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PERSPECTIVES | *The Pathophysiology of COVID-19 and SARS-CoV-2 Infection*

Consideration of Pannexin 1 channels in COVID-19 pathology and treatment

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INTRODUCTION

Pannexin 1 (PANX1) is a ubiquitously expressed, channel-forming protein found in a number of tissues throughout the body (e.g., lung, vasculature, liver, central nervous system, immune system) that is important in many key physiological and immune responses (18, 55). PANX1 channels passively flux ATP (predominantly), multiple metabolites, and likely other small anions (37, 39). PANX1 channels regulate inflammation and host responses to several pathogens, including viruses (36, 42, 53). While there is currently no evidence suggesting novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and PANX1 directly interact, there is an urgent need for therapeutic strategies, especially those targeting the hyperinflammation and cytokine storm that occurs in severe cases of COVID-19 (27, 41). Here we argue that PANX1, and drugs known to target PANX1 (including the FDA-approved drug probenecid), should be the focus of further investigation in the context of SARS-CoV-2 infection and its associated pathology in COVID-19 patients.

REGULATION OF INFLAMMATION BY PANX1 IN THE CONTEXT OF COVID-19

COVID-19 patients frequently present with hypoxemia and dyspnea requiring supportive oxygen therapy before reaching a more severe hyperinflammatory phase of the disease and acute respiratory distress syndrome (ARDS) (4, 11, 26). Control of the early phase of innate immunity, enabling a productive adaptive immune response, is critical to ensure patient recovery (56). It has been suggested that immunosuppression treatment for COVID-19 patients displaying hyperinflammation could limit disease progression as well as limit viral entry (38, 65). There are several lines of evidence demonstrating that PANX1 channel opening (and release of ATP) enhances inflammatory responses, including in the systemic endothelium (lung microvasculature), lung epithelium, olfactory epithelium, and the parenchyma of several tissues throughout the body (13, 17, 22, 29, 31, 35, 51). Multiple studies have shown that PANX1 signaling exacerbates inflammatory responses through: being activated and enhanced by TNFα-receptor signaling, being implicated in the inflammasome, involvement in leukocyte recruitment, and playing a role in the production and secretion of proinflammatory cytokines such as IL-1β and IL-6 by endothelial cells and other cell types (12, 22, 24, 35, 41, 51, 63). Given that disruption (both deletion and inhibition) of PANX1 in endothelial cells significantly reduces inflammation in several injury models (22, 28, 51, 59), PANX1 represents a potential target in reducing inflammatory burden and the damaging effects of the cytokine storm in COVID-19 patients. With intense vascular inflammation observed in severe cases of SARS-CoV-2 infection (14, 66), effective treatments to dampen hyperinflammation represent an urgent treatment need.

Of particular interest to SARS-CoV-2 infection, endothelial PANX1 has been shown to play a key role in regulating lung vascular inflammation and edema in response to ischemia/reperfusion injury (51). Control of pulmonary edema is crucial in limiting the severity of ARDS (58). In this light, TRPV4 channels in the alveolo-capillary unit were recently proposed to be a pharmacologically tractable target for treatment of ARDS associated with COVID-19 (30). In addition to direct control of lung barrier function by TRPV4 inhibitors, there is evidence that TRPV4 channels could induce PANX1 channel activity (45, 49, 50), suggesting that PANX1 inhibitors might improve the efficacy of TRPV4 channel inhibitors. Defining the molecular basis for coordinated regulation of TRPV4 and PANX1

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channels should facilitate the design of therapeutic approaches attenuating this signaling axis in treatment of COVID-19.

In addition to its general role in inflammatory signaling, PANX1 (either directly or pharmacologically) has been implicated in host responses to viral infection and regulation of virus life cycle [e.g., human immunodeficiency virus (HIV), hepatitis B, influenza, vesicular stomatitis virus (VSV), etc.]. PANX1 is expressed in key cells and tissues targeted by SARS-CoV-2, including airway epithelium (46), lung endothelium (34, 51), and neurons (7) (as well as many cell types and tissues throughout the body). In the case of HIV (36, 42, 53), ATP release via PANX1 channels following viral binding stimulates purinergic signaling pathways that enhance viral binding, uptake, and replication. Extracellular ATP has also been linked to viral infection and sequelae: it triggers HIV-1 release from cells (23), is released from cells following VSV infection (64), and is linked to ARDS associated with adenoviral infection (32). How extracellular ATP and purinergic receptors generally regulate entry of viruses has not yet been elucidated, and no links have yet been made between extracellular ATP and coronaviruses. Coronavirus membrane fusion can occur at the plasma membrane or at endosomes (25, 54, 62). Coronaviruses have been shown to enter cells via macropinocytosis, a clathrin- and caveolin-independent process (20, 57). Notably, elevated extracellular ATP can also trigger interactions between PANX1 and P2X7 receptors and their internalization to endosomes through a clathrin- and caveolin-independent process reminiscent of micropinocytosis (6, 8). If direct links between PANX1 and the SARS-CoV-2 life cycle are identified, targeting PANX1 could help mitigate the significant viral titers observed with COVID-19 that result in endothelial damage and neuronal tissue accumulation (33, 44, 68).

