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What's in an ACE: The Role of ACE2 in COVID-19's Tissue Damaging Effects

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Coronavirus disease of 2019 (COVID-19) is the disease state caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the current pandemic. SARS-CoV-2 infects cells via the membrane bound form of angiotensin converting enzyme 2 (ACE2). Aside from its role in viral entry, the role of ACE2 in the morbidity and mortality of severe COVID-19 is often overlooked. ACE2 functions as a member of the renin-angiotensin system (RAS) by cleaving the angiotensin II into angiotensin (1-7). Angiotensin II increases inflammation, thrombosis, and inflammation, whereas angiotensin (1-7) decreases these phenomena, making ACE2 an important balancing agent. ACE2 is downregulated in severe COVID-19 by both viral entry, which internalizes it, and the increased activity of "a disintegrin and metalloprotease 17" (ADAM17), which cleaves it off the membrane, delocalizing it. A meta-analysis was performed to demonstrate the striking resemblance between ACE2 downregulation and severe COVID-19. Sources were examined according to organ, type of ACE2 downregulation, and symptom. The downregulation of ACE2 exhibits similar symptoms in each organ for each pathology. RAS-based treatments of severe COVID-19 attempt to counter the downregulation of ACE2 prevalent in the disease. The most promising is human recombinant soluble ACE2 (hrsACE2; APN01), which both blocks viral entry and

increases the functional ACE2 in the body. It is hoped that this study elucidates what is known and highlights understudied aspects of ACE2 and its role in COVID-19.

keywords and phrases: coronavirus-disease of 2019, angiotensin converting enzyme 2, severe acute respiratory syndrome coronavirus 2, inflammation, fibrosis, thrombosis, ACE2 downregulation

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Introduction

History praises the military prowess of human conquerors, such as Alexander the Great and Genghis Khan. However, disease has proven to be the most successful conqueror of humankind. The dawn of civilization saw people group together in densely populated communities, which made them much more vulnerable to infectious disease.[1] Several of the most notable intersocietal achievements, including trade routes, wars, and colonization, greatly enhanced the spread of diseases, and in return diseases greatly shaped human populations and behavior.[2,3] Cleaner living conditions were found to decrease the transmission of disease, leading such progressions as organized sewer systems, clean water sources, better sanitation availability, and the stigmatization and measures against rodents.[4,5] Quarantine, the isolation of individuals until they prove healthy, has been widely used during pandemics and occasionally during immigration to limit disease spread.[4] Although society has learned many ways to mitigate the risk of disease, pathogens occasionally bypass them and spread within and/or between human populations.

There are many categories of pathogens, including bacteria, viruses, fungi, protozoa, parasites, prions, and viroids. Of these, bacteria and viruses have caused the most detrimental pandemics in history.[2] In recent years, antibiotic drugs have become widely available across the world, especially in developed nations.[6] These drugs have greatly reduced the severity of most bacterial diseases, reduced the likelihood of bacterial pandemics, and raised life expectancy in developed nations.[3,6] The main downside of antibiotic use is that it selects for bacterial strains that develop antibiotic resistance

mechanisms. Some of these strains have spread across the world, gaining notoriety in the medical community.[3,6,7] For example, methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known strain that survives treatment with the antibiotic methicillin; it most commonly infects hospital patients, and some strains of MRSA are also resistant to other antibiotics.[8,9] MRSA is just one of the growing set of "superbugs" that exhibit multi-drug resistance (MDR).[9] However, these antibiotic-resistant bacterial superbugs typically only pose a danger to hospitalized and immunocompromised individuals.[3] Besides these antibiotic resistant bacterial strains, most modern global pandemics are viral, largely due to the lack of broad-spectrum antiviral drugs; antibiotics don't affect viruses, and antiviral drugs typically have too high of a specificity to be useful against multiple viruses.[3,10–12]

