



# The innate immune response, microenvironment proteinases, and the COVID-19 pandemic: pathophysiologic mechanisms and emerging therapeutic targets

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The coronavirus disease 2019 (COVID-19) pandemic, causing considerable mortality and morbidity worldwide, has fully engaged the biomedical community in attempts to elucidate the pathophysiology of COVID-19 and develop robust therapeutic strategies. To this end, the predominant research focus has been on the adaptive immune response to COVID-19 infections stimulated by mRNA and protein vaccines and on the duration and persistence of immune protection. In contrast, the role of the innate immune response to the viral challenge has been underrepresented. This overview focuses on the innate immune response to COVID-19 infection, with an emphasis on the roles of extracellular proteases in the tissue microenvironment. Proteinase-mediated signaling caused by enzymes in the extracellular microenvironment occurs upstream of the increased production of inflammatory cytokines that mediate COVID-19 pathology. These enzymes include the coagulation cascade, kinin-generating plasma kallikrein, and the complement system, as well as angiotensin-generating proteinases of the renin-angiotensin system. Furthermore, in the context of several articles in this Supplement elucidating and detailing the trajectory of diverse profibrotic pathways, we extrapolate these insights to explore how fibrosis and profibrotic pathways participate importantly in the pathogenesis of COVID-19. We propose that the lessons garnered from understanding the roles of microenvironment proteinases in triggering the innate immune response to COVID-19 pathology will identify potential therapeutic targets and inform approaches to the clinical management of COVID-19. Furthermore, the information may also provide a template for understanding the determinants of COVID-19-induced tissue fibrosis that may follow resolution of acute infection (so-called “long COVID”), which represents a major new challenge to our healthcare systems.

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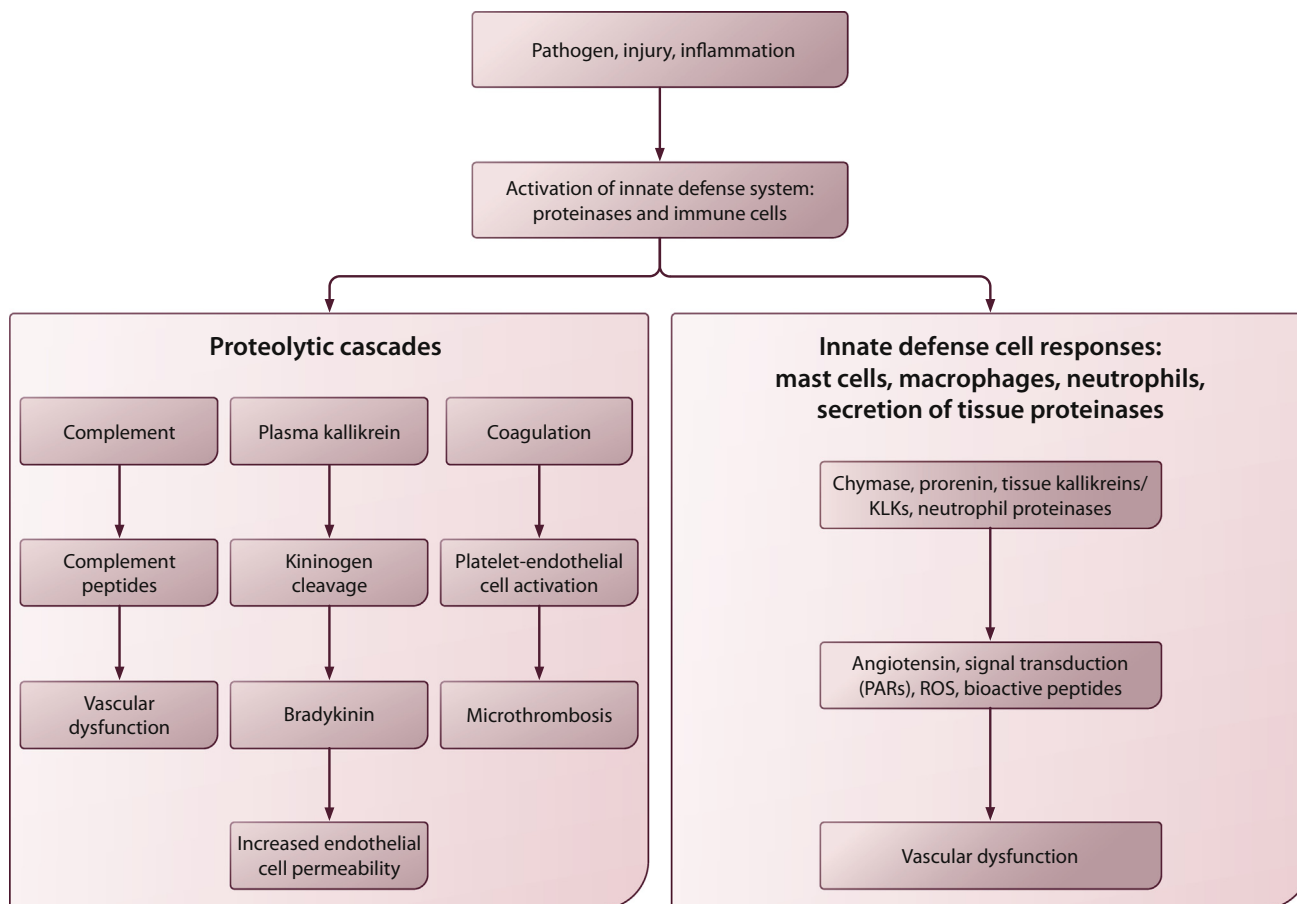
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The current coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is a member of the *Coronavirinae* virus subfamily, has exerted an unprecedented global impact. As of a January 13, 2022 update, the World Health Organization COVID-19 Dashboard had reported 312.173 million cases and 5.501 million deaths worldwide.<sup>1</sup> However, when considering “excess deaths” (the standard method of tracking changes in total mortality), 19.2 million (95% confidence interval 12.0–22.4 million) people worldwide are estimated to have died as a consequence of the COVID-19 pandemic.<sup>2</sup> The pandemic catalyzed an extraordinary effort to develop vaccines to induce specific adaptive immunity, based on virus-neutralizing antibodies and T-cell responses. Since their rollout in late 2020, vaccines have been highly effective in preventing clinically significant COVID-19 morbidity/mortality. Notwithstanding this effect, mutations that have given rise to new variant strains with increased infection transmission rates have emerged to trigger breakthrough infections, even in fully vaccinated individuals.<sup>3,4</sup> This development has markedly altered the landscape for the management of COVID-19, prompting both renewed “lockdowns” that disrupt local and global economies and the revision of estimates of the level of herd immunity necessary to quell disease.<sup>5–7</sup> Pressure has also increased on healthcare systems, because of both the reduced efficacy of currently approved vaccines to stop virus transmission, and the chronic disabling symptoms and residual fibrosis in the lungs, kidneys, and cardiovascular system that affect some individuals who have recovered from acute COVID-19 infection—so-called “long COVID.”<sup>6,8</sup> Consequently, a prudent and, indeed, essential course is to adopt a multipronged strategy that does not place sole reliance on vaccines but instead implements

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**Figure 1 | Overview of the innate defense response.** Schematic representation of potential mechanisms and effectors whereby pathogens, injury, and inflammation promote activation of the innate defense process. The process encompasses 3 proteolytic cascades (*left*: complement, plasma kallikrein, and coagulation) and (*right*) innate defense cell responses. KLK, kallikrein-related peptidase tissue; PAR, protease-activated receptor; ROS, reactive oxygen species.

multiple complementary approaches to quell the impact of disease.

### COVID-19 and the innate immune response: a platform for complementing the adaptive immune response

To date, the majority of attention surrounding COVID-19 therapy has been focused on both the inflammatory cell “adaptive” immune response, which results in the generation of neutralizing antibodies, and the duration of immune protection, as outlined in recent reviews.<sup>9–11</sup> This focus has led to the generation of highly successful vaccines. What has been underappreciated, however, is the role of the “innate” immune response in driving the pathophysiology of COVID-19, as outlined in the following paragraphs.

