A hypothesis for pathobiology and treatment of COVID-19: the centrality of ACE1/ACE2 imbalance

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April 28, 2020

Abstract

Angiotensin converting enzyme-2 (ACE2) is the receptor for the coronavirus SARS-CoV-2, which causes COVID-19. We propose the following hypothesis: Imbalance in the action of ACE1- and ACE2-derived peptides, thereby enhancing Angiotensin-II (ANG II) signaling, a primary driver of COVID-19 pathobiology. ACE1/ACE2 imbalance occurs due to the binding of SARS-CoV-2 to ACE2, reducing ACE2-mediated conversion of ANG II to ANG peptides that counteract pathophysiological effects of ACE1-generated ANGII. This hypothesis suggests several approaches to treat COVID-19 by restoring ACE1/ACE2 balance: 1) ANG II receptor blockers (ARBs); 2) ACE1 inhibitors (ACEIs); 3) Agonists of receptors activated by ACE2-derived peptides [e.g., ANG (1-7), which activates MAS1]; 4) Recombinant human ACE2 or ACE2 peptides as decoys for the virus. Reducing ACE1/ACE2 imbalance is predicted to blunt COVID-19-associated morbidity and mortality, especially in vulnerable patients. Importantly, approved ARBs and ACEIs can be rapidly repurposed to test their efficacy in treating COVID-19.

Abbreviations

ACE: Angiotensin Converting Enzyme; ANG: Angiotensin; ACEI: ACE Inhibitor; ARB: Angiotensin Receptor Blocker; ALI: Acute Lung Injury; ARDS: Acute Respiratory Distress Syndrome; PK: Pharmacokinetics; PD: Pharmacodynamics; AGTR1: Angiotensin II Receptor 1; EMT: Epithelial-to-Mesenchymal transition; RAS: Renin-Angiotensin Signaling; ROS: Reactive oxygen species

Introduction

The SARS-CoV-2 virus infects human cells by first binding via its S protein to its receptor: Angiotensin Converting Enzyme 2 (ACE2), a 120 kDa integral membrane glycoprotein on the surface of cells in the lungs, heart, kidneys, and intestine (Wan et al., 2020; Zhang et al., 2020; Zhou et al., 2020a; Hamming et al., 2004). Binding of SARS coronaviruses to ACE2 is followed by fusion of the viral and plasma membranes, endocytosis and cellular infection of the virus (Inoue at al., 2007; He et al., 2020). ACE2 expression in respiratory epithelium is important in the pathobiology of COVID-19 (Xu et al., 2020a). The infection typically begins in epithelia in the upper respiratory tract, before spreading to alveoli in the lungs (Xu et al., 2020a; Zhou et al., 2020a), pathologic events that are more severe in patients with compromised immune response or ability to combat the spread of infection (Rothan & Byrareddy, 2020). COVID-19 typically (in 80% of patients) causes mild symptoms, Wu & McGoogan, 2020) but severe morbidity in a subset of patients, requiring hospitalization, intensive care and in some cases, death. Increased morbidity and mortality occur in patients with comorbidities, especially ones associated with aging, including hypertension, cardiac disease, diabetes, chronic lung disease, and compromised immunity (Zhou et al., 2020a; Wu & McGoogan, 2020). Many strategies, are being pursued in response to the urgent need for effective therapies of COVID-19 (e.g., Stebbing et al., 2020; Gautret et al., 2020).

The SARS-CoV-2 virus shares many characteristics with the SARS-CoV-1 coronavirus, which caused a pandemic in 2002-2003 (He et al., 2020; Wan et al., 2020). Common features include ˜80% shared sequence identity in their viral genomes (He et al., 2020), the range of tissues that are infected, mortality from acute respiratory distress syndrome (ARDS) and their cellular receptor: ACE2 (Wan et al., 2020; Zhang et al., 2020; Zhou et al., 2020a; Zhou et al., 2020b). Compared to SAR-COV-1, SARS-CoV-2 has ˜4-fold higher affinity for ACE2 (Walls et al., 2020). Due to their similarities, information related to SARS-CoV-1 can aid in development of hypotheses for treatment of SARS-CoV2, including the repurposing of pharmacological agents approved for use in humans.

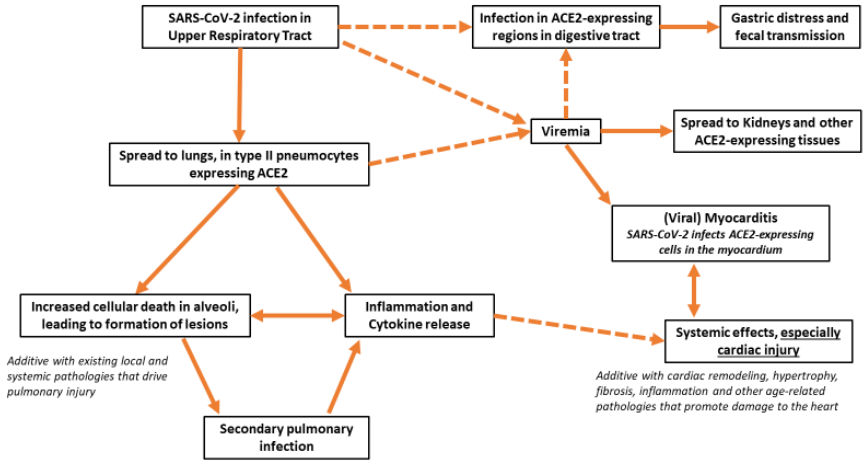
How does SARS-CoV-2 infection cause pathology?

Based on published data for both SARS viruses, we propose a pathophysiologic chain of events for COVID-19 (**Figure 1**). Central to this mechanism is angiotensin (ANG II) signaling, which we elaborate in sections below.

SARS-CoV-2 infection typically begins in the upper respiratory tract by exposure to the virus in aerosolized droplets or from fomites (Bar-On et al., 2020). Oral epithelial cells and other respiratory tract areas have substantial expression of ACE2, explaining their susceptibility to viral entry (Xu et al., 2020a; Zhou et al., 2020a). The virus spreads into the lower respiratory tract (the lungs) where epithelial cells, in particular type-2 pneumocytes, express ACE2 (Hamming et al., 2004; Mossel et al., 2008; Tian et al., 2020; Zhang et al, 2020). Infection in the lungs, and especially damage to the alveolae, is a primary cause of morbidity in COVID-19 (He et al., 2020; Zhou et al., 2020a; Zhou et al., 2020b). In susceptible patients, lesions from viral infection and entry of fluid into alveolar spaces induce respiratory distress. Pulmonary lesions appear to be a hallmark and diagnostic feature of COVID-19 (Pan et al., 2020). Certain patients have severe inflammation and cytokine storm, with overwhelming immune activation that attacks the host (Qin et al., 2020). These events can produce respiratory failure and death, especially if critical care support and mechanical ventilation are unavailable and may ultimately be associated with multiple organ failure and death (Du et al, 2020). The pulmonary pathobiology in COVID-19 is akin to what occurred with SARS-1 infection (Channappanavar & Perlman, 2017).

In addition to pulmonary injury, COVID-19 can cause cardiac complications. Most notable is myocarditis from viral infection of the myocardium, perhaps facilitated by ACE2 on cardiac myocytes (Sun et al., 2020; Chen et al., 2020). Myocardial infection, myocarditis and cardiomyopathy occurred with SARS-1 infection (Oudit et al., 2009) but data for their frequency in COVID-19 are still emerging. Cardiac injury in COVID-19 patients likely occurs from at least two mechanisms: a) cytokine release ("storm") associated with inflammation in the lung (Pedersen & Ho, 2020) that affects the heart and b) infection of the myocardium, most likely via viremia (Huang et al., 2020; Kam et al., 2020). In SARS-1, a strong association was noted between circulating viral loads and disease severity (Hung et al., 2004). This finding suggests that viremia results from alveolar damage and access of the virus to the capillary network in the lung, which can occur in more severe cases. The stage of illness during which viremia occurs in COVID-19 and if viremia correlates with severity/injury are not as-yet well-defined.

SARS-1 and COVID-19 can affect other tissues. A symptom of COVID-19 is gastric distress, which can occur prior to pulmonary symptoms (Rothan & Byrareddy, 2020; Gu et al., 2020). Fecal transmission of SARS-CoV-2 has also been documented (Gu et al., 2020), which confirms the presence of the virus in the gastrointestinal (GI) tract, a site in which ACE2 is expressed (Hamming et al., 2004). The spread of infection to the GI tract may result from viremia or from the mouth and upper respiratory tract, suggesting that the virus survives passage through the stomach. COVID-19 can involve other ACE2-expressing tissues, including the kidneys (Naicker et al., 2020).



The primary causes of COVID-19 morbidity and mortality are: 1) lung injury with associated respiratory distress (and accompanying cytokine storm) and 2) heart failure or other cardiac dysfunction (Guo et al., 2020; He et al., 2020; Zhou et al., 2020a; Zhou et al., 2020b). Therapeutic strategies for COVID-19 seek to prevent/slow viral infection or mitigate injury from the infection. In view of the apparent lack of success thus far with antiviral therapies and the time needed to develop vaccines, we propose strategies that focus on blunting the pathobiology that we hypothesize results from ACEI/ACE2 imbalance and enhanced ANG II signaling that occurs with SARS-CoV-2 infection.