Viruses are tiny infectious particles consisting of a protein capsid that surrounds genetic material. With such a simplistic structure, viruses are windows into the very origins of life.[13] Viruses require outside machinery and materials to proliferate.[14] Cellular hosts fulfill this need, providing the ribosomes that will make viral proteins, the amino acids that will form these proteins, and nucleotides to replicate the viral genome.[15] Almost every cellular organism, whether prokaryote or eukaryote, has viruses that specifically target it by exploiting unique cellular proteins and incorporating them as an essential part of the viral life cycle.[15] For bacteriophages, sometimes shortened to phages, the hosts are bacteria; phages are model organisms for viruses and are the most abundant organisms on the planet; they inject their genomes into bacteria from the outside in an attempt to replicate by hijacking the bacteria's resources.[16] Another group of viruses called virophages can infect other viruses, depending on both their helper-virus and its

cellular host to complete their reproductive cycle.[14] Regardless of the host, viruses utilize cellular machinery to multiply at much higher rates than any cells.[3] The high-speed viral replication can contribute to carcinogenesis, the formation of cancer.[17]

Several reproduction strategies exist for viruses, depending on the type of genetic material the virus uses: RNA or DNA, how many strands of genetic material it has: single- or double-stranded, and whether it contains genetic exons directly encoding viral proteins or requires a complementary strand: positive- or negative-sense, respectively.[13] In general, viruses utilize two reproduction cycles, each having different consequences for the host. In the lytic cycle, a virus infects a cell, reproduces in large enough numbers to burst or lyse the host cell, then the new viruses search for a new host cell, leaving the dead host cell behind.[18] In the lysogenic cycle, a virus infects a cell and integrates its genome into that of the host, leading to the production of more viruses.[18] The lysogenic cycle does not immediately kill the host cell, and can even provide it with evolutionary advantages via "moron genes."[18,19] These beneficial genes often protect the host from infection by other viruses, adding a symbiotic element to the virus-host relationship.[19]

All viruses, regardless of their reproductive strategies, must generate mRNA from which the host cell ribosomes can create new viral proteins.[13] All viruses copy their genomes using host cell resources and encapsidate the produced genomes within the protein capsids produced.[13] DNA viruses use the host cell transcription enzymes to create mRNA, and some are capable of being incorporated into the host cell genome.[13] RNA viruses either use RNA-dependent RNA-polymerase to both copy their genomes and make enough mRNA to hijack most of the host cell ribosomes, or use reverse transcriptase to make complementary DNA that is integrated into the host genome to be

transcribed into mRNA; the latter RNA viruses are called retroviruses.[13,15] Endogenization, the integration of viral genes into host genome, is a process that all viruses of eukaryotes have potential for; retroviruses such as HIV utilize endogenization as an essential part of their lifecycle.[15]

Due to endogenization and the incorporation of foreign DNA into their hosts, viruses have shaped the genomes of modern species.[15,20] At least 8% of the human genome can be traced to retroviruses.[20] Viruses can trade genes among themselves and with their hosts in the process of horizontal gene transfer; they can also infect cells that aren't their optimal host in the process of zoonosis.[15] These two processes, combined with rapid reproduction, make the creation of new viruses not only possible but expectable.

In general, RNA viruses are more likely to cause pandemics than DNA viruses because they mutate more quickly, which results in higher variant quantity and the potential for higher fitness.[3] With complex modern trade and travel routes, the lack of broad-spectrum antiviral drugs, the high rates of proliferation and mutation in viruses, and the possibility of zoonosis, cross-species viral transmission due to the close proximity of humans to animals, the modern world is particularly vulnerable to viral pandemics. An RNA virus that recently evolved successfully exploited this vulnerability.

In December of 2019, a new sickness causing respiratory symptoms emerged in people residing near an animal market in Wuhan, China.[21] The virus responsible for the pandemic is named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), while the disease state it causes is termed coronavirus disease 2019 (COVID-19).[22] The number of COVID-19 cases grew exponentially, and the virus proved to spread more

quickly than influenza.[21,22] In a matter of months, COVID-19 became an global pandemic.[21] There have been 153,505,262 reported cases of COVID-19 in 219 countries (May 3, 2021).[23] 3,216,599 of these cases resulted in death (May 3, 2021).[23]