The above snapshot of PANX1 regulation of inflammatory cascades and viral pathologies supports the need for further study to explore potential direct links to COVID-19. From a therapeutics standpoint for COVID-19, PANX1 also has some intriguing preexisting ties and potential. For instance, nucleotide antiviral drugs, of which remdesivir has shown some effectiveness, may exhibit anti-PANX1 activity. Moreover, probenecid is an FDA-approved drug that blocks PANX1, often recapitulating the effects of *PANX1* deletion. Given the evidence described below, we argue PANX1 blockers should be considered in COVID-19 preclinical drug repurposing studies.

PANX1 AND NUCLEOTIDE ANALOG ANTIVIRALS

Nucleotide analog antivirals are designed to compete for incorporation into newly synthesized viral nucleic acid chains thereby disrupting virus life cycles. In preliminary analyses of ongoing clinical trials (1, 10), remdesivir, first described in the treatment of Ebola, has shown modest effects in reducing time to recovery (2). With respect to PANX1, tenofovir (not under consideration for COVID-19 but a nucleotide analog antiviral), used in the treatment of hepatitis B and HIV, inhibited PANX1-mediated ATP release in a mouse macrophage cell line (RAW264.7 cells) and a human liver cell line (HepG2 cells) (19). It is possible the inhibitory action of tenofovir on PANX1 could be via an intracellular mechanism, due to the fact that the drug is metabolized into a nucleotide analog inside the cell. However, high concentrations of extracellular ATP inhibit PANX1 and lead to PANX1 internalization (8), raising another possibility: that the active form of the drug might somehow be released into the extracellular space and block the channel from an external site, like ATP. In light of these prior findings, it could be valuable to determine whether, like tenofovir, remdesivir also impacts both PANX1 channel activity and possibly PANX1-associated inflammatory signaling in COVID-19. This work would be facilitated by the recent advances in our understanding of the PANX1 structure, including identification of key extracellular regulatory residues (16, 39). Remdesivir's effects on PANX1 channels are not known but would merit investigation in light of the blocking effect of the related drug, tenofovir, and the established role of PANX1 in inflammation and regulation of virus life cycles.

REPURPOSED FDA-APPROVED DRUGS THAT BLOCK PANX1 FOR COVID-19 TREATMENT?

Probenecid (commercially known as Probalan, Benemid, or Benuryl) is an FDA-approved treatment for gout that is also a well-established PANX1 inhibitor (61). Influenza A viral infection and lung viral load were attenuated following probenecid treatment both in vitro and in vivo (43). Probenecid also decreased inflammasome-dependent IL-1β secretion from macrophages in vitro (15), reduced the inflammatory response in sepsis (55), and suppressed hyperinflammation resulting from severe influenza A infection in mice (48). Additionally, probenecid treatment lowered the required dose of another antiviral medication, oseltamivir (43), likely due to probenecid's ability to increase plasma levels of the antiviral drug (47). Note that probenecid also inhibits P2X7 receptors (3), which is also likely to contribute to the antiviral and anti-inflammatory activity. Probenecid has also been shown to have a protective effect in ischemia/reperfusion injury by inhibiting secretion of the lysosomal cathepsin proteases (60). This suggests a mechanism of protection from SARS-CoV-2 infection, since cathepsins promote coronavirus infection through proteolytic cleavage of the spike protein (67). Thus, considering probenecid is