Figure 1. A schema identifying the tissues impacted by SARS-CoV-2 infection and COVID-19 pathobiology . Dashed arrows identify events whose role is as-yet unclear

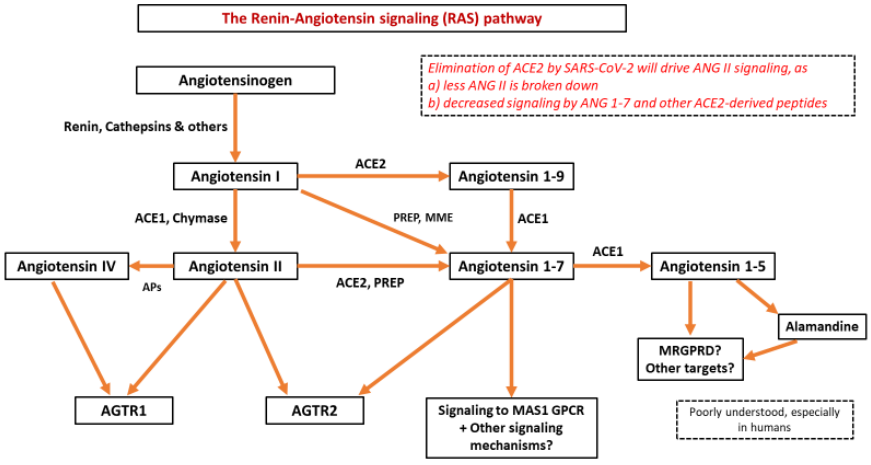


Figure 2. Renin-Angiotensin signaling (RAS), Angiotensin (ANG II) signaling, the balance between ACE1 and ACE2, and in red, the impact of COVID-19. APs: Aminopeptidases; PREP: prolyl endopeptidase; MME: membrane metalloendopeptidase

The Renin-Angiotensin signaling (RAS) pathway

Figure 2 shows a schema of the RAS pathway and is based on data for angiotensin signaling from a range of sources (Santos, 2014; Zhuo et al., 2013; Tikellis and Thomas, 2012; Karnik et al., 2017, Santos et al.,

2019), including the Guide to Pharmacology database (GtoPdb; https://www.guidetopharmacology.org/) (Armstrong et al., 2020). In the "traditional"/canonical RAS pathway, renin is secreted from the kidney and angiotensinogen (AGT) is produced and secreted by the liver. Renin cleaves AGT to form Angiotensin 1 (ANG 1), which in turn generates ANG II or ANG 1-9, primarily from the actions of ACE1 and ACE2, respectively. ANG II is cleaved by multiple enzymes, most importantly ACE2, to form ANG (1-7). ANG II also forms ANG IV via the action of aminopeptidases (APs). Both ANG II and ANG IV act primarily via AGTR1 (a GPCR). ANG (1-7) acts primarily via the MAS1 (a GPCR), and forms ANG (1-5), which signals via MRGPRD (a GPCR). ANG (1-7) can also act via the AGTR2 receptor but with much lower affinity than ANG II (refer GtoPdb entry).

As discussed below, the lung and heart express components of the RAS system, i.e., 'local' RAS signaling (e.g., Uhal et al., 2012; Forrester et al., 2018), that can contribute to tissue injury. For example, activated lung fibroblasts and injured epithelial cells express AGT and other RAS components (e.g., Uhal et al., 2012; Wang et al., 2000).

ACE2, a carboxypeptidase (zinc metalloprotease), is the primary enzyme responsible for ANGII degradation, thus regulating signal transduction by ANGII. The conversion by ACE2 of ANG II to ANG (1-7) (and its signaling via MAS1) produces effects that oppose those of ANG II (Karnik et al., 2017, Santos et al., 2018; Santos et al., 2019). ACE2, in particular its catalytic ectodomain, can be shed from cells, an action mediated by the metalloprotease ADAM17, into the circulation as soluble ACE2, levels of which can be increased by Ang II via its ability to increase ADAM17 activity (Lambert et al., 2005; Patel et al., 2014).

The RAS signaling pathway (**Figure 2)** thus relies on a "yin/yang relationship" between ACE1 and ACE2: ACE1 generates Angiotensin II (ANG II) and in turn, signaling by the GPCRs AGTR1 and AGTR2 while ACE2 generates peptides whose receptors act to oppose responses mediated by AGTR1. AGTR1 is highly expressed and mediates ANGII signaling and effects (including inflammation, apoptosis, pro-fibrotic signaling and tissue remodeling) in pulmonary and cardiac tissue (Forrester et al., 2018). By contrast, the role of AGTR2, which is expressed in the lung and at low levels in the heart, is more controversial (Forrester et al., 2018; Santos et al., 2019; GtoPdb). AGTR2 can mediate effects that oppose those of AGTR1 but other data suggest that AGTR2 promotes effects such as apoptosis. In subsequent section that focus on the pathophysiology of COVID-19, we emphasize the role of ACE1-generated AGTR1 as the key mediator of ANG II actions and the opposing actions of ACE-derived peptides.

SARS viruses and Angiotensin signaling

Infection of cells by SARS viruses that bind ACE2 results in two effects: inhibition of ACE2 activity and decrease of ACE2 expression in infected cells (Kuba et al., 2005; Haga et al., 2008; Glowacka et al., 2010; Dijkman et al., 2012; Zhang et al., 2020). Indirect evidence for the latter response in COVID-19 is data showing elevation in circulating ANG II with viral infection and increased circulating ANG II peptide with higher viral loads (Liu et al., 2020b).

Our hypothesis is founded on the following ideas that derive from data in reports cited in ensuing sections:

1. SARS viruses decrease ACE2 activity and expression.
2. The decrease in ACE activity creates an imbalance in signaling by ACE1 and ACE2 products
3. This imbalance increases ANG II/AGTR1 signaling and is superimposed on concurrent pathology (e.g., chronic lung disease, cardiac remodeling in the lung and heart, respectively). ANG II is a pivotal mediator of injury in both tissues; enhancement of its effects together with signaling from comorbidities can increase severity of COVID-19.
4. In patients more susceptible to the damaging effects of ANG II, the decrease in ACE2 activity by SARS viruses can unleash a cascade of injurious effects through a heightened imbalance in the actions of the products of ACE1 versus ACE2.
5. The proposed imbalance in the signaling and actions of products of ACE1/ACE2 implies the potential of pharmacological approaches that redress this imbalance via: a) decrease in ACE1 activity, b) blockade of AGTR1, and/or c) increase in ACE2-mediated signaling by affinity-trapping the SARs virus,

enhancement of ACE2 expression/activity, or d) by agonists for receptors of ACE2-derived peptides

We provide data from the literature in support of this hypothesis, in particular as related to the lungs and the heart, and regarding potential therapeutic approaches that address the imbalance of ACE1/ACE2 signaling and resultant actions involved in the pathobiology and severity of COVID-19.

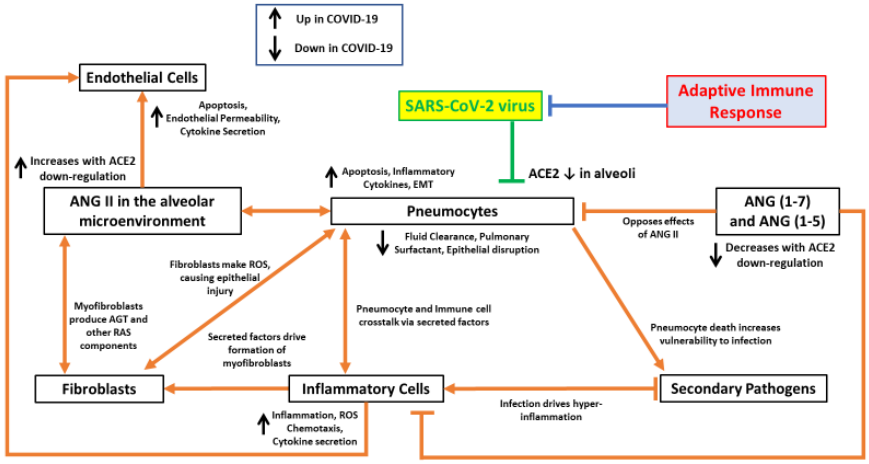


Figure 3 . Hypothesized model of cell-cell communication and pathobiology in pulmonary infection from SARS-CoV-2 and the role of ACE1- and ACE2-derived peptides in mediating these effects on several different cell types.

A model for how imbalance in the RAS pathway produces COVID-19 injury in the lungs

ACE2 is highly expressed in the lung parenchyma, particularly in type II pneumocytes (type II alveolar cells) (Zou et al, 2020). Type II cells synthesize and release pulmonary surfactant, phospholipids that lower surface tension, which is necessary to maintain alveolar structure (Andreeva et al., 2007). Type II cells also can differentiate to become type I alveolar cells (which form the structure of alveoli), a mechanism for replacement of type 1 cells that are damaged. The SARS-CoV-2 and SARS-CoV-1 viruses perturb alveoli to produce the major pathology in the lung, with increased fluid entry, cell death and inflammation along with reduction in gas exchange and levels of surfactant (Hui et al., 2005; Gralinksi & Baric, 2015; Xu et al., 2020b).