The virus spreads through the air in aqueous aerosols and droplets, which can originate from the coughs and breathing of infected individuals, whether they are symptomatic or not.[24,25] Knowledge that SARS-CoV-2 spreads through the air has led to the widespread use of protective masks, which decrease the intake and output of the virus during breathing.[25] Sanitation of surfaces can neutralize virus particles and also help limit transmission.[25] Another preventative measure involves testing sick people and travelers for the disease.[26,27] Quarantining those who test positive limits the amount of people they can transmit the disease to, and in certain cases quarantining entire populations is necessary to halt the spread.[27]

Symptoms of COVID-19 vary from person to person, but there are some well documented patterns, with 3 standing out: mild, severe, and asymptomatic.[24] Most reported cases of COVID-19 are mild, though still unpleasant.[28] In the first few days of COVID-19, there are little to no symptoms.[24] After an incubation period of 4-5 days, symptoms may appear.[24] Symptom experience varies between cases; common symptoms include, but are not limited to, fever, cough, headache, fatigue, anosmia: loss of smell, sore throat, myalgia: muscle soreness, seemingly chilblained red/purple “COVID toes,” and gastrointestinal symptoms such as nausea, vomiting, and diarrhea.[21,24,29] People with certain pre-existing conditions, such as type 2 diabetes, cardiovascular disease (CVD), chronic respiratory disease, and cancer, tend to have worse outcomes with COVID-19.[30] More severe symptoms may begin to appear at an average of 5 to 8 days after initial

symptoms, typically beginning with breathlessness, then ranging from respiratory problems such as severe pneumonia, acute lung injury, and acute respiratory distress syndrome (ARDS) to other problems, such as kidney damage, heart problems, and multi-organ failure.[24,30] For severe COVID-19 cases, hospitalization is necessary. Treatments include oxygen masks and, in more life-threatening cases, mechanical ventilators.[30] Though many COVID-19 symptoms and nuances seem unrelated to each other from a macroscopic level, they make much more sense by looking more closely how SARS-CoV-2 interacts with its host.

SARS-CoV-2 enters cells through the membrane-bound form of an enzyme called angiotensin-converting-enzyme 2 (ACE2), which is internalized during viral entry.[31] Cells infected by SARS-CoV-2 lose functionality, leading to many of the minor symptoms of COVID-19, such as anosmia.[32] The relationship of SARS-CoV-2 and ACE2 goes beyond a simple virus-receptor interaction.[33] Extensive infection by SARS-CoV-2, as seen in severe cases of COVID-19 where the viral infection and/or immune response occur at dangerous levels, reduces the amount of ACE2 in areas with a high viral presence, having major consequences.[33] ACE2 has anti-inflammatory, anti-fibrotic, and anti-thrombotic effects as a part of the renin-angiotensin system (RAS).[33] The downregulation of ACE2 by SARS-CoV-2 in severe COVID-19 has inflammatory, fibrotic, and thrombotic effects in the lungs, the cardiovascular system, and the kidneys. We performed a meta-analysis to demonstrate the relationships between ACE2, COVID-19, and organ failures. Examining SARS-CoV-2 and the RAS in close detail will help to explain this relationship.

SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes coronavirus disease 2019 (COVID-19). Although SARS-CoV-2 is a novel coronavirus, it follows many of the well-studied patterns seen in other coronaviruses. Coronaviruses are enveloped in a lipid-protein envelope and contain positive sense single-stranded RNA genomes.[34] Their microscopic appearance is that of a membrane surrounded by a crown, which is why they are called coronaviruses: *corona* is Latin for crown.[35] Coronavirus genomes are around 30,000 base pairs long, making them the largest RNA viruses known.[35] SARS-CoV-2 has about 29,900 bases in its genome.[36] The first two thirds of coronavirus genomes encode a polyprotein that is proteolytically cleaved into smaller proteins with virus-creating functions, while the final third encodes structural proteins that protect the genome and facilitate entry via a host cell receptor.[34]