In contrast to the adaptive immune response, the innate immune response must be poised to respond immediately to tissue injury or invading pathogens such as COVID-19.<sup>10</sup> The innate immune response that is mounted to counter an invading pathogen subtends many pathways, including the production of interferon caused by internalized virus and the central contribution of neutrophils, macrophages, and mast cells.<sup>12–14</sup> Although pathogen-associated and damage-

associated molecular patterns (which activate pattern-recognition receptors, such as membrane-bound Toll-like receptors) typically are thought to play a key role in innate immunity, their involvement in the rapid innate response to COVID-19 infection is not yet fully understood.<sup>14</sup> We suggest that extracellular proteinases (the terms “proteinase” and “protease” are used interchangeably throughout this text) in the tissue environment (such as complement, plasma kallikrein, and the coagulation cascade) and cell-secreted proteinases (including renin–angiotensin system [RAS] proteinases) are key drivers and amplifiers of the rapid innate immune response to COVID-19, and set the stage for the subsequent adaptive immune response. The interactions of these proteinase-mediated cascades and innate immune defense cells (e.g., platelets, neutrophils, macrophages, and mast cells) in the rapid innate immune response, which have not been generally appreciated, even in recent overviews,<sup>14</sup> are illustrated in [Figure 1](#).

We hypothesize that, together, extracellular proteinases, cell-secreted proteinases, and the innate immune defense cells amplify the inflammatory processes implicated in the acute and chronic responses to COVID-19 infection. This involves

the proteolytic activation of proteinase-activated receptor-1 (PAR1) and PAR2,<sup>15,16</sup> via mechanisms including thrombin-mediated PAR1 activation secondary to macrophage-derived tissue factor-mediated activation of the coagulation cascade<sup>12,17</sup> and mast cell-derived chymase-mediated and tryptase-mediated PAR activation.<sup>13,17</sup> Further amplification may involve chymase-mediated generation of angiotensin from its precursors.<sup>18</sup> Activation of PARs leads to the production of proinflammatory mediators, including cytokines and neurokinins,<sup>16,19</sup> which in turn attract additional immune cells, including monocytes and macrophages, to the site of infection.<sup>9</sup> These innate immune cells enter the tissue rapidly, and establish a proinflammatory feedback loop, which may lead to elimination of the virus via innate immune mechanisms that remain to be elucidated.<sup>10</sup>

A compelling need exists for development and implementation of new therapeutic approaches, in addition to vaccines, to counter the long-term sequelae of COVID-19,<sup>8,20</sup> as well as to improve clinical management of acute COVID-19 in patients with impaired immunity, such as the elderly, in whom immune senescence (aging-associated alterations in the immune system) may predispose them to COVID-19 infection and more-severe disease.<sup>21</sup> We propose that the lessons garnered from a greater understanding of the roles of microenvironment proteinases in triggering the innate immune response in the setting of COVID-19 pathology should identify potential therapeutic targets and inform new approaches to the clinical management of both acute and chronic COVID-19, in addition to other viral and bacterial infections that cause acute respiratory distress syndrome (ARDS).

In order to highlight such complementary approaches to therapy, our article critically reviews the determinants of the innate immune response and its relevance to COVID-19, with an emphasis on proteolytic enzyme cascades underlying the immediate response to invading pathogens. Building on this platform, we review aspects of the innate immune responses that may enable novel therapeutic opportunities.

### COVID-19 and its pathogenesis

**COVID-19 infection: the roles of TMPRSS2 and ACE2.** Before exploring the innate immune response in detail, an understanding of the overarching pathophysiologic process of COVID-19 infection is important. The infection is triggered when the SARS-CoV-2 spike(S)-protein is proteolytically cleaved by a membrane-tethered serine protease (transmembrane serine protease 2 [TMPRSS2]), to reveal its sequence that can bind to angiotensin-converting enzyme-2 (ACE2).<sup>22,23</sup> ACE2, a membrane-bound peptidase highly expressed in the heart, respiratory tract, kidney, and gastrointestinal tract, then enables viral entry to infect the cell. A similar pathogenesis has been identified for the predecessor to SARS-CoV-2, SARS-CoV.<sup>24</sup> ACE2 overexpression in cells that are otherwise resistant to the virus facilitates viral entry and replication.<sup>25</sup> Additionally, the serine proteinase catalytic moiety of TMPRSS2 can be released into the extracellular milieu to stimulate cell signaling.<sup>26</sup> Parallel to cellular

internalization, the virus triggers the innate defense system, including activation of several proteolytic cascades and responses of innate-system immune cells, which are illustrated in Figure 1 and discussed in detail below.

**COVID-19 pathogenesis is mediated by inflammatory pathways, reactive oxygen species, and proteolytic enzymes.** In addition to direct viral damage, uncontrolled inflammation enhances disease severity in COVID-19.<sup>27</sup> Elevated levels of inflammatory cytokines and chemokines have been documented extensively in patients with severe COVID-19.<sup>28–31</sup> However, cytokine and chemokine levels do not reach those associated with a “cytokine storm,”<sup>32</sup> nor are they as high as those seen in critically ill patients with ARDS or sepsis.<sup>32,33</sup> Instead, the elevation in plasma inflammatory cytokines seen in patients with severe COVID-19 is comparable to that observed in patients with cancer treated with chimeric antigen receptor T-cells.<sup>29</sup>

In addition to cytokine production, another key driver of the inflammatory response to pathogens or injury, as alluded to by Tay *et al.*,<sup>9</sup> is a dysfunctional innate immune response that generates reactive oxygen species (ROS) and leads to the activation of microenvironment proteolytic enzymes, either individually or as amplified cascades, including the coagulation and complement systems. This rapid response adds to the immediate signaling triggered by internalized viruses that stimulate signaling cascades inducing type I and III interferons and proinflammatory cytokines, as summarized by Kolodny *et al.*<sup>34</sup> In turn, the interferons produce an antiviral state in the infected cells and neighboring tissues.

**Key proteolytic cascades in COVID-19 pathophysiology: the importance of the RAS.** To understand the biology of the COVID-19 pandemic more fully, an important factor to consider is the participation of the RAS, involving the proteolytic generation of peptide agonists in addition to other extracellular proteolytic processes (Figure 1).<sup>35</sup> Angiotensin signaling not only affects vascular tension, but also triggers fibrosis-enabling genes that impair the cardiovascular system.<sup>36</sup>

The complex relationship between SARS-CoV-2-induced alterations to RAS pathway components and viral pathogenicity is not fully delineated, and many questions remain unanswered. As highlighted in our published editorial,<sup>37</sup> early speculative reports during the COVID-19 pandemic focused on ACE2 and proposed that the continued use of medications that block the RAS, including ACE inhibitors and angiotensin-receptor blockers (ARBs), may influence both the severity and mortality of COVID-19.<sup>38</sup> Mechanistic evidence suggests the following 2 competing hypotheses<sup>39–44</sup>: (i) RAS blockade decreases the proinflammatory activity of angiotensin II, thereby decreasing the risk of ARDS, myocarditis, or mortality in COVID-19, and (ii) RAS blockade increases ACE2 expression, thereby enabling viral entry to infect the cell, promoting SARS-CoV-2 virulence in the vascular system, lungs, and heart that progresses to ARDS, myocarditis, and death.

Many recent studies have provided compelling evidence that RAS blockers do not enhance susceptibility to COVID-19, or adversely affect the clinical course of COVID-19–

infected patients.<sup>39–43</sup> Although ACE inhibitors do not directly modulate ACE2, they indirectly lead to increased ACE2 expression.<sup>37</sup> As mineralocorticoid receptor antagonists (aldosterone antagonists) and neprilysin inhibitors block the ability of matrix metalloproteinase (MMP) to degrade extracellular peptide agonists, which are also closely related to RAS activation, interest is increasing in whether these drugs have any impact on ACE2.