Figure 3 depicts a hypothetical framework for this process and the cell types involved. The SARS-CoV-2 virus infects alveolar pneumocytes by binding to ACE2, leading to a decrease in ANG II conversion to ACE2-derived peptides, e.g. a reduction in ANG (1-7) and its actions that counteract effects of ANG II (**Figure 2)** . Hence, ANG II levels increase in the alveolar microenvironment, with potential effects on multiple cell types. Below, we provide evidence in support of this framework and the role of ACE1/ACE2 imbalance in the lung injury of COVID-19.

ANG II has a pro-apoptotic action on pulmonary epithelial cells (Wang et al., 1999a; Wang et al., 1999b; Wang et al., 1999c; Wang et al., 2000; Papp et al., 2002), a response that is consistent with the pathology from SARS viruses, i.e., widespread epithelial damage and alveolar damage and cell death (Zhou et al., 2020a; Zhou et al., 2020b). In addition, ANG II promotes and Ang (1-7) suppresses epithelial-to-mesenchymal transformation (EMT), whereby epithelial cells acquire a more fibrotic phenotype (Buckley et al., 2010; Rodrigues-Díez et al., 2008; Lee at al., 2013; Shao et al., 2019), a mechanism that may contribute to the formation of pulmonary lesions. ANG II also decreases the clearance of alveolar fluid (Deng et al., 2012a; Deng et al., 2012b; Ismael-Badarneh et al., 2015). Apoptosis and EMT in alveolar epithelial cells are accompanied by an increase in secretion of pro-inflammatory cytokines (e.g., IL1-β, IL-6, IL-8, MCP-1 and

TNF-α) (Aumiller et al., 2013; Ma et al., 2010; Kode et al., 2006; Pires-Neto et al., 2013; Pedersen & Ho, 2020). Epithelial cells engage in crosstalk with immune cells, in particular during infection and apoptosis (Rzepka et al., 2012; Herold et al., 2012; Chuquimia et al., 2012). These effects are compounded by the secretion of RAS components by activated myofibroblasts and epithelial cells undergoing apoptosis (Wang et al., 1999a; Wang et al., 1999b; Wang et al., 2000; Uhal et al., 2012), thereby amplifying ANG II signaling in a positive-feedback loop.

ANG II also has pro-fibrotic effects on fibroblasts that reside in interstitial spaces around alveoli (Marshall et al., 2000; Uhal et al., 2007; Uhal et al., 2012b) and increases apoptosis of endothelial cells and endothelial permeability in the surrounding capillary network (Watanabe et al., 2005; Bodor et al., 2012), which can increase fluid entry and immune infiltration into regions of the lung. ANG II also affects various types of immune cells (Forrester et al., 2018), increasing macrophage infiltration, ROS production and release of pro-inflammatory cytokines. Besides immune cells, pathological ROS production occurs in pulmonary fibroblasts, driven by ANG II stimulation and inhibited by the actions of ACE2/ANG (1-7) (Meng et al., 2015). ROS production from activated fibroblasts is a key driver of epithelial injury in models of pulmonary fibrosis (Sakai & Tager, 2013).

Crosstalk among epithelial cells, fibroblasts and immune cells suggests a role for macrophages, which are activated by pulmonary epithelial cells in injury settings and can create positive feedback that promotes inflammation (Uhal et al., 2007; Rzepka et al., 2012; Herold et al., 2012; Chuquimia et al., 2012; Bhattacharya & Westphalen, 2016). Such effects can occur in response to ANG II (Uhal et al., 2007). Ang II can also regulate the function of other immune cells (e.g., dendritic cells and neutrophils) that can promote lung injury (Grommes & Soehnlein, 2011; Florez-Sampedro et al., 2018). Importantly, as summarized below, ANG (1-7) has opposing effects that counteract this pathology; these protective effects are blunted by the SARS-CoV-2 virus.

ANG II thus promotes a range of pro-apoptotic, inflammatory, fibrotic and edema-associated processes in the alveolar microenvironment. As cell death occurs, the epithelium, a critical component of innate immunity as a barrier to pathogens and via its formation and release of surfactant (Wright, 2003; Eisele and Anderson, 2011) becomes compromised, paving the way for secondary infection (e.g., bacterial pneumonia). As a result, immune response is further enhanced, greater inflammation ensues (**Figure 3**) and alveolar damage is increased, thereby enhancing lung injury and edema. Patients who recover may have severe tissue damage, potentially with tissue fibrosis. Indeed, studies in patients who recovered from SARS-1 document the presence of fibrosis and chronic lung damage in severe cases (Ketai et al., 2006). Lung tissue from COVID-19 patients shows evidence of a) epithelial injury and disruption, in particular of type-2 pneumocytes, b) invasion of macrophages and neutrophils and c) initiation of fibrosis (Luo et al., 2020; Tian et al., 2020; Dhama et al., 2020; He et al., 2020).

Benefits of ACEI/ARB use in reducing pulmonary injury

Numerous studies have assessed the effects of inhibition of ANG II signaling by the administration of ACEIs or ARBs as ways to mitigate lung injury in a range of experimental models, including ARDS and pulmonary fibrosis. Bleomycin, a natural product-derived peptide used for cancer chemotherapy, is a widely used model in rodents for the study of pulmonary fibrosis. Bleomycin damages the pulmonary epithelium, which then initiates a fibrotic response; the pulmonary injury and fibrosis can be mitigated by treatment with ACEIs or ARBs (e.g., Li et al., 2003; Wang et al., 2000; Otsuka et al., 2004; Yao et al., 2006; Uhal et al., 2012). Other models of lung fibrosis, e.g. γ-irradiation, also show deceased injury and fibrosis in response to ARBs (Mohammadi-Karakani et al., 2006; Molteni et al., 2007; Uhal et al 2012). ACEIs/ARBs appear to blunt the degree of apoptosis of epithelial and other cell types, indicating that initiating events of lung injury require ANG II signaling to drive a pathological response.

How might this occur? As shown in **Figures 2 and 3** , increase in local RAS signaling within the lung, i.e., injury-induced production of RAS signaling components by epithelial cells and activated fibroblasts, promotes pulmonary injury and helps explain the efficacy of ACEIs/ARBs in blunting this pathology. Further evidence

for this mechanism is the reduction in bleomycin-induced injury and fibrosis by antisense oligonucleotides against AGT (Li et al., 2007).

Data from other in-vivo models supports the utility of ACEIs/ARBs in blunting lung injury, especially of the epithelium, thus further implicating the role of ANG II in mediating these effects. Examples include acute lung injury induced by oleic acid in rats (in which use of the ACEI captopril reduced alveolar damage/epithelial disruption, endothelial damage and infiltration of neutrophils (He et al., 2007), the protective effect of ACEI/ARB treatment in reducing pneumocyte death in surfactant-depleted rat lungs (Lukkarinen et al., 2005), and the efficacy of ACEIs in the treatment of radiation-induced lung injury (Medhora et al., 2012). This benefit has also been shown in human studies, for example, a reduction in pulmonary-related mortality by captopril administered to patients receiving total body irradiation prior to hematopoietic stem cell transplantation (Cohen et al., 2012). ACEI administration also reduces radiation-induced pneumonitis in lung cancer patients (Kharofa et al., 2012).

Further evidence for effects of sepsis has been obtained from animal models of acute lung injury (ALI) induced by lipopolysaccharide (LPS): treatment with the ARB losartan reduced pro-inflammatory cytokine secretion and lung injury and improved survival, while also reducing ANG II production in the lungs (Shen et al., 2009). The protective effects of losartan in LPS-induced ALI are associated with reduced contribution by dendritic cells to inflammation (Liu et al., 2012). Captopril was protective in a rat LPS-induced ALI model, reducing immune cell infiltration, edema, and hemorrhage in alveoli (Li et al, 2015). The latter study also showed that the ratio of ACE1/ACE2 expression increased in injury and that captopril attenuated injury and decreased that ratio. These findings were replicated in a study in which captopril reduced inflammatory cytokines and monocyte infiltration in bronchoalveolar fluid (Boskabadi et al., 2019). In addition, in a murine H5N1 influenza infection model. treatment with losartan improved survival and reduced edema, lung injury and immune cell infiltration (Yan et al., 2015). Other studies showed that ACEIs or ARBs inhibit ventilator-induced lung injury (Jiang et al., 2007, Wösten-van Asperen et al., 2008, Jerng et al., 2007, Chen et al, 2014). Also, treatment of rats with losartan in chronic cigarette-smoke-induced injury model reduced pulmonary remodeling and pulmonary arterial hypertension (Han et al., 2010).

Data from retrospective studies in humans show that ACEIs can prevent or reduce the severity of pneumonia (e.g., Mortensen et al., 2005; van de Garde et al., 2007; Mortensen et al., 2012 and Caldeira et al., 2012). In addition, reatment with ACEIs/ARBs in COPD (chronic obstructive pulmonary disease) reduces inflammation, comorbidities and disease complications (Shrikrishna et al., 2012). Retrospective analyses also suggest that ACEI/ARB treatment may reduce mortality in ARDS/ALI (Noveanu et al., 2010) and mitigate the effects of radiation pneumonitis (Harder et al., 2015). However, evidence is lacking from prospective clinical studies with ACEIs/ARBs in these types of pulmonary injury.