The coronavirus family is divided into four genera: alpha and beta coronaviruses infect primarily mammals, gamma coronaviruses infect primarily birds, and delta coronaviruses can infect both mammals and birds.[35,37] Though there are many viruses in the coronavirus family, only seven are known to infect humans: HKU1, NL63, OC43, 229E, Middle East respiratory syndrome (MERS), severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2.[37,38] HKU1, NL63, OC43, and 229E each cause mild symptoms consistent with the common cold.[37] On the other hand, SARS-CoV, MERS, and SARS-CoV-2 have higher infection and mortality rates.[37]

Coronaviruses arise in humans through zoonosis, a host-species-switching process where viruses from one animal species infect another animal, typically from genome mutations and close physical proximity that select for this change.[39,40] To confidently identify the immediate ancestor of a virus, another virus with nearly identical genome must be identified (>99% similarity).[41] Bats and birds are a major reservoir for coronaviruses, but they are not the only animals involved in transferring coronaviruses to humans.[40] It is widely accepted that bat coronaviruses gave rise to MERS, SARS-CoV, and now SARS-CoV-2.[36,42] However, with MERS and SARS-CoV, intermediate hosts were also involved that accepted bat coronaviruses and gave rise to human coronaviruses.[39,42] For MERS, the intermediate host was the dromedary camel.[39] For SARS-CoV, the intermediate host was the civet cat.[36,39] The previous hosts for NL63 and 229E were bats.[39] Bovine and rodent coronaviruses gave rise to OC43 and HKU1, respectively.[39,43] The zoonotic origins of SARS-CoV-2 are not yet fully understood, but bats are the most likely source, with the bat coronavirus RaTG13, found in China, having a 96.21% genome similarity to SARS-CoV-2.[41,44] Some scientists are skeptical of RaTG13's legitimacy, but other scientists have found similar strains of bat coronaviruses in nearby countries such as Thailand.[41,44] Climate change may have affected bat population density in Southeastern Asia so as to promote coronavirus zoonoses.[45] Pangolins are also being considered as potential zoonotic sources, as certain pangolin coronaviruses have similar receptor-binding domains on their spike proteins to that of SARS-CoV-2.[41,44] Previously discovered human coronaviruses are not suspected of mutating into SARS-CoV-2, as they are too dissimilar. SARS-CoV is the most similar, sharing only 79.5% genomic homology with SARS-CoV-2.[36] MERS has only about 50%

genomic homology with SARS-CoV-2.[36] Identifying the zoonotic source of SARS-CoV-2 is crucial to preventing similar catastrophic zoonoses in the future.

Coronaviruses share several structural proteins that differ in sequence but not function. The general structure for SARS-CoV-2 is shown in **Figure 1**. Membrane (M) proteins organize as 2-dimensional lattices to form most of the outer virion, or virus structure, both initially as a frame to build the rest of the virion and in the completed virion.[46] Envelope (E) proteins are also part of the external virion structure; they oligomerize-combine with other E proteins-to form pores that may disrupt ion gradients within the endoplasmic reticulum or the Golgi bodies, leading to the exocytosis of the virion.[46] When both M and E proteins are produced, spherical structures are produced in combination with the endoplasmic reticulum membrane.[46]