A key outcome of activation of the RAS is the upregulation of aldosterone production. Aldosterone, as well as glucocorticoids, can activate mineralocorticoid receptors. A key downstream effect of aldosterone on COVID-19 is its impact on catalyzing an array of profibrotic mechanisms. One illustrative example of aldosterone's effect on fibrosis is via an aldosterone-upregulated gene, neutrophil gelatinase-associated lipocalin (lipocalin-2/NGAL). This gelatinase/MMP-lipocalin-associated protein stimulates inflammatory and profibrotic markers in human cardiac fibroblasts and, therefore, may play a role in the longer-term fibrotic impact of COVID-19.<sup>45,46</sup> Significantly, transcription driven by the mineralocorticoid receptor can be amplified by a Rac1 signaling pathway.<sup>47</sup> Rac1 is also activated by G-protein-coupled receptors. These include PAR1 and PAR2, both of which are activated by the coagulation pathway. This signaling can, in principle, amplify the impact of the renin-angiotensin-aldosterone system on NGAL-mediated fibrosis in the absence of concomitant changes in ambient aldosterone levels. Of note, aldosterone also promotes oxidative injury through increasing the expression of the NADPH oxidase subunits Nox2, p47(phox), and p22(phox) and by stimulating Rac1 activation.<sup>48,49</sup> The aldosterone-mediated increase in ROS could, in principle, stimulate myofibroblast fibrosis pathways and would also in turn impair endothelial function through a reduction in endothelial nitric oxide synthase phosphorylation/activation.<sup>50–54</sup>

Whether transient use of a Rac1-targeted inhibitor might be of use therapeutically remains an open question. A recently discovered inhibitor of the action of NGAL, GPZ614741, has been demonstrated to block its proinflammatory and profibrotic effects in murine models.<sup>55</sup> This inhibitor, in concert with a mineralocorticoid receptor antagonist, might work to diminish and reduce the fibrotic response, both acutely and in the chronic phase of COVID-19 infection, as discussed in the following section.

**The interplay of the RAS with other proteolytic enzyme systems.** In addition to the interplay of SARS-CoV-2 and the RAS cascade, related enzyme pathways participate in the pathogenesis of COVID-19. These enzymes<sup>56</sup> include tissue kallikreins (e.g., prostate-specific antigen, also known as kallikrein-related peptidase-3 [KLK-3]) and the plasma kallikrein-kinin system,<sup>57</sup> as well as coagulation pathway proteinases (e.g., thrombin and plasmin) and enzymes of the complement system. These enzymes play a key role in pulmonary and cardiovascular microvascular thromboses that exacerbate COVID-19 pathophysiology.<sup>58–60</sup> The plasma kallikrein-bradykinin system in particular has become a

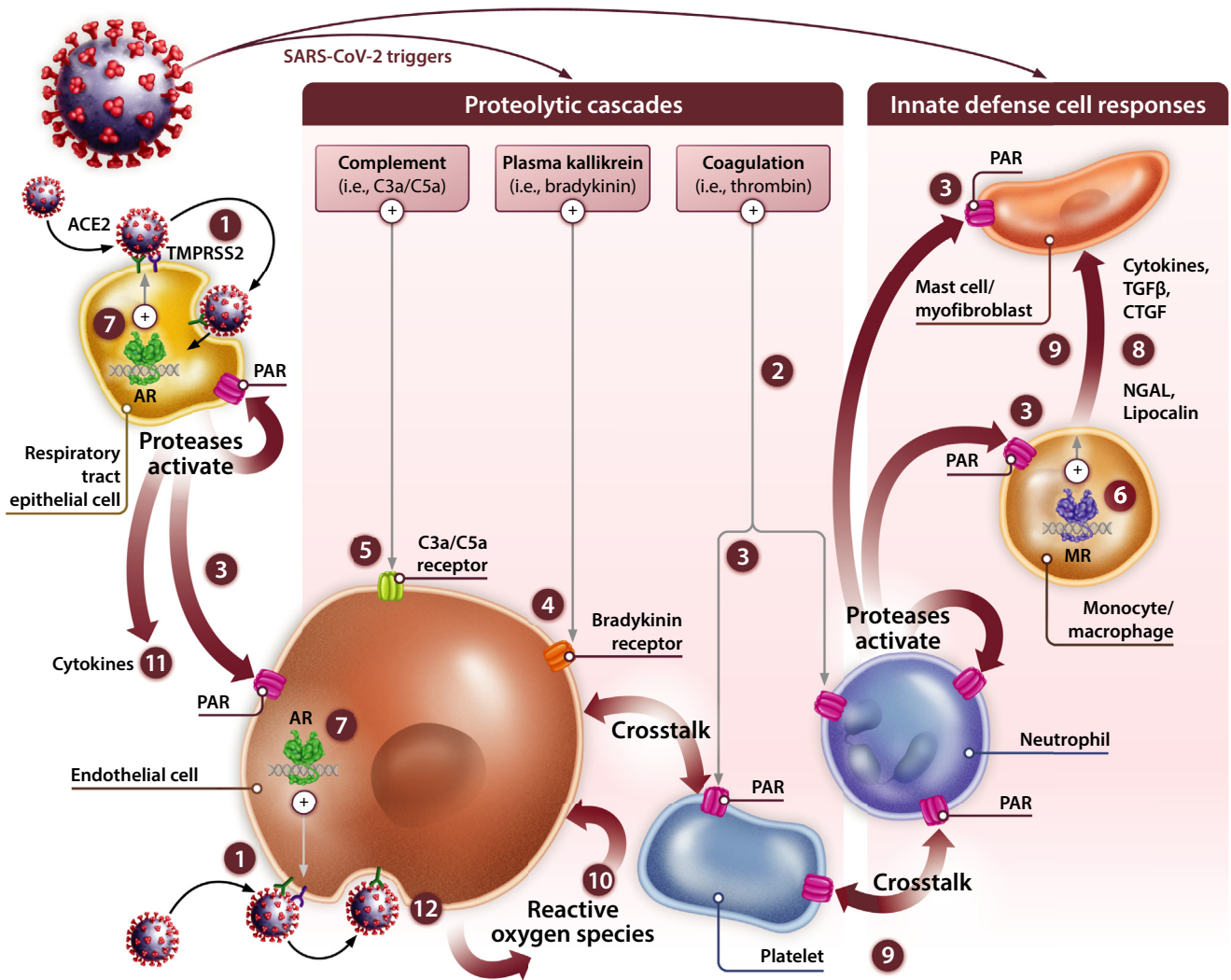
major clinical and investigative focus, constituting a platform for elucidating rational clinical management of patients with COVID-19 and providing newer treatment paradigms that may interdict COVID-19 progression.<sup>61</sup>

**The plasma kallikrein system, kinins, and chymase microenvironment proteinases.** As described elsewhere in this Supplement in the article by Ferrario *et al.*, the plasma kallikrein-kinin system is closely linked to the RAS cascade.<sup>18</sup> Regrettably, the possibility that the kallikrein-kinin system and chymase may play a role in mediating the more severe symptoms of COVID-19 has thus far received scant investigative attention.<sup>62</sup> Of note, chymase, potentially released from resident mast cells, not only generates angiotensin II, but also potentially can regulate vascular vasodilator function, renal function, and endothelial permeability by activating endothelial PAR2.<sup>63</sup> This action of chymase may exacerbate the vascular dysfunction observed in COVID-19 in addition to the vascular actions of bradykinin (BK; Figure 1, right-hand panel).