Cell-based assays provide additional support for the importance of ANG II in mediating lung injury. ANG II induces apoptosis in a human epithelial cell line (A459) and in rat type-II pneumocytes, effects that are blocked by treatment with ACEIs or ARBs (Wang et al., 1999b, Wang et al., 1999c). Such findings were replicated using losartan in the same cell types, results that confirmed AGTR1 as the receptor that promotes apoptosis in pulmonary epithelial cells (Papp et al, 2002). Moreover, treatment of human and rat lung epithelial cells with FAS protein (an apoptosis-inducing ligand) induced apoptosis by increasing AGT and ANG II expression/secretion, effects blocked by ANG II antibodies (Wang et al., 1999b). The latter authors further showed that pro-fibrotic human lung fibroblasts induce epithelial cell apoptosis by producing ANG II and that these myofibroblasts express the components necessary to drive a local RAS signaling cascade (Wang et al., 1999a; Wang et al., 1999c). In addition, the ARB telmisartan blunts ANG II-promoted EMT of A549 cells (Buckley et al., 2010).

Thus, the available data provide consistent evidence indicating that ANG II signaling, via AGTR1, has a central role in lung injury. Blunting this signaling by ACEIs or ARBs has beneficial effects in modulating such damage, including in the context of acute pulmonary injury caused by infection.

ACE2 and ANG (1-7) reduce pulmonary injury

An extensive literature has emerged describing the protective effects of ACE2 and ANG (1-7) in mitigating pulmonary injury by acting in opposition to the effects of ANG II. The approaches include treatment with soluble ACE2 that ultimately converts ANG II to ANG (1-7), whose protective role is most likely mediated by MAS1.

Data from rodent models indicate that ACE2/ANG (1-7) can mitigate fibrosis induced by agents such as bleomycin. For example, in a mouse bleomycin model, treatment with recombinant ACE2 reduced epithelial injury, pro-fibrotic cytokine release, activation of fibroblasts and inflammatory cell infiltration, thereby prominently reducing the extent of lung injury (Wang et al., 2015). Studies in a bleomycin model in rats have revealed that the protective effects by ANG (1-7) may occur by inhibition of signaling cascades that involve MAP Kinase and NFkB (Meng et al., 2014). The authors also found the ANG (1-7) or ACE2 overexpression had antifibrotic effects via inhibition of MAP Kinase and NFkB in human lung fibroblasts. Other studies confirm a protective role by ACE2/ANG (1-7) in bleomycin-treated rats (Wu et al., 2014). Lung samples from patients with idiopathic pulmonary fibrosis (IPF) or from bleomycin-treated mice and rats have decreased ACE2 expression in association with lung injury and fibrosis (Li et al., 2007). Fibrosis (collagen accumulation) was also enhanced in mice treated with ACE2 siRNA or ACE2 inhibition but treatment with recombinant ACE2 reduced bleomycin-induced fibrosis (Li et al., 2008b). In a cigarette smoke-induced model of lung injury in mice, Zhang et al., (2018) showed that treatment with ANG 1-7 reduced lung inflammation and fibrosis.

ACE2/ANG (1-7) also have a protective effect in ALI/ARDS models induced by LPS stimulation. In LPS-induced ARDS models in rats, treatment with ANG (1-7) or an ARB reduced lung injury and inflammation and improved lung function (Wösten-van Asperen et al., 2011; (Chen et al., (2013). He et al., (2015) reported that mesenchymal stem cells (MSCs) engineered to overexpress ACE2 had strong protective effects in an ALI model, improving endothelial barrier integrity and reducing lung injury and inflammation. Inhibition of ACE2 increased lung injury, IL-17 signaling and inflammation with infiltration by neutrophils in a murine model of bacterial (Pseudomonas) lung infection but the converse occurred in mice treated with recombinant ACE2 (Sodhi et al., 2019). ACE2 not only mitigated lung injury but also improved response to the infection. Ex-vivo experiments with mouse lung organoids confirmed the effects of ACE2 limiting IL-17 signaling.

In-vitro data with human cells support the idea that ACE2/ANG (1-7) can protect cells from ANG II or bleomycin-induced apoptosis. It has been suggested that endoplasmic-reticulum (ER) stress induces apoptosis, which can be eliminated by treatment with ANG (1-7) via MAS1 receptor activation (Uhal et al., 2011; Uhal et al., 2013). TGF-β1 treatment promotes EMT in human airway epithelial cells, a response associated with a reduction of ACE2 expression and elevation of migration and expression of myofibroblast markers; treatment with ANG (1-7) blocked TGF-β1-induced EMT and activation of targets downstream of TGF-β1 (Shao et al., 2019).

Effects of the MAS1 receptor in modulating immune response have been reported in a number of studies. These include effects of MAS1 on neutrophil influx in models of arthritis in mice and rats (Silveira et al., 2010) and on the ability of macrophages to phagocytose neutrophils that have undergone apoptosis (Barroso et al., 2017). MAS1 knockout mice have greater inflammatory cell infiltration, lung remodeling and inflammatory cytokine production in models of allergic pulmonary inflammation (Magalhães et al., 2016) and altered macrophage function that contributes to a range of inflammatory pathology (Hammer et al., 2016).

Thus, considerable data document a protective role for ACE2/ANG (1-7) by opposing effects of ANG II in lung injury. ACE2/ANG (1-7) have many such actions, which include blunting of alveolar epithelial apoptosis, infiltration of inflammatory cells, activation of fibroblasts and endothelial disruption.

ACE2 in SARS-CoV infections of the lung

In a landmark study, using mouse models of acid-respiration or LPS, the Penninger group initially showed a protective role of ACE2 in ARDS (Imai et al., 2005) and demonstrated that ACE1-mediated signaling promotes ARDS but ACE2 exerts strong protective effects. ACE2 knockout (KO) mice had dramatically increased lung injury, effects that were reduced by treating with recombinant human ACE2. Conversely,

ACE-deficient mice had reduced severity of injury, supporting the concept that ACE1/ACE2 balance is a central mediator of lung injury.

The Penninger group subsequently used ACE2 KO mice and found that ACE2 expression is necessary for SARS infection (Kuba et al., 2005). The authors hypothesized that RAS signaling blockade or treatment with recombinant ACE2 would protect from SARS-induced injury. A crucial observation was that SARS-CoV-infected mice resembled ACE2 KO mice in their susceptibility to lung injury (Kuba et al., 2006). Penninger and coworkers have extended these ideas to COVID-19, advocating for the use of ACE2 as a target in SARS-CoV-2, including by providing soluble ACE2 (Zhang et al., 2020). The Penninger group recently showed the efficacy of recombinant human ACE2 in drastically reducing the infectivity of SARS-CoV-2 in ex-vivo models, including with organoids (Monteil et al., 2020).

What is the contribution of AGTR2 to pathobiology in the lung and COVID-19?

Few studies have assessed the actions of AGTR2 in the lung. A protective role for AGTR2 in was implied by findings indicating that AGTR2-null mice and those treated with an AGTR2 inhibitor were more vulnerable to ARDS Imai et al., (2005). However, subsequent studies related to the lungs using selective inhibitors of AGTR2 to distinguish its effects from AGTR1, reach the opposite conclusion: in general, it appears AGTR2 drives pathology synergistically with AGTR1, in particular in cell types relevant to the hypothesis presented above.

Examples of such studies include animal models of fibrosis (Königshoff et al., 2006; Waseda et al., 2008) and *in-vitro* data from human lung fibroblasts (Königshoff et al., 2006). AGTR2 also promotes apoptosis in pulmonary endothelial cells (Lee et al., 2010), rat alevolar epithelial cells (Bechara et al., 2006) and human alveolar epithelial cell lines (Pickel et al., 2010). Consistent with animal data on fibrosis are histological data from patients with systemic sclerosis and lung fibrosis, showing elevated alveolar AGTR2 expression with disease; higher AGTR2 expression is associated with increased mortality Parra et al., (2014).

Reviews that discuss AGTR2 action in other tissues (e.g., Forrester et al., 2018; Lemarie & Schiffrin, 2010; Jones et al., 2008; Kaschina, & Unger, 2003) also note disagreement regarding AGTR2 action (e.g., effects on vasodilation, protection from ischemic injury), but all concur that AGTR2 exerts pro-apoptotic effects in various cell types. A pro-fibrotic role for AGTR2 that promotes injury in concert with AGTR1 has also been noted in lung fibroblasts Uhal et al., (2012b). We conclude that AGTR2 may act in parallel with AGTR1 in driving cell death and tissue damage in the schema depicted in **Figure 3** .

How is the pathobiology of lung injury related to ACE1/ACE2 imbalance counteracted?