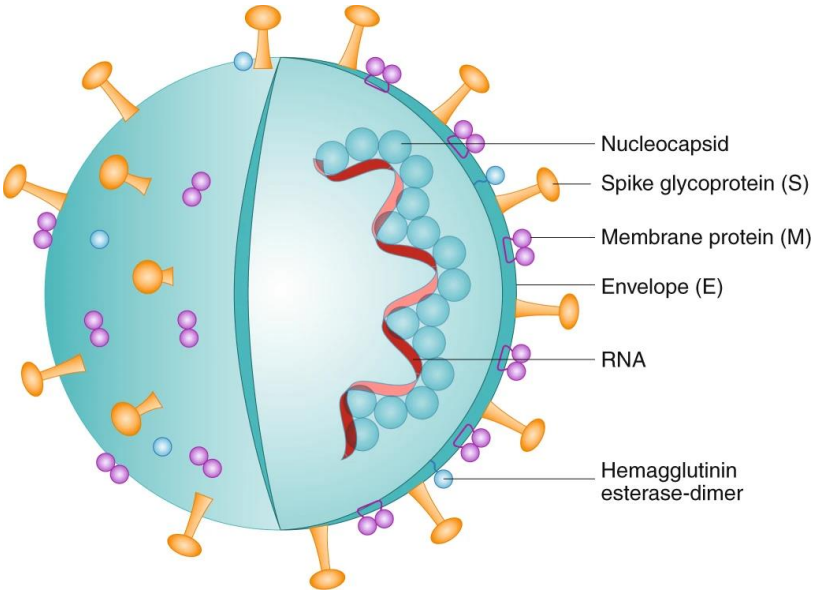

Figure 1: SARS-CoV-2 structural diagram. The nucleocapsid protein and RNA lie within the virus. The outer envelope contains envelope proteins, spike proteins, membrane proteins, and hemagglutinin esterase. Image from Florindo et al., 2020.[47]

The third structural protein on coronaviruses is the nucleocapsid (N) protein. N proteins are highly expressed in coronaviruses and associate with their RNA within the capsid through electrostatic interactions.[46] N proteins aid in viral entry, influencing cellular processes, and facilitating RNA conformational shifts as RNA chaperones.[46,48] They can even inhibit certain immune responses such as interferon production, increasing susceptibility to the virus.[46]

The fourth and largest structural protein all coronaviruses express is the spike (S) glycoprotein.[48] The spike is highly glycosylated and initiates viral entry into cells.[48] The spike is a homotrimer with two subunits.[48] The S1 subunit contains a receptor-binding domain (RBD) that initiates entry upon binding a host receptor.[48] The S1 subunit can have an active "up" conformation or an inactive "down" conformation.[46] After binding a host receptor, the S1 subunit dissociates from the S2 subunit.[46] The S2 subunit then facilitates entry by either fusion or endocytosis, depending on the specific coronavirus and/or the host's characteristics.[49] The spike protein extends the furthest away from the center of the virus, and is thus the optimum target for adaptive immunity, whether by vaccination or direct exposure to SARS-CoV-2. It is also the characteristic that gives rise to the crown-like appearance of coronaviruses, as seen in **Figure 2**. Although each coronavirus expresses spike proteins, their respective spikes can have different targets. The MERS spike targets dipeptidyl peptidase 4.[35] 229E targets aminopeptidase N.[50] OC43 and HKU1 spikes bind to sugars called 9-O-acetylated sialic acids.[35,51] NL63, SARS-CoV, and SARS-CoV-2 all bind membrane-bound angiotensin converting enzyme 2 (ACE2), despite NL63 having a much different spike protein from SARS-CoV and SARS-CoV-2.[35,52]

Figure 2: SARS-CoV-2 particles isolated from a patient, viewed with transition electron microscopy.[53] Image captured and colored by the National Institute of Allergy and Infectious Disease (NIAID) Integrated Research Facility (IRF) in Fort Detrick, Maryland.[53]

Some coronaviruses express a fifth structural protein called hemagglutinin esterase (HE). HE is only expressed by beta coronaviruses, which for humans include OC43, HKU1, MERS, SARS-CoV, and SARS-CoV-2.[48] It is another component of the viral envelope for these viruses. HE is thought to have receptor destroying properties in OC43 and HKU1, but it is understudied, especially in the case of SARS-CoV-2.[43,48]

Although SARS-CoV-2 follows the structural patterns outlined, it also has some unique features not seen in other human coronaviruses. Although the genomes of the