Many COVID-19 hallmark features mimic the known actions of the BK system, including promotion of proinflammatory and proliferative actions, coupled with enhanced leakage of blood vessels, edema formation, and general fatigue.<sup>61,64</sup> Therefore, it is tempting to postulate that the interplay of BK/BK B-1 receptor/BK B-2 receptor and RAS are substantively involved in COVID-19 pathogenesis. In this context, it is conceivable that the ACE2 depletion that occurs upon SARS-CoV-2 infection enhances [des-Arg9]-BK levels, promoting inflammation and coagulopathy via the BK B-1 receptor.<sup>57,65,66</sup>

Recently, Garvin *et al.* proposed that the kallikrein-kinin system may accelerate cytokine elevation in severe COVID-19 cases,<sup>61</sup> based on comparison of gene expression data from cells in bronchoalveolar lavage fluid from patients with COVID-19 (that were used for viral sequencing) and from control subjects.<sup>61</sup> A critical imbalance in the RAS was observed, characterized by decreased ACE expression combined with increases in ACE2, renin, angiotensin, key RAS receptors, kininogen, both BK receptors, and the plasma kallikrein enzyme that generates BK receptor-activating kinins. This atypical pattern of the RAS is predicted to elevate BK levels in multiple tissues and systems and may promote vascular dilation, vascular permeability, and hypotension. Several investigators postulate that BK-driven outcomes may contribute to the vascular pathophysiology observed in COVID-19.<sup>61,66,67</sup> Triggering of the plasma kallikrein-kininogen and complement systems, both of which represent enzymatic cascades that generate inflammatory peptides affecting endothelial cell function, is tightly linked to activation of the coagulation cascade. Therefore, it is essential to explore also the roles of the coagulation system, endothelium, and microthrombi, and the complement system in promoting COVID-19 pathophysiology.

**The coagulation cascade and microvascular thrombosis.** The respiratory system epithelium is a key point of entry for COVID-19 infection. It does not, however, play the major role in the ensuing hypoxia. Rather, the



**Figure 2 | A summary of the expansive panoply of mediators and effectors promoting the pathophysiology of coronavirus disease 2019 (COVID-19).** The schematic designates sites of potential therapeutic intervention that are denoted by Arabic numerals, corresponding to those in Table 1. The figure summarizes the (upper left) COVID-19 infectious process that triggers (middle panel) the activation of proteolytic cascades and cellular responses that drive disease progression, as described in the text. (Upper left) Infection commences with transmembrane protease serine 2 (membrane-tethered serine proteinase that unmasks COVID-19 ACE2 binding site; TMPRSS2) enabling COVID-19 binding/internalization via angiotensin-converting enzyme 2 (ACE2) into respiratory tract epithelial cells. Concurrently, (right-hand panel) virus infection triggers innate defense proteolytic cascades in the microenvironment, including the complement, plasma kallikrein, and coagulation systems. COVID-19-infected respiratory tract epithelial cells generate both cell-regulating proteinases that cause inflammation via protease-activated receptor (PAR) activation on epithelial cells and other targets and via upregulated cytokines (e.g., interleukin [IL]6 and tumor necrosis factor [TNF]-alpha) that amplify the influx of innate defense inflammatory cells. Proteinase-mediated signaling is upstream of the increased production of inflammatory cytokines by the epithelial cells that mediate COVID-19 pathology. The proteinases include the angiotensin-generating enzymes of the, renin-angiotensin system, as well as enzymes involved in complement activation, plasma kallikrein-kinin generation, and the coagulation cascade. The interplay and intersection of these multiple cascades act in a complementary manner to promote acute inflammation and long-term fibrosis. An understanding of the roles of microenvironment proteinases in triggering the innate immune response to COVID-19 pathology will identify potential therapeutic targets, as indicated by the Arabic numbers, and inform approaches to the clinical management of other viral and bacterial infections. Each of the depicted trajectories is discussed in detail in the text. AR, androgen receptor; CTGF, connective tissue growth factor; MR, mineralocorticoid receptor; NGAL, neutrophil gelatinase-associated lipocalin; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TGFβ, transforming growth factor beta.

pulmonary microvascular compartment is compromised by microthrombi, thereby leading to the mismatch between lung perfusion and oxygenation.<sup>60</sup> Furthermore, microthrombi are also a major cause of cardiac injury in COVID-19.<sup>58,59,68,69</sup> Cardiovascular system damage, including stroke,

has emerged as an unusual presentation of COVID-19, prompting treatment with anticoagulants. These agents have had limited, albeit encouraging, success in treating a subset of patients with COVID-19 to reduce overall mortality.<sup>70-75</sup> However, anticoagulation alone is not sufficient to

minimize the impact of microthrombi. Essential to limiting microthrombus formation is a focus on the interactions between the endothelium and proteinases of the coagulation cascade, both of which are disrupted in the setting of COVID-19.

**Endothelial cell dysfunction: a target underlying acute COVID-19 pathophysiology.** The potential role of the endothelium as a key determinant of vascular dysfunction in COVID-19 has been elaborated upon by a number of publications.<sup>69,76,77</sup> Like the lung epithelium, microvascular endothelial cells (representing approximately one-third of lung cells)<sup>78</sup> are susceptible to COVID-19 viral infection, presumably because of their expression of ACE2.<sup>68</sup> As summarized in Figure 2,<sup>15,55,58,61,64,66,73,79–108</sup> dysregulation of many of the above-cited pathways and cascades converge on the vascular endothelium, acting as a common pathway to promote severe COVID-19 vasculopathy.<sup>109</sup> Infection and activation of the endothelium, as well as its response to increased oxidative stress attributable to COVID-19–elevated cytokines, can trigger the coagulation cascade by promoting platelet adherence/activation and stimulation of platelet thrombin receptors (PAR1 and PAR4), which can also be proteolytically activated at the site of platelet–endothelium interactions by proteinases other than thrombin.<sup>110</sup> Platelet neutrophil interactions also play an important role, with neutrophil–platelet interactions stimulating an increased secretion of proteinases such as neutrophil elastase and cathepsin-G into the microenvironment.<sup>111</sup> These neutrophil-secreted proteinases amplify the coagulation cascade and signal via the PARs in the tissue.<sup>111,112</sup> Thus, the coagulation cascade and fibrin formation, as well as the disruption of endothelial cell function, lead to microthrombi.

Because of the central role played by the endothelium, it is not surprising that endothelial dysfunction is a preexisting common denominator for COVID-19 patients who have poor clinical outcomes. For example, patients with type 2 diabetes, metabolic syndrome, cardiovascular disease, or kidney disease—all of which share endothelial dysfunction—are at enhanced risk of increased morbidity in the setting of COVID-19, as summarized in depth in recent reviews.<sup>68,69</sup> Consequently, therapeutic strategies that target the endothelium, the coagulation cascade, and the platelet–endothelium interaction process that triggers microvascular thrombosis are of central importance in defining the treatment strategy for managing such individuals.

In summary, the endothelium, which will be affected by the generation of kinins caused by the plasma kininogen process outlined earlier and by microenvironment proteolytic enzyme–PAR activation, will also be dysregulated by COVID-19 infection *per se* and by the concurrent interaction between platelets and coagulation cascade enzymes. Here again, microenvironment proteinases generated by the innate immune defense system can be seen to play critical roles. The implication of this mechanism is that to prevent coagulopathy, simply blocking fibrinogen generation will not suffice, as for example with the use of heparin or direct thrombin

inhibitors such as dabigatran. Rather, what is needed is simultaneous blocking of the activation of the platelet and endothelial cell receptors, such as the PARs or other cell activators. In this regard, inflammation-stimulated ROS that can also promote platelet–endothelium–mediated thrombosis are important. Furthermore, upregulation of endothelial interleukin 1-beta caused by inflammatory cytokines and proteinase signaling can affect thrombosis, possibly by regulating endothelial inducible nitric oxide synthase. Consequently, agents that minimize both platelet–endothelium signalling and ROS may prove valuable in COVID-19 treatment.