The data described above leads us to propose a model for pulmonary pathology based on imbalance in RAS signaling that influences multiple cell types (**Figure 4**) and has feedback loops that amplify pathobiology. Most COVID-19 patients suffer minor illness and avoid severe tissue injury. We propose that this occurs via an effective adaptive immune response that eliminates infection and prevents the damage induced by the RAS pathway and associated feedback mechanisms. By contrast, patients with underlying pathologies suffer more severe disease because of amplified RAS-driven mechanisms. These mechanisms cause acute injury that exceed the capacity of the protective immune response. The damaging effects of the virus via RAS/Ang II are thus competing with the effectiveness of the immune response (**Figure 4**).

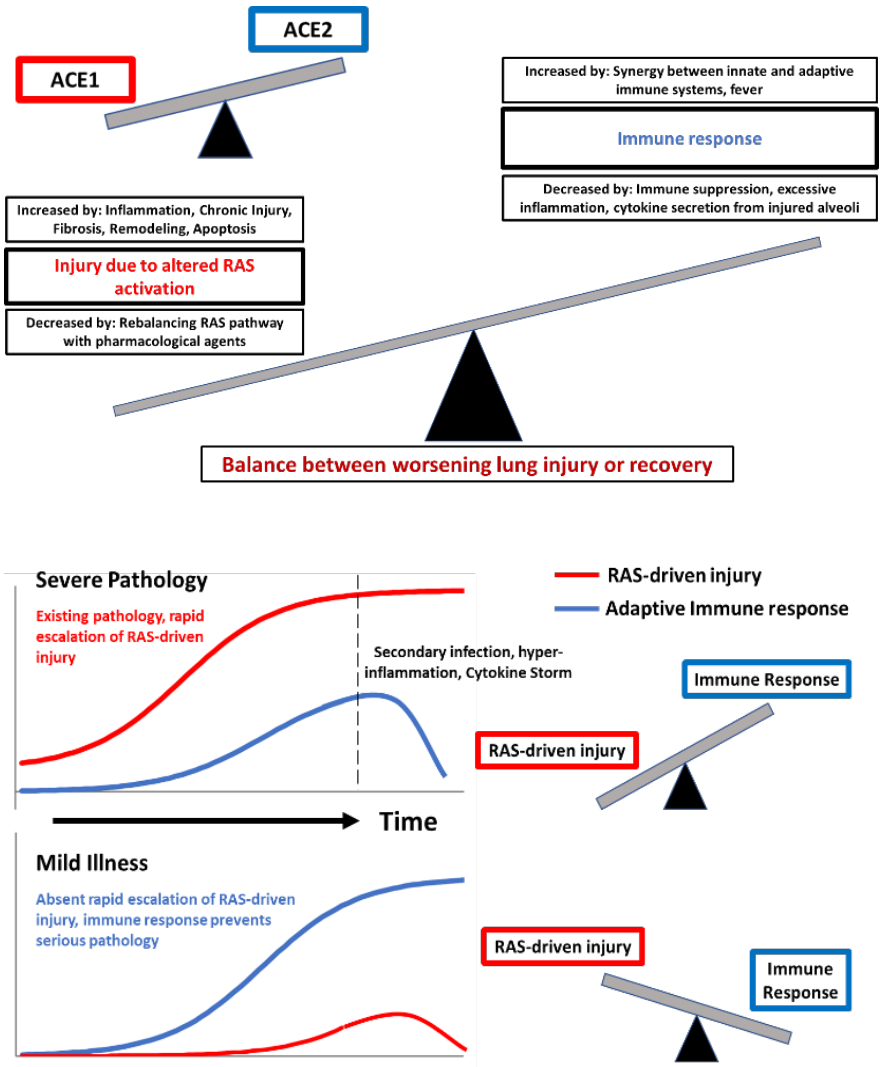


Figure 4. ACE1/ACE2 imbalance, lung injury, and factors that determine severe pathology or mild illness. (A.) A model for the course of COVID-19 that links ACE1/ACE2 imbalance to lung injury via the RAS pathway and the protective role of the immune response. **(B.)** An illustration of the predicted course of COVID-19 in two settings: **top** : Severe pathology, in patients who lack appropriate immune response or who have prior conditions that enhance ACE1/ACE imbalance (and RAS-induced injury). The result is increased injury that overwhelms the adaptive immune response; **bottom** : Mild illness, in which patients lack underlying conditions and can mount an appropriate immune response, so that RAS-induced injury is less serious, an effective immune response occurs and the infection is resolved.

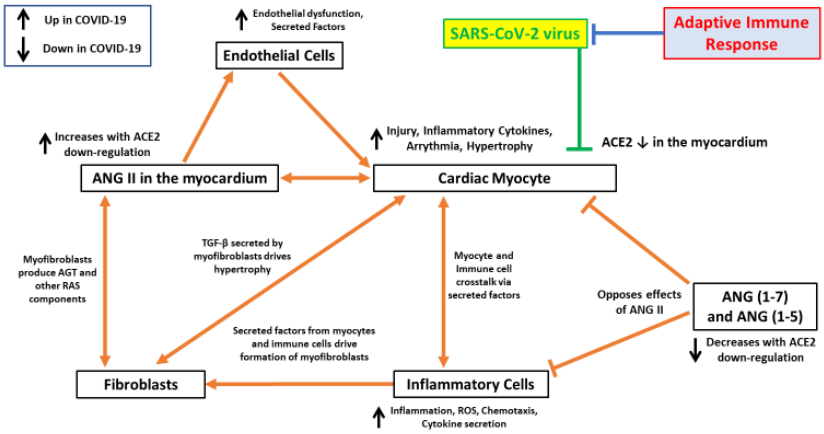
Early insights into the immune response to COVID-19 have been reported (To et al., 2020, Thevarajan et al., 2020 and Zhou et al., 2020) along with the proposed models of this response (Li et al., 2020; Prompetchara et al., 2020). Analysis of the immunopathology of SARS-1 and MERS (Channappanavar and Perlman, 2017) is consistent with such data and models. Prior reviews describe the importance of T cell-mediated adaptive immune response to coronaviruses, especially SARS (Channappanavar et al. 2014) and Li et al., 2009) and provided insight into roles of macrophages, dendritic cells, B cells and T cells in SARS-CoV-1 infection (Zhao et al., 2009; Yasui et al., 2014).

Based on these studies, the following framework describes the interplay between pathological—as opposed

to protective– immune response and acute pulmonary injury in COVID-19, in part extrapolating from information and ideas related to SARS-CoV-1 (Channappanavar and Perlman 2017).

1. During early stages of infection, inflammatory cells (macrophages, neutrophils and dendritic cells) infiltrate into the alveoli in response to cytokine secretion by alveolar cells. Immune infiltration is greater in subjects who experience more rapid RAS-induced injury and epithelial cell death (i.e., a more severe imbalance between ACE1 and ACE2), thereby establishing a positive feedback loop: accumulation of large numbers of inflammatory cells further promotes cell death in alveoli.
2. Human coronaviruses express proteins that suppress the production by immune cells of interferons (IFNs), which suppress viral replication. This IFN response is exacerbated and injury is increased in conditions of 'hyper-inflammation' that undermine protective innate immune responses. Such hyper-inflammation is more likely to occur in conditions of escalated RAS-driven injury, due to greater ACE1/ACE2 imbalance.
3. Seroconversion begins early, within ˜5 days from the onset of symptoms, with increasing titers of IgM antibodies, and promotes an adaptive immune response, mediated by T helper cells, cytotoxic T cells and antibody production by B cells, recruited by T helper cells. Hyper-inflammation results from extensive injury that overwhelms this adaptive response, with accumulation of activated macrophages that can suppress effective antigen presentation by dendritic cells and the recruitment of T cells.
4. In patients with mild pulmonary injury, effective recruitment of T cells leads to clearance of the virus, followed by tissue repair and restoration of homeostasis. Transition occurs from IgM to IgG antibodies. In the case of severe pathology, a vicious cycle of inflammation and cell death leads to widespread epithelial disruption and onset of pneumonia. Such patients are more vulnerable to bacterial infection with the decrease in surfactant production and loss of an intact epithelial barrier.
5. Mild disease (with less severe RAS-driven effects) shows a progressive decline in viral loads and improvement in symptoms and signs of infection, generally by ˜7 days from initial symptoms. In progressive disease (with more severe RAS-driven effects), greater pulmonary injury (perhaps with secondary pneumonia) necessitates critical care, including assisted ventilation. ARDS can develop if inflammation, cell death and infection continue. Cardiac complications may exacerbate this, especially if myocarditis and further pulmonary edema occur. The cytokine storm stemming from ARDS also has the potential to exert systemic effects, raising the risk of multiple organ failure (Pedersen & Ho, 2020).

The outcome of COVID-19 infection is thus determined by the competing actions of different elements that promote (the RAS pathway) or blunt (immune response) lung injury (**Figure 4**). In severe cases, a series of injurious effects unfold and overwhelm protective immune responses. Greater imbalance in the effects if ACE1 and ACE2 in the RAS pathway is predicted to exacerbate pathology, making it more likely that the immune response will be overcome. Factors/comorbidities that increase the ACE1/ACE2 imbalance are discussed below.



A model for how imbalance in the RAS pathway produces COVID-19 injury in the heart

Figure 5 . Hypothesized model of cell-cell communication in myocardial infection from SARS-CoV-2 and the influence of ACE1/ACE2 imbalance on a variety of cell types involved in cardiac injury.