human coronaviruses differ among all the proteins that they encode, the spike protein is where these differences stand out. The SARS-CoV-2 spike protein RBD binds ACE2 with higher affinity than the SARS-CoV spike RBD, despite both binding to the same region on ACE2.[38,54] Also, certain strains of SARS-CoV-2 have mutations in the spike and its RBD that increase affinity for ACE2 beyond the original Wuhan strain.[55] An example of this acquired advantage via spike mutation is the strain first identified in South Africa (lineage B.1.351).[55] The differing affinities for ACE2 among SARS-CoV, SARS-CoV-2, and mutant SARS-CoV-2 spike proteins are due to key residue differences in the RBDs of these spike proteins.[38,55] Another unique feature of the SARS-CoV-2 spike protein is the presence of a furin cleavage site, an RRAR insertion where the SARS-CoV spike has just an R, between the S1 and S2 subunits.[38] The presence of a furin cleavage site is associated with higher infectivity and a larger host range; the furin cleavage site allows the S1 and S2 subunits to separate upon being acted on by a compatible protease like furin, after which the S1 subunits float away and the S2 trimer facilitates fusion.[38] For SARS-CoV-2, the proteases that work the best at facilitating this cleavage are TMPRSS2, a cell surface serine protease, and lysosomal proteases such as cathepsin.[56] These same proteases also help activate fusion in SARS-CoV by activating its spike, although the furin cleavage site is absent.[56] A recent study that removed the furin cleavage site on SARS-CoV-2 found that pathogenesis was reduced.[57] Interestingly, exposure to SARS-CoV-2 with this furin cleavage site removal provided adequate immunity to fight wild-type SARS-CoV-2.[57] Thus, the SARS-CoV-2 spike binds ACE2 with high affinity at the RBD and has a furin cleavage site, both of which contribute to a higher infectivity than that seen in SARS-CoV.

The entry mechanism of SARS-CoV-2 is becoming highly researched but remains contentious in certain aspects; still, enough is known for a general outline of the process. The infection cycle of SARS-CoV-2 begins when its RBD on S1 binds to membrane-bound ACE2.[56] Upon this binding, depending on the presence or absence of the TMPRSS2, two entry pathways exist (**Figure 3**).[49,58] If TMPRSS2 is present on the cell surface, it cleaves the spike protein at the S1/S2 border.[49] S1 dissociates from the virus, floating away in the extracellular fluid likely with its receptor, ACE2.[54,56] The S2 subunit facilitates membrane fusion with the cell, after which the viral RNA is released into the cytosol.[49] It is unknown whether this membrane fusion creates a temporary pore or a permanent and complete fusion.[49] Since TMPRSS2 expression is higher in men, as its expression depends on androgen levels, excess in this entry pathway potentially contributes to the differing severities of COVID-19 between the sexes.[59] Alternatively, if TMPRSS2 is not present, endocytosis occurs.[49] This typically but not necessarily involves clathrin, an endocytic protein.[49] Once the virus is taken into an endosome, the endosome travels to and fuses with a lysosome.[49] Within this lysosome, proteases such as cathepsin and furin facilitate the fusion event by cleaving the S1/S2 cleavage site, releasing the S1 subunit and ACE2 into the lysosome.[49,60] S2 then facilitates the fusion, and the viral RNA is released into the cytosol.[49] Once the viral RNA is released into the cytosol, the two pathways converge.