**The complement system and COVID-19.** Increased complement activation has been documented to prevail in individuals with severe COVID-19, in association with both hypercoagulability and endothelial cell injury.<sup>91</sup> As in many disease states,<sup>113</sup> the complement system plays an important role in COVID-19. Of significance, there is interplay of all 3 proteolytic cascades depicted on the left in Figure 1, working simultaneously to promote the thromboinflammatory process. Thus, the coagulation pathway, acting in concert with the complement system and the plasma kallikrein system, can cleave and activate the complement C3 peptide.<sup>93,114</sup> The tissue kallikrein family is also able to activate complement.<sup>115</sup> This cleavage by the 3 proteolytic cascade members is amplified by the COVID-19 spike protein itself, which upon binding to cell-surface glycosaminoglycans, causes the activation of the alternative pathway of complement.<sup>93</sup> In summary, the participation of the complement system, in concert with the plasma kallikrein and coagulation cascade, converge to promote endothelial cell dysfunction, which may constitute the core pathophysiology of COVID-19. Consequently, inhibitors of the complement cascade and the receptors for complement C3 and C5 have potential value in the setting of COVID-19 treatment (see Therapeutics section, below).<sup>88,90,92</sup> A recent study documented the activation of the complement pathway in COVID-19 sepsis. Lam *et al.* demonstrated enhanced C3b and C4d deposition on erythrocytes in both COVID-19 and non-COVID sepsis patients, compared with healthy controls.<sup>116</sup> The data support the role of complement in sepsis-associated organ injury. Of interest, the data suggest that erythrocyte analysis may provide a tool for individualizing the approach to sepsis and may have diagnostic value in monitoring complement dysregulation in both non-COVID and COVID-19 sepsis. The analysis of complement-tagged erythrocytes in the setting of sepsis can thus identify complement activation, so as to single out patients who may benefit from complement-targeted therapies.<sup>116</sup>

**Pathogenetic mechanisms that link proteolysis signaling to engaging profibrotic pathways implicated in long COVID.** All of the articles in this Supplement<sup>18,46,53,54,117–121</sup> focus on 2 overriding mechanisms: inflammation and fibrosis. Indeed, several of the articles elucidate and detail the trajectory of diverse profibrotic pathways. Therefore, it is of great interest that fibrosis and profibrotic pathways participate in important ways in the pathogenesis of COVID-19. A clear finding is that,

following recovery from the acute phase of COVID-19, many individuals develop long COVID, a chronic phase involving both peripheral and central nervous system sequelae, and in which tissue fibrosis plays a substantive role.<sup>8</sup>

The National Institutes of Health refers to long-term COVID-19 symptoms as “post-acute COVID-19 syndrome,” or PACS for short.<sup>8,122,123</sup> For as long as 4 months after discharge, individuals with either moderate or severe COVID-19 have been observed to have persistent symptoms and radiologic evidence of lung dysfunction, consistent with fibrosis.<sup>124</sup> The overall duration of these abnormalities has not yet been established.<sup>125,126</sup> A recent cross-sectional observational study of 156 patients attending a major academic PACS clinic in New York, NY, reported that persistent symptoms associated with PACS appear to impact physical and cognitive function and health-related quality of life. Reported persistent symptoms included fatigue ( $n = 128$ ; 82%), brain fog ( $n = 105$ ; 67%), and headache ( $n = 94$ ; 60%).<sup>127</sup> These alarming findings emphasize the need to clarify further the relationship between COVID-19 infection and PACS symptoms, to identify the underlying mechanisms, and to investigate the treatment options, which we detail later in this review. We suggest that both in the lung and elsewhere in the periphery, including the heart and kidney, persistent and progressive fibrosis can play a pathophysiologic role with a long-term residual impact. This possibility is currently being investigated in convalescent individuals with COVID-19.<sup>128,129</sup> This progressive pathology is precisely what may be amenable to therapeutic intervention if treatment is started early enough following discharge from the intensive care unit.

Three main mechanisms can be posited to be driving progressive fibrosis,<sup>130</sup> summarized as follows: (i) activation of PAR1, 2, and 4 by the coagulation pathway<sup>15,86,131,132</sup>; (ii) gene induction by the RAS, which promotes the mineralocorticoid-mediated upregulation of NGAL<sup>94,133</sup>; and (iii) triggering of the transcription of a set of Rho/myocardin-related transcription factor/serum response factor-mediated fibrosis-related genes.<sup>100</sup>

The activation of PARs as playing a key fibrotic role has been well summarized by Chambers and colleagues.<sup>85–87</sup> PAR activation that upregulates fibrosis pathways can be caused by either coagulation pathway enzymes (thrombin, activated protein C) or inflammatory cell-derived enzymes.<sup>15</sup>

Gene induction by the RAS promotes mineralocorticoid receptor activation, transducing an array of profibrotic mediators, as detailed by Luther and Fogo<sup>54</sup> and Bauersachs and Lother.<sup>46</sup> These effects include stimulating plasminogen activator inhibitor-1 expression in multiple tissues, including the heart, kidney, and aorta. Increased plasminogen activator inhibitor-1 expression contributes to progressive kidney injury by inhibiting plasmin activation, which results in decreased MMP activation and increased extracellular matrix accumulation and fibrosis. Transforming growth factor- $\beta$ 1 promotes fibrosis by stimulating cell transformation of fibroblasts and epithelial to mesenchymal cell differentiation. Another pathway encompasses upregulation of NGAL, the

MMP-9/lipocalin-2 complex that mediates the profibrotic effects of aldosterone in the vasculature.<sup>134,135</sup>

As already mentioned, signal pathways involving Rac1 can enhance mineralocorticoid receptor-mediated gene induction in diverse targets, including immune cells.<sup>47,136</sup> The immune cells in the inflammatory environment are thought to play an important role in the profibrotic action of NGAL.<sup>137</sup> This Rac1-signaling mechanism can be amplified considerably, for not only the mineralocorticoid receptor but also a variety of G-protein-coupled receptors such as PAR1, known to affect Rac1 signaling and to regulate cellular microtubule function. This activation of PAR1 can be stimulated by neutrophil-derived proteinases in the microenvironment.<sup>138</sup>

The role of the enzymatic MMP activity *per se* of the lipocalin-associated gelatinase/MMP, NGAL, is not clear. Thus, an MMP inhibitor may not block the profibrotic actions of NGAL. Consequently, blockade of the mineralocorticoid receptor and a Rac1-targeted inhibitor that would block the upregulation of NGAL can be considered as a potential treatment for attenuating the profibrotic process. A common denominator of the profibrotic mechanisms appears to be the generation of transforming growth factor- $\beta$  and the production of ROS.<sup>139</sup> Furthermore, these profibrotic mechanisms involve the actions of microenvironment proteinases,<sup>139</sup> as reviewed in depth in this article. Consequently, further elucidation of these potential mechanisms that can drive persistent fibrosis may inform potential therapeutic interventions, as discussed below.<sup>139</sup>

**The influence of patient sex on proteolytic enzyme systems and COVID-19 pathophysiology: androgen-dependent microenvironment proteinases.** Whereas it may not be readily evident at initial consideration, or indeed surprising, an individual's sex constitutes an important determinant of the contributions of proteinases and proteinase receptors in the context of COVID-19. Apart from the increased vulnerability to COVID-19 of individuals with preexisting conditions that affect the vasculature, an increased severity COVID-19 has been observed in male, as compared with female, patients.<sup>97,98,140</sup> What may not be widely appreciated is that males and females differ in their immunologic responses to foreign and self-antigens and manifest differences in innate and adaptive immune responses.<sup>141</sup> This difference may involve androgen-upregulated genes—these include kallikreins such as KLK3/PSA (a well-known prostate cancer marker),<sup>56</sup> as well as the “COVID-19-priming membrane-tethered proteinase,” TMPRSS2. We postulate that androgen-mediated upregulation of proteinases that are secreted into the tissue microenvironment can impact and modulate COVID-19 pathology. Likewise, Wambier and colleagues<sup>98</sup> have posited that androgen sensitivity, leading to an upregulation of TMPRSS2, may correlate directly with the severity of COVID-19 disease in male patients. Important to note is that TMPRSS2 can affect the inflammatory component of COVID-19 not only by priming the virus for ACE2-mediated cell infection but also by activating inflammatory cell signaling in its environment by cleaving and activating PAR2.<sup>26</sup> At present,

**Table 1 | Treatment paradigms focused on targeting the proteolytic signaling system, mineralocorticoid receptor, and bradykinin, together with newly emerging and repurposed agents for management of the complications of COVID-19 illness**