By analogy with our model for lung injury from COVID-19 (**Figure 3**) and based on data in the literature, **Figure 5** describes a model for COVID-19 pathology in the heart. Below, we discuss details of this model.

ACE2 is highly expressed in the myocardium in particular on cardiac myocytes but also in endothelial cells and fibroblasts (Patel et al., 2016; Santos et al., 2018). The binding of SARS-COV-2 to ACE2 from Infection of the myocardium decreases ACE2 activity (Oudit et al., 2009) and results in increased ACE1/ANG II driven signaling and a decrease in effects of ACE2-derived peptides, including ANG (1-7). ANG II alters the function of multiple cell types in the heart, including cardiomyocytes, fibroblasts, endothelial cells and inflammatory cells, in particular macrophages. ANG (1-7) action occurs on cardiomyocytes, inflammatory cells and cardiac fibroblasts.

Akin to the lung, the heart has a local RAS that can contribute to cardiac pathology (Dostal and Baker, 1999; De Mello and Danser, 2000; Reyes et al., 2017; Forrester et al., 2018). ANG II has direct effects on cardiomyocytes, increasing hypertrophy and contractility, altering heart rate and rhythm, and enhancing secretion of cytokines that help facilitate cardiac remodeling (Baker et al., 1992; Wen et al., 2012; Reyes et al., 2017; Forrester et al., 2017). ANG II converts cardiac fibroblasts to a more pro-fibrotic myofibroblast phenotype and enhances secretion of factors (in particular TGF-β and RAS signaling components) that promote hypertrophy via crosstalk with cardiomyocytes (Dostal and Baker, 1999; Singh et al., 2008; Frieler and Mortensen, 2016; Forrester et al., 2018). ANG II also exerts effects on endothelial cells, inducing endothelial dysfunction and cytokine secretion, in particular of endothelin-1, which enhances myocyte hypertrophy (Schmermund et al., 1999; Forrester et al., 2018). RAS signaling can promote pro-inflammatory effects on inflammatory cells, in particular cardiac macrophages, which can further increase myocyte hypertrophy and fibroblast activation (Frieler and Mortensen, 2016; Forrester et al., 2018). ANG II signaling in multiple cardiac cell types increases oxidative stress and reactive oxygen species (ROS) (Wen et al., 2012; Kurdi and Booz, 2012; Forrester et al., 2018).

Signaling in the heart by ACE2-derived peptides, in particular ANG (1-7), opposes effects of ANG II (Patel et al., 2016; Santos et al., 2018). Via those actions, the ACE2- ANG (1-7)-MAS1 axis mitigates hypertrophic, fibrotic, oxidative stress and remodeling effects of ANG II. Protective effects via ACE2 on cardiac myocytes are likely particularly important in cardiomyopathy and heart failure (e.g., Flores-Muñoz et al., 2011).

ANG II thus alters multiple cell types in the heart and promotes a pro-inflammatory, hypertrophic state via a range of mechanisms. These mechanisms are counteracted by ACE2-derived products, in particular ANG (1-7), implying an alteration in ACE1/ACE2 balance as a contributor to the resultant cardiac phenotype. The SARS-CoV-2-promoted increase in ACE1/ANG II actions resulting from a decrease in ACE2-derived peptides is predicted to unleash inflammatory, oxidative stress and remodeling events and potentially myocyte apoptosis, depressed myocardial function, heart failure, arrythmia, and cardiac fibrosis.

Enhanced ACE1/ACE2 imbalance in comorbidities that influence COVID-19 morbidity and mortality

Why do some patients, with comorbidities have increased susceptibility to morbidity and mortality from COVID-19? Our model for pulmonary and cardiac injury implies a role for crosstalk between ACE2-expressing cells infected by SARS viruses and other cell types, especially inflammatory cells (e.g., macrophages and neutrophils) and fibroblasts. In patients with underlying disease, one or more of these cell types may be dysfunctional. Superimposed SARS-CoV-2 infection further amplifies ANG II signaling (which promotes injury) and suppresses ANG (1-7) signaling (which opposes injurious effects), thus increasing pathobiology. Multiple clinical conditions may be associated with elevated vulnerability to COVID-19. Examples include:

Chronic Lung Injury/Disease . Lung injury (e.g., from fibrotic disease, smoking or radiation) is associated with increased local inflammatory signaling, predisposing the epithelium to ANG II-promoted injury. In these settings, epithelial cells and fibroblasts can have elevated a net increase in pro-inflammatory ANG-mediated responses. The addition of SARS-CoV-2 further increases this imbalance, thereby enhancing lung injury.

- *Cardiac hypertrophy and remodeling* . ANG II regulates cardiac remodeling in multiple settings, including hypertension. The elevation in ANG II signaling, derived in part from cardiac RAS, increases effects of ANG II in the heart (Forrester et al., 2018). Patients with cardiac pathologies associated with remodeling are thus particularly susceptible to an acute imbalance in the RAS pathway caused by myocardial SARS-CoV-2 infection. Decreased cardiac function, especially in patients with left heart failure, may also increase the likelihood of pulmonary edema, accompanying pulmonary infection and complications.
- *Diabetes, obesity, metabolic syndrome and chronic inflammatory disease* . Advances in the understanding of the immune system and chronic inflammation have led to the concept of 'inflammageing', whereby aging is associated with the advent of chronic inflammation, and the presence of inflammation-associated illnesses, including Type-2 diabetes (Ferruci and Fabbri, 2018; Fulop et al., 2018; Castelo-Branco, 2013). Chronic inflammation is also predicted to rise with obesity (Ferruci and Fabbri, 2018), a risk factor for COVID-19 morbidity. Inflammation is a key mechanism by which elevated ANG II signaling and ACE1/ACE2 imbalance causes injury (**Figures 3-5**). Certain patients with Type 2 diabetes and obesity also have hypertension and hypercholesterolemia; together, these features characterize the metabolic syndrome. The metabolic syndrome is associated with chronic inflammation, which may be a causative feature of this syndrome (Monteiro & Azevedo, 2010; Donath et al., 2019). Increased RAS activity appears to be a pathogenic factor in metabolic syndrome (Skov et al., 2014). Patients with the metabolic syndrome are 'pre-sensitized' to RAS-mediated effects and hence, more vulnerable to dysregulation of RAS by COVID-19.
- *Weakened adaptive immune response* . Within the inflammageing paradigm, aging-associated chronic inflammation via the innate immune system is coupled with a weakened adaptive immune response. The adaptive immune system thus has a reduced ability to establish a defense against SARS-CoV-2, allowing greater viral infectivity and opportunities for tissue injury. Immune-compromised subjects from other causes (e.g. those on immune-suppressive medications) would also be more vulnerable to SARS-CoV-2/COVID-19.
- *ACE polymorphisms* . ACE1 insertion/deletion polymorphisms (I/D) have been widely studied. The D-allele is associated with higher ACE1 activity. Patients with the D allele, especially those with the D/D genotype, are at higher risk of morbidity and mortality from ARDS (Adamzik et al., 2007) and certain cardiac, pulmonary and inflammatory conditions (Gard, 2010; Zhou et al., 2010). The ACE1/ACE2 imbalance hypothesis predicts that patients with the D allele of ACE1, in particular the D/D genotype,

will have elevated severity of COVID-19, as was seen in patients with SARS-1 (Itoyama et al., 2004).

Therefore, the ACE1/ACE imbalance model for SARS-CoV-2 pathobiology can help explain how/why patients with certain underlying conditions/comorbidities are at greater risk if infected with SARS-CoV-2/COVID-19. Elderly individuals are at particular risk, since many of the comorbidities are age-associated. Those with health conditions such as immune deficiencies, diabetes, or cardiac disease will likely be at greater risk for more severe COVID-19 infections and ACE1/ANGII-mediated pathology. By contrast, children (who lack comorbidities associated with ACE1/ACE2 imbalance) are predicted to have less morbidity and mortality from COVID-19.

Opportunities to target the Angiotensin pathway

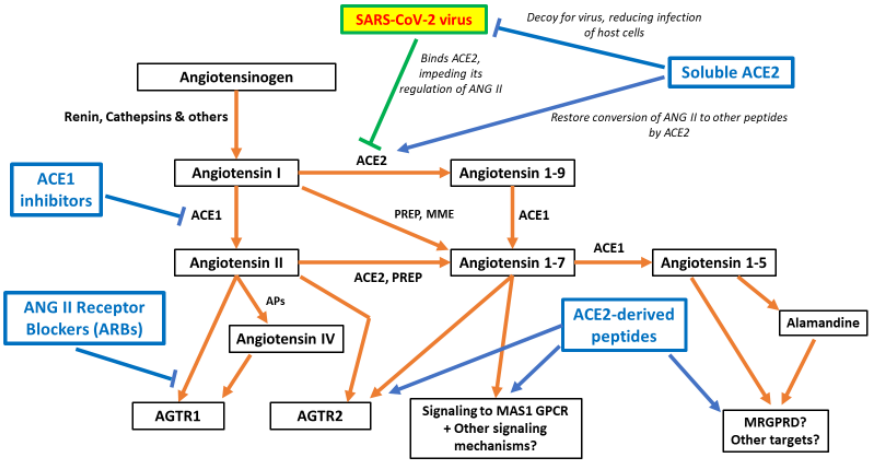


Figure 6. Pharmacological agents that target the RAS pathway and counteract effects of SARS-CoV-2 infection.