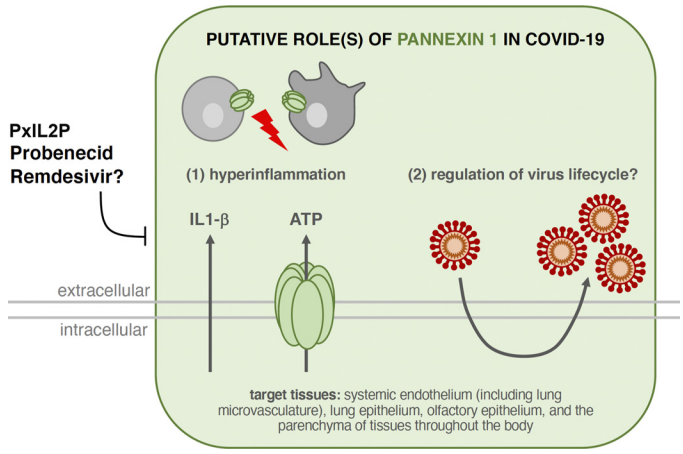

Fig. 1. Possible roles for Pannexin 1 (PANX1) in COVID-19 pathology. Pannexin intracellular loop 2 peptide (PxIL2P) could potentially be commercialized for specific PANX1 inhibition of inflammatory cues (e.g., IL-1β). Probenecid, an FDA-approved drug used in the treatment of gout, could help dampen the hyperinflammation observed in COVID-19 and could also have an impact on the life cycle of the virus. In addition to affecting the life cycle of the virus, remdesivir could potentially impact inflammation through blocking PANX1.

relatively well tolerated, has demonstrated action on viral infection-associated inflammation, and decreases required doses of other drugs, it could be of interest to investigate its potential use for COVID-19.

Another PANX1 channel-inhibiting FDA-approved drug with potential for the treatment of COVID-19 is spironolactone, an aldosterone antagonist used initially as a diuretic for the treatment of high blood pressure (21). In addition to its potential benefit as a PANX1 blocker in the context of SARS-CoV-2 infection, it was recently suggested that spironolactone may be useful in COVID-19-associated ARDS patients with hypertension (9). It was postulated that spironolactone might selectively increase plasma levels of the spike protein receptor ACE2 (27, 52), increasing the proportion of circulating to lung-endothelial cell-membrane-associated ACE2 levels (thereby minimizing lung infection), as a safer mechanism of action than ACE inhibitors that target cell-bound ACE2 (9). Further investigation is needed to determine whether this is the case and to determine whether there are benefits of the PANX1-inhibiting action of spironolactone in the context of COVID-19.

A POTENTIAL FOR THE DIRECT TARGETING OF PANX1 CHANNELS

Finally, if PANX1 is found to be involved in the primary regulation of SARS-CoV-2 infectivity and inflammatory responses, it may also be worth considering direct targeting of the channel functions. Currently there are no FDA-approved PANX1-specific blockers, although a PANX1-specific inhibitor peptide, pannexin intracellular loop 2 peptide (PxIL2P), has shown promise in reducing inflammatory responses in vitro and in vivo (35, 63). PxIL2P contains a short mimetic sequence for the IL2 region of PANX1 attached to an HIV-TAT transactivation protein (5) that binds to the second intracellular loop of PANX1 and blocks channel release of ATP, altering intracellular Ca^{2+} flux (35, 63). Using PxIL2P in cultured endothelial cells blocks PANX1-regulated expression and release of cytokines including IL-1β and CxCL10 and limits monocyte adhesion in the vasculature (35, 63). Thus direct targeting of the PANX1 channel may have functionality in reducing SARS-CoV-2 infectivity and vascular inflammatory responses in COVID19 patients.

CONCLUSION

Although there are currently no direct lines of evidence linking PANX1 to COVID-19, the central role of PANX1 in regulating inflammation, and more generally viral infection, provides a rationale supporting preclinical investigation of PANX1 and repurposing of approved PANX1-targeting drugs like probenecid as potential treatments. A summary of our perspective is illustrated in Fig. 1. We postulate that evaluating current COVID-19 treatment protocols for their effects on PANX1 may lead to improved combination therapeutic approaches by including specific PANX1 inhibitors as part of a treatment regimen.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.A.S. and B.E.I. conceived and designed research; L.A.S., S.R.J., C.S.N., J.C.S.-A., M.E.G., S.P., A.W.L., A.G.W., V.E.L., M.K., and B.E.I. analyzed data; L.A.S. and B.E.I. interpreted results of experiments; L.A.S. and B.E.I. drafted manuscript; L.A.S., S.R.J., C.S.N., J.C.S.-A., M.E.G., S.P., A.W.L., A.G.W., V.E.L., M.K., and B.E.I. edited and revised manuscript; L.A.S., S.R.J., C.S.N., J.C.S.-A., M.E.G., S.P., A.W.L., A.G.W., V.E.L., M.K., and B.E.I. approved final version of manuscript.

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