Agent/compound	Target	Therapeutic site of action (see number in Figure 2)	References
Proteinase inhibitors (camostat, nafamostat, and newly synthesized inhibitors)	<ul style="list-style-type: none"> <li>Inhibit TMPRSS2 and other inflammatory proteinases</li> <li>Block spike protein processing</li> </ul>	1	<ul style="list-style-type: none"> <li>Bittmann <i>et al.</i>, 2020<sup>79</sup></li> <li>Boras <i>et al.</i>, 2020<sup>80</sup></li> <li>El Amri, 2021<sup>81</sup></li> <li>Gunst <i>et al.</i>, 2021<sup>82</sup></li> <li>Mahoney <i>et al.</i>, 2021<sup>83</sup></li> <li>Shapira <i>et al.</i>, 2021<sup>84</sup></li> </ul>
Anticoagulants (low-molecular-weight heparin, direct inhibitors of factor Xa or thrombin)	<ul style="list-style-type: none"> <li>Prevent vascular microthrombosis</li> </ul>	2	<ul style="list-style-type: none"> <li>McFadyen <i>et al.</i>, 2020<sup>58</sup></li> <li>Tang <i>et al.</i>, 2020<sup>73</sup></li> </ul>
PAR inhibitors: PARs 1, 2, 4	<ul style="list-style-type: none"> <li>Block platelet activation</li> <li>Inhibit platelet aggregation–secretion</li> <li>Inhibit neutrophil activation</li> <li>Block endothelium dysfunction</li> <li>Attenuate profibrotic pathways</li> </ul>	3	<ul style="list-style-type: none"> <li>Chambers, 2008<sup>85</sup></li> <li>Jose <i>et al.</i>, 2014<sup>86</sup></li> <li>Mercer and Chambers, 2013<sup>87</sup></li> <li>Ramachandran <i>et al.</i>, 2012<sup>15</sup></li> </ul>
Plasma kallikrein inhibitors and bradykinin receptor antagonists	<ul style="list-style-type: none"> <li>Reduce kinin-mediated endothelial cell permeability</li> </ul>	4	<ul style="list-style-type: none"> <li>Garvin <i>et al.</i>, 2020<sup>61</sup></li> <li>Han <i>et al.</i>, 2002<sup>88</sup></li> <li>Kulkarni and Atkinson, 2020<sup>89</sup></li> <li>Marceau <i>et al.</i>, 2020<sup>64</sup></li> <li>McCarthy <i>et al.</i>, 2021<sup>66</sup></li> </ul>
Complement pathway antagonists	<ul style="list-style-type: none"> <li>Block C3a/C5a receptors to improve endothelial cell function</li> </ul>	5	<ul style="list-style-type: none"> <li>Kulkarni and Atkinson, 2020<sup>89</sup></li> <li>Li <i>et al.</i>, 2015<sup>90</sup></li> <li>Ma <i>et al.</i>, 2021<sup>91</sup></li> <li>Thomson <i>et al.</i>, 2020<sup>92</sup></li> <li>Yu <i>et al.</i>, 2020<sup>93</sup></li> </ul>
Mineralocorticoid receptor antagonists	<ul style="list-style-type: none"> <li>Inhibit NGAL/Lipocalin production</li> <li>Attenuate profibrotic pathways</li> </ul>	6	<ul style="list-style-type: none"> <li>Bonnard <i>et al.</i>, 2021<sup>55</sup></li> <li>Tarjus <i>et al.</i>, 2015<sup>94</sup></li> <li>Wilcox and Pitt, 2021<sup>95</sup></li> </ul>
Androgen receptor antagonists	<ul style="list-style-type: none"> <li>Block upregulation of spike protein-activating proteinase, TMPRSS2</li> </ul>	7	<ul style="list-style-type: none"> <li>Cadegiani <i>et al.</i>, 2021<sup>96</sup></li> <li>Goren <i>et al.</i>, 2021<sup>97</sup></li> <li>Wambier <i>et al.</i>, 2020<sup>98</sup></li> <li>Wambier and Goren, 2020<sup>99</sup></li> <li>Wilcox and Pitt, 2021<sup>95</sup></li> </ul>
Lipocalin inhibitors	<ul style="list-style-type: none"> <li>Block fibrosis pathways</li> </ul>	8	<ul style="list-style-type: none"> <li>Bonnard <i>et al.</i>, 2021<sup>55</sup></li> </ul>
Fibrosis inhibitors	<ul style="list-style-type: none"> <li>Block fibrosis transcription pathways</li> </ul>	9	<ul style="list-style-type: none"> <li>Kahl <i>et al.</i>, 2019<sup>100</sup></li> </ul>
Sulforaphane	<ul style="list-style-type: none"> <li>Upregulates antioxidant genes to reduce endothelial ROS</li> </ul>	10	<ul style="list-style-type: none"> <li>El-Daly <i>et al.</i>, 2018<sup>101</sup></li> <li>Pulakazhi Venu <i>et al.</i>, 2019<sup>102</sup></li> </ul>
Cytokine-targeted monoclonal antibodies	<ul style="list-style-type: none"> <li>Block interleukin-6 action</li> </ul>	11	<ul style="list-style-type: none"> <li>Jones and Hunter, 2021<sup>103</sup></li> <li>Malgie <i>et al.</i>, 2021<sup>104</sup></li> </ul>
Metformin	<ul style="list-style-type: none"> <li>Minimizes endothelial oxidative stress</li> <li>Improves endothelial cell function</li> <li>Reduces fibrosis</li> </ul>	12	<ul style="list-style-type: none"> <li>Mather <i>et al.</i>, 2001<sup>105</sup></li> <li>Park <i>et al.</i>, 2012<sup>106</sup></li> <li>Pulakazhi Venu <i>et al.</i>, 2021<sup>107</sup></li> <li>Rangarajan <i>et al.</i>, 2018<sup>108</sup></li> </ul>

COVID-19, coronavirus disease 2019; NGAL, neutrophil gelatinase-associated lipocalin; PAR, proteinase-activated receptor; ROS, reactive oxygen species; TMPRSS2, transmembrane serine protease 2. The table illustrates a wide array of possible treatment paradigms that may have utility in attenuating the multi-organ complications of COVID-19. Their potential sites of action are indicated numerically, as illustrated in Figure 2. The proposed therapeutic approaches/agents are not mutually exclusive, but they may be implemented in a complementary manner as appropriate.

the data are conflicting regarding the androgen sensitivity of lung TMPRSS2. One study reported that androgen antagonists do not affect TMPRSS2 expression and SARS-CoV-2 infectivity in cultured human lung cell lines, but another reported the opposite; differences in cell lines are hypothesized to account for the differing results.<sup>142,143</sup>

Of note, the PAR1 receptor that regulates platelet and endothelial cell function also has an upstream androgen promoter sequence.<sup>144</sup> Consequently, the potential impact of

androgens on TMPRSS2, kallikreins, and PAR1 may represent only the tip of the iceberg, supporting the hypothesis that androgens can regulate and affect pathology in the setting of COVID-19 by upregulating microenvironment proteinases,<sup>96,97</sup> and this action may contribute to sex-based immunologic differences that affect susceptibility to infectious diseases and responses to vaccines in males and females.<sup>141</sup> Consequently, the relative roles and interplay of several mechanisms that lead to sex disparity in the impact of COVID-19



constitute an investigative target of interest that remains to be elucidated.<sup>145</sup> Of note in this regard, spironolactone can act as an antagonist for not only the mineralocorticoid receptor but also the androgen receptor. Wilcox and Pitt have recently reviewed the evidence suggesting that spironolactone may be the preferred combined mineralocorticoid–glucocorticoid receptor inhibitor to increase protease nexin 1 and decrease TMPRSS2, furin, and plasmin activities, and thereby reduce viral cell binding, entry, and infectivity, and, ultimately, tamp down adverse outcomes.<sup>95</sup>

### Next steps—potential therapeutic implications

**Putative therapeutic targets.** Based on the pathophysiologic considerations discussed above, several therapeutic strategies to minimize the impact of COVID-19 infection may be potentially efficacious and should be investigated to reduce the effects of microenvironment proteinase signaling in several areas. These therapeutic possibilities are summarized in Table 1, depicted schematically in Figure 2, and enumerated below. These therapeutic areas related to mechanisms that can be targeted for interventions include the following:

- (i) processing of the S-protein to interact with ACE2;
- (ii) activation of the coagulation cascade to affect endothelial/platelet interactions;
- (iii) triggering of the kallikrein-kinin cascade that generates endothelium-acting kinins;
- (iv) activation of the complement cascade that generates peptides that affect endothelial function;
- (v) impact of RAS-generated vasoactive peptides along with the generation of aldosterone-mediated profibrotic agonists of vascular remodeling;
- (vi) the upregulation of COVID-19–enabling proteases by androgens;
- (vii) the COVID-19–induced generation of cytokines that elevate vascular ROS (as a consequence, ROS impair endothelial cell function); and
- (viii) the effect of protease-inflammation-stimulation of chronic profibrotic signaling pathways.