The concepts and data discussed above suggest a variety of approaches that may decrease the pathobiology of SARS-CoV2 infection resulting from the imbalance in signaling by peptides generated by ACE1 and ACE2 (**Figure 6**). Several complementary strategies might improve the clinical status and outcome of patients with COVID-19. The goal of these approaches is to restore ACE1/ACE2 balance in favor of ACE2-derived peptides. The proposed therapies, discussed below, are directed at: 1) Inhibition of ACE1; 2) Inhibition of the binding of SARS-Cov-2 to ACE2; 3) Agonism of receptors activated by ACE2-derived peptides; and 4) Inhibition of AGTR1 (ARBs) (**Table 1**).

Inhibition of ACE1

The ability of ACE1 to generate ANG II is a critical step in providing the "driver" for multiple features of the pathophysiology of COVID-19. ACE1 (also known as dipeptidyl carboxypeptidase 1) is a zinc metalloenzyme that removes a dipeptide from the C-terminus of certain peptides, including His-Leu from the inactive decapeptide ANG I to generate ANG II, the active octapeptide. ACE inhibitors approved for clinical use are competitive peptide antagonists that blunt ANG II formation but also the cleavage by ACE1 of the peptide bradykinin. We have found no studies that assessed the efficacy of ACE1 inhibitors in SARS-1 infections. The decrease in ANG II formation would likely be beneficial in such infections. However, since a substantial proportion (˜20%) of patients administered ACEIs develop cough as a side effect (generally attributed to an increase in bradykinin) and dry cough is a feature of COVID-19 infection, this is a potential drawback to this approach.

Inhibition of the binding of SARS-Cov-2 to ACE2

As ACE2 is the key cellular receptor for SARS-Cov-2, approaches that block its interaction with the virus have the potential to maintain ACE2 activity and its generation of products, such as ANG (1-7) that counteract pathological actions of ANG II. One such approach is to use soluble ACE2 as a decoy to bind the virus and spare cellular ACE2 (Zhang et al., 2020). A recombinant human ACE2, GSK2586881, has been tested and was well-tolerated in Phase 1 studies and a Phase 2 trial with ARDS patients (Khan et al., 2017). GSK2586881 was shown to reduce circulating levels of ANG II and Interleukin-6 (IL-6). However, this study was not powered to verify effects on clinical endpoints and direct therapeutic effects on the lungs were unclear. A clinical trial investigating use of recombinant human ACE2 in COVID-19 was initiated in China, but enrollment was subsequently withdrawn (Clinical Trials.gov identifier NCT04287686). Linkage of the extracellular domain of the ACE2 protein to human immunoglobulin G Fc domain is a strategy that could have advantages for treatment of COVID-19, including prolongation of the half-life of ACE2 (Kruse, 2020; Liu P et al, 2018). A related "decoy approach" is the use of the receptor binding domain (RBD) of the SARS S protein that interacts with ACE2 (Wong et al., 2004; Han et al., 2006); this approach has not yet been tested in patients. Small molecules have been identified as inhibitors of coronavirus binding to ACE2 (Adedeji et al., 2013) but their efficacy has only been evaluated in limited pre-clinical studies (Adedeji & Sarafianos, 2014). All these approaches, which block viral entry into cells, maintain ACE2 activity and thus would be expected to blunt disease pathobiology. By contrast, agents that block later steps in viral infectivity (e.g., cellular entry, endosomal inhibition, etc.) will likely yield decrease in ACE2 activity, which may be counterproductive with respect to the generation of beneficial ACE2-derived peptides.

Agonism of receptors activated by ACE2-derived peptides

Ang (1-7) is considered the major ACE2-derived peptide but ACE2 can also generate other peptides, including Ang (1-9) and the heptapeptide alamandine. Ang (1-7) primarily acts via the Mas-1 receptor (**Figure 2**) while response to Ang (1-9) may also involve AT2R; alamandine has effects similar to Ang (1-7) via interactions with the Mas-related GPCR, member D (MrgD) (Alexander at al., 2019). The actions of the ACE2-Ang (1-7)-Mas-1 receptor, ACE2-Ang (1-9)-AGTR2 and ACE2-alamandine-MrgD pathways generally oppose those of the Ang II-AGRT1 pathway, though this remains controversial for AGTR2, as discussed earlier in this text. Activation of the former pathways, in particular via GPCRs that mediate their effects, is thus predicted to blunt COVID-19 pathobiology. Signaling by ANG (1-7) via Mas-1 can increase activity of MAP kinase, phosphoinositide 3-kinase/Akt and NADPH oxidase and subsequently activate effectors that include forkhead box protein 01 (FOXO1), cyclcooxygenase 2 (COX2) and generation of prostanoids and nitric oxide (NO) (Santos et al., 2019; Povlsen et al., 2020). Less is currently known regarding alamandine signaling and action. Additional peptides may be generated as part of non-canonical ("non-classic") Ang II signaling (Santos et al, 2019), Efforts are underway to discover and develop drugs that mimic the physiological peptides in their actions on Mas-1 and Mrgd receptors. Initial clinical studies have tested an oral formulation of hydroxypropyl-β-cyclodextrin-ANG-(1-7) on muscle injury (Becker et al., 2018). As recently reviewed (Wang et al., 2019), targeting of non-canonical Ang signaling pathways has also been initiated in studies of ARDS. Such approaches should be highly suitable for testing in patients with COVID-19.

Inhibition of AGTR1 (ARBs) and potentially post-AGTR1 signaling mechanisms

Antagonism of AGTR1, the primary mediator of Ang II-promoted tissue injury in SARS infections, is an attractive means to improve the course/outcome of patients with COVID-19 by preventing and/or decreasing such injury. This prediction is supported by data from studies of ventilator-induced lung injury and ARDS (Wang D et al., 2019) and other settings of tissue injury (e.g., Arumugam et al., 2016). A recent preprint reported less morbidity and mortality in elderly COVID-19 patients with hypertension treated with ARBs prior to hospitalization (Liu Y et al, 2020). Since numerous ARBs are approved drugs for other indications (e.g., hypertension, heart failure, renal disease), such ARBS could be rapidly tested as therapeutics on a compassionate use basis and in trials to assess their efficacy for COVID-19. Although the use of ARBs is potentially effective in decreasing lung and cardiac injury from COVID-19, possible side effects, including systemic hypotension, may occur in patients receiving those drugs.

Table 1 summarizes information (including the pros and cons) of each of those types of therapeutic approaches. Systemic administration of drugs will likely be the focus of therapies for COVID-19 but should administration by inhalation be considered for direct delivery to the lungs (albeit collapsed alveoli in COVID-19 patients might limit such delivery)? An important advantage of such an approach is to reduce effects/complications from systemic administration. Clinical studies have shown that inhaled NO or prostacyclin can improve oxygenation in ARDS, but without improvement in mortality (Attaway et al., 2017). We speculate that other inhaled agents might offer benefit in the lungs by increasing cellular cAMP (e.g., agonists of β-adrenoceptors or other Gs-linked GPCRs), cGMP (e.g., guanylyl cyclase activators) or both cyclic nucleotides (phosphodiesterase inhibitors). Given the importance of inflammation to COVID-19 pathobiology, as discussed above and in Pedersen & Ho, (2020), inhalational administration of other immunomodulatory/anti-inflammatory drugs may also have utility. Multiple ACEIs and ARBs are available in solution and thus are potential candidates for use with nebulizers. Such an approach might minimize complications from systemic administration of those agents, of particular importance in hypotensive patients or those at risk for hypotension. Of note, both ACEIs and ARBs have been administered experimentally via inhalational methods (Godugu et al., 2013; Suk et al., 2019; Kim et al., 2020).

Much recent debate has occurred regarding the use of ACEIs and ARBs in COVID-19 patients (Gurwitz, 2020; Danser et al., 2020; Diaz, 2020; Fang et al., 2020; Bozkurt et al., 2020; Phadke and Saunik, 2020; Lewis, 2020; Patel and Verma, 2020; Vaduganathan M et al., 2020). Some authors emphasize potential harms of these medications (e.g. Fang et al., 2020, Diaz, 2020) whilst others argue against this idea (e.g. Danser et al., 2020; Bozkurt et al., 2020) or hypothesize benefits of these drugs (e.g. Gurwitz 2020; Phadke and Saunik, 2020; Patel and Verma, 2020). Most of these articles are brief correspondences with limited supporting evidence. As a result, health providers and patients have been confused regarding the administration of ACEIs and ARBs to patients with COVID-19.

Articles with concern regarding the potential harms of ACEI/ARB use in COVID-19 generally cite articles that report administration of ACEI/ARBs may increase ACE2 expression, thereby possibly increasing the risk and spread of infection. We recently analyzed results from animal and human studies and concluded that no consistent, replicable data provide evidence of a relationship between ACEI/ARB use and ACE2 expression (Sriram and Insel, 2020), Here, we focus on the potential benefits of ACEI/ARB and other ways to target the RAS pathway in COVID-19, an idea that others share, e.g., Zhang et al., (2020), who suggested ACE2 as a target.

