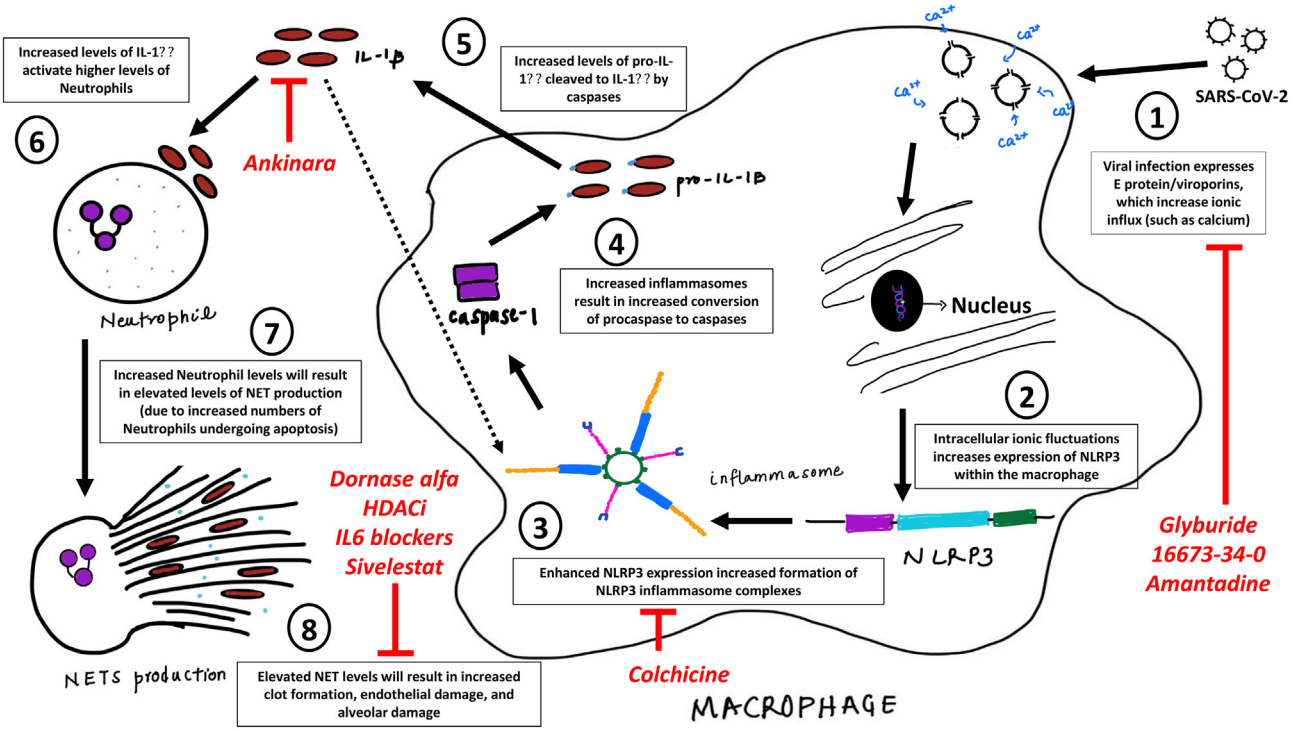

Fig. 1. Schematic visualisation of the proposed mechanism underlying increased Neutrophil Extracellular Trap (NET) production in response to SARS-CoV-2 infection, mediated via NLRP3 inflammasomes and elevated production of IL-1β, presented chronologically (steps 1–8). Viral infection expresses E-protein/viroporins, which increase ionic influx, enhancing NLRP3 expression and increased formation of NLRP3 inflammasome complexes within the macrophage. Elevated levels of inflammasomes cause an increase in cleavage of pro-IL-1β to IL-1β via elevated production of caspases. The increased levels of IL-1β will enter a 'feedforward loop' with the NLRP3 inflammasome (dotted line), and further activate higher levels of Neutrophils, resulting in elevated levels of NET production. Such highly increased NET levels will result in increased clot formation, endothelial damage, and alveolar damage associated with COVID-19. Potential drug candidates (red text) are displayed in appropriate areas where inhibition (red lines) of this proposed mechanism could take place. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

virus are investigated.

Declaration of Competing Interest

Ahmed Yaqinuddin and Junaid Kashir declare that there is no conflict of interest either financial or personal relationships with other people or organisations that could inappropriately influence (bias) our work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.109906.

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