Over the long term, these pathways can lead to progressive tissue fibrosis and other long-term sequelae after recovery from the initial acute COVID-19 infection. These therapeutic interventions should be considered as being complementary to currently established treatment paradigms encompassing blocking viral infection by antibodies that inhibit the interaction of COVID-19 with its cellular ACE2 target of internalization and cellular infection. Additionally, agents already used to treat other conditions in individuals who then contract COVID-19, such as metformin for patients with pulmonary inflammation and fibrosis,<sup>106,108</sup> and statins, have been found to play a role in reducing disease severity.<sup>146,147</sup> Surprisingly, neutralizing a key cytokine upregulated early in COVID-19 infections, interleukin-6, has had only minimal, if any, impact on the outcome of the disease.<sup>103,104</sup> Thus, contemplating new approaches focused on new targets is clearly warranted. We explore these possibilities in the sections that follow.

(i). **Targeting proteinases that cleave the COVID-19 spike protein and serve to unmask its ACE2 binding sequence.** Because extracellular MMPs and serine proteinases represent a driving force for COVID-19 pathophysiology,<sup>148</sup> one can suggest that selective inhibition of the enzymes (e.g., thrombin) or blocking receptor signaling by the substrate-released receptor-activating peptides (such as the kinins) would be of therapeutic value. As discussed, blocking the PARs or the kinin receptors is already a consideration for treating COVID-19. However, in an inflammatory setting such as COVID-19, many microenvironment enzymes with common catalytic mechanisms (e.g., serine vs. cysteine vs. metalloproteinases) can be activated; and specifically targeting a single enzyme may miss the therapeutic mark because of catalytic redundancy in the system. This redundancy exists because several different enzymes of the same proteinase family can, in principle, cleave the same substrates to generate pathogenic signaling. Therefore, an alternative approach is to use broad-spectrum inhibitors, but in a localized and time-restricted way, to combat COVID-19 infection and inflammation. For instance, the clinically used serine protease inhibitors camostat and nafamostat are potent inhibitors of TMPRSS2.<sup>149</sup> Thus, these agents can very effectively block processing of the COVID-19 S-protein and prevent ACE2-mediated cellular infection. In this context, current efforts are focused on developing highly potent TMPRSS2 inhibitors, which in principle should be of considerable promise.<sup>79–84</sup> Further, MMP inhibitors, including newly developed non-antimicrobial tetracycline-related agents, are able to block tissue inflammation.<sup>150</sup> A possible outcome is that if these broad-spectrum protease inhibitors are used early on in COVID-19 infections, the burden of disease might be diminished. Of particular utility, the intranasal administration of such inhibitors can be suggested to have prophylactic potential, by preventing the initial viral infection in the upper airway that appears to be a principal route of COVID-19 infection. This same strategy may work for other viral pathogens that depend on nasopharyngeal infection to cause disease.

(ii). **The coagulation cascade, platelets, and thromboinflammation.** A common theme for many of the suggested areas for therapeutic intervention is the impact of proteolytic cascades, as outlined in Figure 1. An underlying process that leads to the thrombotic pathophysiology of COVID-19, as emphasized by McFadyen and colleagues<sup>58</sup> and outlined above, involves the coagulation cascade–mediated “thromboinflammation” that is well recognized in the context of sepsis. Thromboinflammation involves the complex interplay between the coagulation cascade and the pathophysiology of vascular inflammation, with platelets playing a central role. This interplay involves not only the action of thrombin but also the cellular signaling responses of platelets and neutrophils, and interaction with the endothelium.<sup>151,152</sup> As already discussed, a key feature of thromboinflammation is endothelial dysfunction leading to dysregulation of coagulation, complement, platelet activation, and leukocyte recruitment in the

microvasculature.<sup>76,77,152</sup> Upfront in this cascade is the thrombin-mediated activation of PAR1 and PAR4 on human platelets<sup>15</sup> and on the endothelium, along with the generation of thrombin-mediated proteolysis products from fibrin and osteopontin.<sup>151</sup> Consequently, the finding that anticoagulation with either low-molecular-weight heparin or direct oral anticoagulants exerts only a limited, albeit beneficial, effect in the COVID-19 setting was not anticipated.<sup>153</sup> This result should not be surprising, however, as several microenvironment proteinases, including trypsin, chymase, MMPs, and tissue kallikreins, can also activate PARs at the site(s) of vascular inflammation, and the complex platelet signal pathways stimulated by these enzymes via the PARs can greatly amplify the local inflammatory response involving the influx of neutrophils and their secretion of inflammation-stimulating PAR-regulating proteinases.<sup>17,138,154</sup>

Thus, in addition to the inhibition of the coagulation pathway by indirect (heparin) or direct thrombin or Factor Xa inhibitors (e.g., direct thrombin inhibitors such as dabigatran or direct Factor Xa inhibitors such as rivaroxaban), we suggest that a potentially useful consideration is the addition of inhibitors of PARs 1 and 4, as well as inhibitors of platelet function, possibly such as clopidogrel, that block the procoagulant action of platelet-released adenosine diphosphate triggered by thrombin action.

**(iii, iv). The plasma kallikrein and complement cascades.** As discussed, in addition to the coagulation cascade, the plasma kallikrein and complement systems also affect platelet and endothelium function by the generation of active peptides in the setting of COVID-19. Therapeutic intervention is also possible using agents that block BK production or signaling (e.g., danazol or icatibant).<sup>61,64</sup> Similarly, agents that inhibit the production or action of active peptides generated by the complement system (e.g., eculizumab, which inhibits complement C5 cleavage and a C5a inhibitor, vilobelimab/IFX-1) have been assessed for their impact on COVID-19 pathology, and found to have somewhat positive effects.<sup>89,155</sup>

**(v). RAS-targeted agents.** As discussed above, the receptor on host cells that promotes virus uptake, through attachment of its S-protein, as depicted in Figure 2 (upper left), is ACE2.<sup>37,43,156</sup> In 2003, when ACE2 was discovered to be the host receptor for SARS-CoV, *in vitro* experiments with native soluble ACE2 showed that this protein can neutralize the S-protein of SARS-CoV.<sup>25</sup> In 2020, Batlle *et al.* hypothesized that soluble recombinant ACE2, by acting as a decoy, might constitute a therapeutic approach for SARS-CoV-2 infection.<sup>157</sup> Of significance, the idea that ARBs, such as the “sartans,” might be a compromising factor for COVID-19 infection, because of the potential upregulation of ACE2, has now been discounted.<sup>37</sup> Indeed, angiotensin II generated locally, for example by microenvironment chymase, may be a stimulus for a fibroproliferative response to COVID-19 infections.<sup>158</sup> Thus, apart from their potential action to upregulate ACE2, ARBs may be of benefit long term for COVID-19-infected individuals.

Recent *in vitro* studies have investigated ACE2 itself as a decoy to neutralize SARS-CoV-2.<sup>159,160</sup> However, *in vivo*

studies in suitable animal models (to the best of our knowledge) are as yet lacking. That being said, Monteil *et al.* demonstrated that native soluble ACE2 can neutralize SARS-CoV-2 infectivity in human organoids.<sup>161</sup> This soluble protein is currently being studied in a clinical trial (NCT00886353), in which it is administered intravenously to patients who are seriously ill with COVID-19.