As listed below several clinical trials for COVID-19 have been initiated that target the RAS pathway. These studies are mostly of small size and are yet to begin enrollment. The targeting of the RAS for therapeutic benefit has thus as-yet not received the attention given to other approaches.

- NCT04335123: Study of Open Label Losartan in COVID-19. Phase-1 trial to evaluate losartan safety in COVID-19; University of Kansas Medical Center
- NCT04312009: Losartan for Patients With COVID-19 Requiring Hospitalization; University of Minnesota
- NCT04335136: Recombinant Human Angiotensin-converting Enzyme 2 (rhACE2) as a Treatment for Patients With COVID-19 [APN01-COVID-19]; Apeiron Biologics and multiple academic collaborators in Europe
- NCT04332666: Angiotensin-(1,7) Treatment in COVID-19: the ATCO Trial (ATCO); Erasme University Hospital in Belgium

Therapeutic approach	Pros	Cons	Comments
ACE Inhibitors (ACEIs)	Mitigates effects of ANG II in relevant cell types/tissues involved in COVID-19 pathobiology Well-established clinical utility Well defined PK and PD Large amount of preclinical animal data Oral administration. Possible inhalable administration Generic Drugs, with established production, supply chain and handling	Does not salvage the protective effects of ACE2 inhibited by SARS-CoV-2 Dry cough is common side effect May not be effective in tissues with high Chymase expression (e.g., heart) Challenges with drug interactions, hypotensive patients, other side effects	Potential for rapid (compassionate) use in at-risk patients and for rapid (repurposing) clinical trials.
Angiotensin Receptor Blockers (ARBs)	Mitigates effects of ANG II (via AGTR1) in relevant cell types/tissues involved in COVID-19 pathobiology Long track record of clinical use Well defined PK and PD Large amount of preclinical animal data Oral administration. Possible inhalable administration Generic Drugs, with established production, supply chain and handling	Does not salvage the protective effects of ACE2 inhibited by SARS-CoV-2 Challenges with drug interactions, hypotensive patients, other side effects Will not impede possible pathological effects of AGTR2	Potential for rapid (compassionate) use in at-risk patients and for rapid (repurposing) clinical trials.
Recombinant soluble ACE2	'Rescues' ACE2 activity inhibited by SARS-CoV-2 infection via action as a 'decoy' for the virus Mitigates effects of ANG II in relevant cell types/tissues involved in COVID-19 pathobiology Substantial preclinical data in animals	Limited data from human studies; unknowns regarding safety Limited information regarding dosing, target engagement, PK, PD etc. Higher costs + more complicated handling, new supply chain Infusion is required	Additional preclinical studies needed, in parallel with early-phase trials

Therapeutic approach	Pros	Cons	Comments
ANG (1-7)	Engages protective MAS1 signaling, mitigating harmful effects of ACE2 inhibition by SARS-CoV-2	Very limited data from human studies Less available preclinical data than for the other options Limited information regarding dosing, target engagement, PK, PD etc., all Indirectly inhibits pathological ANG II signaling Higher costs + more complicated handling, new supply chain Infusion is required	Additional preclinical studies needed. Unclear that human trials are justified.

Table 1 . The pros and cons of different tools for targeting the RAS pathway. PK: Pharmacokinetics; PD: Pharmacodynamics

Summary and Conclusions

The COVID-19 pandemic has sparked an urgent search for effective therapeutics, with little clear success at the time we have prepared this article (early April, 2020). Targeting the RAS pathway has received limited attention even though it is an important component of COVID-19 pathobiology with implications for therapeutics that could ameliorate tissue injury, disease progression and improve morbidity and mortality. A key reason for the limited attention on the RAS system as a therapeutic target may stem from the lack of mechanistic insight regarding the potential benefit of targeting this pathway.

To address this gap, we propose an overriding hypothesis:*imbalance of ACE1- and ACE2-mediated signaling as a primary driver of tissue pathobiology in COVID-19, impacting the phenotypes of multiple interacting cell types in infected tissue, leading to feedback loops that promote inflammation and injury* . Tissue damage from the infection is a consequence of enhanced ANG II/AGTR1 signaling and decreased signaling by ANG (1-7) and perhaps other ACE2-derived peptides. In the lungs and heart (**Figures 3, 5**), the imbalance in the RAS pathway and positive feedback loops can establish a vicious cycle of events mediated by communication among cell types that produce COVID-19 pathology. Similar mechanisms may also occur in other organs.

Numerous studies and findings corroborate this hypothesis, including results from animal models, clinical data in humans and in-vitro findings with human and rodent cells. Moreover, it provides a parsimonious explanation for key features of the disease and for the contribution of comorbidities to adverse outcomes in COVID-19.

The hypothesis also leads to several testable predictions for COVID-19: a) susceptibility for adverse outcomes in those with specific ACE1 genotypes and perhaps other genetic variants in RAS pathway elements; b) potential for patients with a range of illnesses to have worse outcomes; c) adjusting for other clinical variables, patients administered ARBs (and perhaps ACEIs) should have improved outcomes; d) counteracting the imbalance in RAS signaling with agents discussed above should modulate clinicopathological effects of the SARS-CoV-2 virus; e) early administration of RAS-targeted agents may yield maximum benefit, preventing ALI and ARDS by mitigating the damage from the imbalance in ACE1- and ACE2-derived peptides and their signaling.

Given the severity of the COVID-19 crisis, what types of studies (besides clinical trials) might help test our hypothesis? We envisage several possibilities: a) in-vitro studies using human alveolar epithelial cells, perhaps in 3-D organoid models in co-cultures with other key cell types (e.g. fibroblasts, endothelial cells,

immune cells) to determine if cellular injury by SARS-CoV-2 infection is inhibited by ACEIs/ARBs/soluble ACE2. Monteil et al., (2020) have demonstrated efficacy of recombinant ACE2 in blunting infectivity of SARS-CoV-2 in such models but more detailed analyses and further studies are needed. b) In vivo studies to test efficacy of ACEIs/ARBs/soluble ACE2 in animal models [e.g., mice, ferrets and rhesus monkeys, with preference for primate models, e.g., Sutton & Subbarao, (2015); Gretebeck & Subbarao, (2015)] of SARS-1 infection. Ferrets may be useful to assess SARS-CoV-2 infectivity but infected animals appear to have few pathological features (Shi et al., 2020); rhesus monkeys with SARS-CoV-2 infection also show signs of clinical disease (Bao et al., 2020). c) epidemiological data from COVID-19 patients to define associations between ACEI/ARB use/dose and disease severity/mortality. Certain data of this type have been obtained (Liu et al., 2020) but more are needed, especially because of confounding factors in such analyses, including comorbidities relevant to COVID-19. A multi-country database that assessed usage of ARBs and ACEIs prior to hospital admission with clinical outcomes from COVID-19 might be very helpful for such analyses and to define possible differences from patients in different countries.

The hypothesis thus implies that those being treated for approved indications with ACEIs and ARBS should maintain their use of these drugs and in addition, that those drugs may have therapeutic utility in treating patients who develop COVID-19, in particular those most vulnerable to this viral infection (e.g., those >70 years old and/or with comorbidities). ACEIs and ARBs have well known safety profiles, making these drugs well-suited for repurposing. In addition, a rationale exists for testing the possible therapeutic effects of soluble ACE2 or perhaps ACE2 peptides. The current and likely future challenge of treating seriously ill COVID-19 patients argues for aggressive approaches. We urge that these approaches include ones that seek to restore ACE1/ACE2 imbalance.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design & Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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Figure Legends

Figure 1. A schema identifying the tissues impacted by SARS-CoV-2 infection and COVID-19 pathobiology . Dashed arrows identify events whose role is as-yet unclear

Figure 2. Renin-Angiotensin signaling (RAS), Angiotensin (ANG II) signaling, the balance between ACE1 and ACE2, and in red, the impact of COVID-19. APs: Aminopeptidases; PREP: prolyl endopeptidase; MME: membrane metalloendopeptidase.

Figure 3 . Hypothesized model of cell-cell communication and pathobiology in pulmonary infection from SARS-CoV-2 and the role of ACE1- and ACE2-derived peptides in mediating these effects on several different cell types.

Figure 4. ACE1/ACE2 imbalance, lung injury, and factors that determine severe pathology or mild illness. (A.) A model for the course of COVID-19 that links ACE1/ACE2 imbalance to lung injury via the RAS pathway and the protective role of the immune response. **(B.)** An illustration of the predicted course of COVID-19 in two settings:**top** : Severe pathology, in patients who lack appropriate immune response or who have prior conditions that enhance ACE1/ACE imbalance (and RAS-induced injury). The result is increased injury that overwhelms the adaptive immune response; **bottom** : Mild illness, in which patients lack underlying conditions and can mount an appropriate immune response, so that RAS-induced injury is less serious, an effective immune response occurs and the infection is resolved.

Figure 5 . **Hypothesized model of cell-cell communication in myocardial infection from SARS-CoV-2 and the influence of ACE1/ACE2 imbalance on a variety of cell types involved in cardiac injury.**

Figure 6. Pharmacological agents that target the RAS pathway and counteract effects of SARS-CoV-2 infection.