ACE2 is highly expressed within the kidneys.<sup>162</sup> Acute kidney injury is becoming increasingly recognized to be a severe potential complication of COVID-19.<sup>163</sup> Attempts to target the kidney RAS using native ACE2 to treat kidney disease are hindered by its large molecular size (100 kDa), which precludes glomerular filtration and subsequent tubular uptake. Wysocki and colleagues have bioengineered soluble ACE2 variants that are shorter than native soluble ACE2<sup>164,165</sup> and that may be more suitable for the treatment of kidney disease because they are amenable for glomerular filtration.

**(vi). Anti-androgens.** As already pointed out, the striking differences for COVID-19 pathology in male compared with female patients can be attributed to the androgen-mediated upregulation of TMPRSS2, the proteolytic enzyme that unmasks the ACE2 interacting site on the COVID-19 spike protein.<sup>97,98</sup> Additionally, ACE2 itself, as well as another spike protein-cleaving enzyme, furin, has been found to be upregulated by androgens.<sup>166</sup> Therefore, compounds that either reduce active androgen levels, such as the alpha-reductase inhibitor dutasteride, or are androgen receptor antagonists such as bicalutamide, appear to be potential agents to mitigate COVID-19 infection.<sup>97</sup> However, apart from androgen-mediated upregulation, TMPRSS2 can be upregulated in the lung and potentially other tissues via an androgen-independent mechanism.<sup>142</sup> Further, the effects of simply reducing active androgen levels (dutasteride) may differ from direct androgen receptor antagonism (enzalutamide).

As detailed in a recent review by Wilcox and Pitt,<sup>95</sup> the mineralocorticoid receptor antagonist spironolactone has off-target effects to block the androgen receptor. Thus, treatment with spironolactone that will block both the androgen receptor and the mineralocorticoid receptor, as outlined in detail elsewhere,<sup>95</sup> merits further intensive investigation. In principle, spironolactone could block both the upregulation of COVID-19-targeted proteinases (e.g., TMPRSS2) as well as the fibrosis promoted by mineralocorticoid receptor activation. In some individuals, such as those undergoing androgen deprivation to treat prostate cancer or benign prostatic hypertrophy, chronically reduced androgen levels may prove protective. However, in the acute-disease setting, the hope is that upfront treatment with androgen receptor antagonists would reduce disease intensity, making chronic treatment unnecessary.

**(vii). Cytokine-mediated ROS.** Oxidative stress, as occurs in response to inflammatory cytokines or hyperglycemia, leads to endothelial dysfunction and an increased risk of cardiovascular events.<sup>167,168</sup> As mentioned above, COVID-19 causes innate immune cell dysfunction that results in increased tissue ROS.<sup>9</sup> A consequence of increased ROS in COVID-19, as in other settings such as hyperglycemia, is to impair endothelial cell function and lead to thromboinflammation. Thus,

minimizing ROS can improve vascular function.<sup>101,102</sup> To this end, sulforaphane, which induces intracellular antioxidant genes, and metformin, which reduces increased ROS in endothelial cells, have been found to protect the endothelium from ROS-induced dysfunction.<sup>101,102,107</sup> These data suggest that minimizing endothelial ROS in COVID-19–infected individuals may attenuate the thromboinflammatory response and preserve vascular function in many tissues, such as the lung, heart, and kidney. Consequently, agents that reduce endothelial ROS, including sulforaphane and metformin, may prove to be of value in treating COVID-19.

**(viii). Proteases and profibrotic signaling.** Evidence has been found of progressive lung dysfunction and fibrosis upon recovery from COVID-19 infection; and a greater decline in pulmonary function is associated with longer duration of mechanical ventilation in those patients who required it in the acute phase.<sup>124</sup> Microenvironment proteases are likely to play a key role in this chronic progression of fibrosis. For instance, in a murine bleomycin model of lung fibrosis, procoagulant signaling mechanisms involving the triggering of PARs represent a key mechanism stimulating fibrosis.<sup>85,87</sup> Therefore, targeting the PARs with available thrombin and receptor inhibitors offers opportunities for pharmacologic intervention.<sup>132</sup> Apart from thrombin, MMP1–PAR1 activation may also regulate signaling in the setting of sepsis and fibrosis.<sup>169</sup> Thus, the use of non-antimicrobial MMP inhibitors that can prevent tissue damage in the setting of inflammation may be considered as a candidate to reduce fibrosis.<sup>150</sup>

Several therapeutic possibilities are available to diminish both the acute and chronic impact of COVID-19 on the tissue fibrosis response. The commonly prescribed biguanide metformin has many effects, apart from that of glycemic control in patients with diabetes.<sup>105,170</sup> Metformin has been demonstrated to reduce fibrosis in a murine bleomycin fibrosis model.<sup>108,171</sup> Furthermore, metformin has been found to improve vascular endothelial function in individuals with type 2 diabetes<sup>105,172</sup> and to minimize endothelial dysfunction in a type 1 diabetes model.<sup>107</sup> Consequently, an avenue of interest may be to investigate the potential of metformin as an agent to prevent acute and chronic fibrosis in the setting of COVID-19, particularly in individuals with compromised endothelial function, including individuals with type 2 diabetes, and hypertensive individuals with renal disease. Indeed, a clinical trial, for which the data will be reported, is assessing the potential impact of metformin treatment in the setting of COVID-19 (NCT04510194). In addition, a new gene transcription inhibitor has been developed that, in analogy with metformin, blocks fibrosis-associated signal pathways.<sup>100</sup> Further, as alluded to above, the ability of angiotensin II to promote a fibroproliferative response in the lung<sup>158</sup> points to the possible use of ARBs to diminish lung fibrosis. These proposed treatment strategies would complement currently suggested approaches for the treatment of post–COVID-19 pulmonary fibrosis, employing many of the agents that have been successfully evaluated for the treatment of idiopathic pulmonary fibrosis.

## Conclusions

Clearly, the proteolytic cascades of the innate immune defense system (Figure 1) play substantive, albeit complex roles in the pathophysiology of COVID-19 (Figure 2). A central role has been postulated for microthromboses that can affect the lung, heart, and kidneys. The endothelium also plays a pivotal role as a target for platelet–neutrophil interactions, signaling via the PARs, and activation of endothelial G-protein receptors by plasma kallikrein-generated BK and complement receptor-stimulating peptides. In addition to endothelial cells as central mediators of COVID-19 pathology, tissue myofibroblasts may also promote fibrosis, as discussed elsewhere in this issue.<sup>46,54</sup> These cells are affected by signaling stimulated by protease–PAR activation, G protein–coupled receptor agonist peptides, cytokines, and steroid hormone signaling pathways (Figure 2). The participation of these multiple pathogenetic pathways may suggest potential therapeutic targets worthy of investigation and interrogation, as summarized in Table 1 and illustrated in Figure 2. However, countering any single target is unlikely to suffice to repress the acute phase of the disease. Even though vaccination will ultimately attenuate the pandemic, dealing with chronic COVID-19 sequelae will remain a challenge. Furthermore, the lessons garnered from the roles of the innate immune response to COVID-19 pathology will inform approaches to the clinical management of other viral and bacterial infections that result in acute respiratory distress syndrome.

In January 2022, the future for COVID-19 survivors remains uncertain; if this virus circulates among us for years to come, long-term effects are highly likely to accumulate exponentially. Consequently, our hope is that the present consideration of the array of pathogenetic cascades discussed herein will constitute a platform for evidence-based management and potentially therapeutic interventions that will prove beneficial and succeed in helping define future treatment paradigms to mitigate acute and chronic infectious diseases that can begin in the lungs or elsewhere and can affect other tissues. We adhere to the proposition that a multipronged approach must be embraced and implemented to tamp down the florid manifestations of COVID-19, with an important focus on long-haul COVID-19.

## DISCLOSURE

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