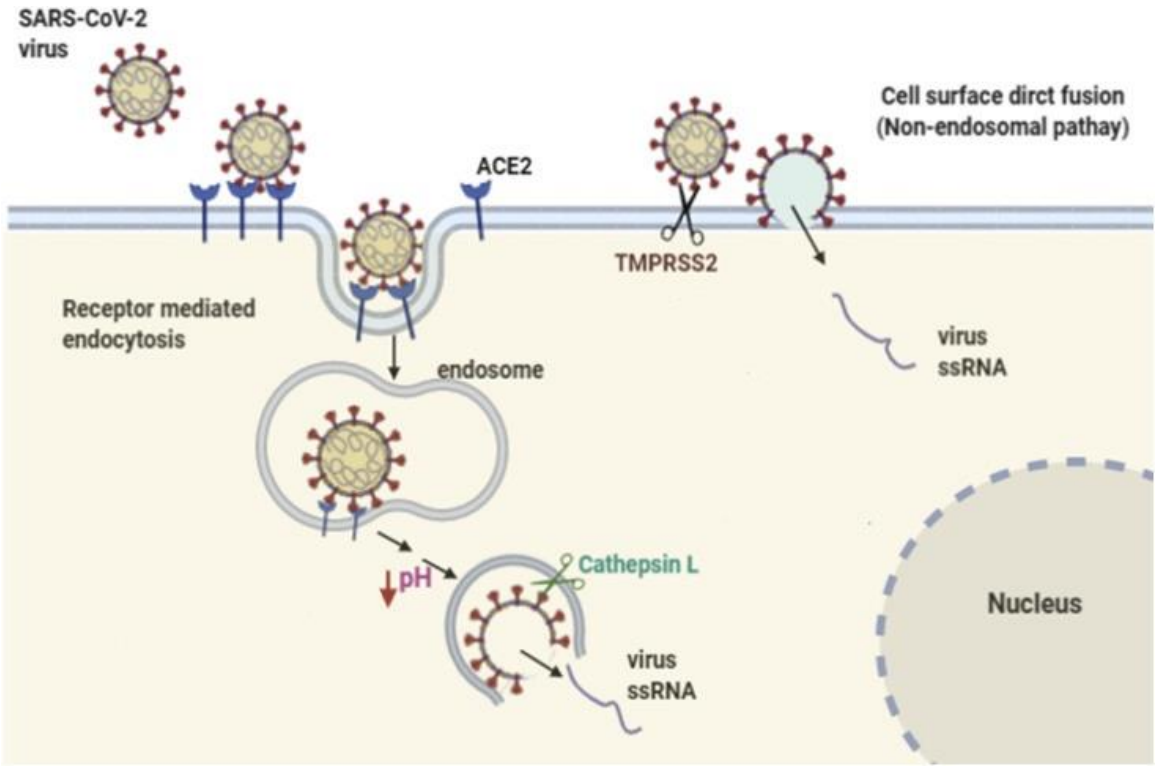

Figure 3: SARS-CoV-2 entry occurs by either endocytosis or direct fusion. Diagram modified from Mahmoud et al., 2020.[58]

Once in the cytosol, the viral RNA travels to and binds the host ribosomes and translation occurs, producing proteins and polyproteins that divide into several functional proteins that help in the virus-making process.[61] Perhaps the most important of these functional proteins is RNA-dependent RNA polymerase (NSP12), which copies and greatly amplifies the amount of viral RNA within the cell.[61] The RNA in the cell accumulates, and ribosomes start making lots of viral structural proteins. The M, S, E, N, and HE proteins form virions, many of which successfully contain a viral RNA strand. The completed viruses are then ready to leave the cell. There are two main mechanisms for leaving the cell. Extrusion, or budding, is a form of exocytosis in which the virus is neatly released to the interstitium.[62] Lysis occurs when the cell builds so many viruses

that the pressure bursts the cell, releasing all of the viruses and killing the cell. Regardless of the way the viruses are released, they immediately search for new cells to infect by blindly extending and retracting their new spike proteins, relying on Brownian motion to connect them with ACE2. But ACE2 is more than just a receptor for SARS-CoV-2.[63] Its function is critical, and when its levels are insufficient, the effects are devastating.

Renin-Angiotensin System

Angiotensin converting enzyme 2 (ACE2) is involved in a complex pathway called the renin-angiotensin system (RAS). The RAS governs many aspects of the body's organs and cardiovascular system, and research continues to reveal new functions and mechanisms of the RAS.[64] The first RAS component was discovered in 1898 by Robert Tigerstedt and his student Per Bergman.[64,65] They noticed that a molecule from the renal cortex caused blood pressure to be raised, and called that molecule renin.[64,65] Renin was forgotten about until its rediscovery the 1930's, and by 1970's many more RAS components were identified.[64]

Around that time, the RAS was considered to consist of a sequential string of reactions occurring by enzymes from various organs in the circulation.[64] This classical RAS pathway goes as follows: renin from the kidneys cleaves angiotensinogen from the liver into angiotensin I, which is then turned into angiotensinogen II by angiotensin-converting enzyme (ACE) mostly in the lungs.[64,66] The angiotensin II then causes the release of the hormone aldosterone from the adrenal cortex, and blood pressure is raised.[64,66] Although all of this is true, the RAS has now been proven to be much more extensive in its participants, its functionally, and where it occurs in the body.[64]

One popular modern model of the RAS focuses on the existence of two connected pathways, the "classical pathway" and the "alternative pathway," that work in opposition to each other (**Figure 4**).[67] Although more complex models exist that outline more reactants, receptors, and effects, the dual pathway model is adequate to describe the

physiological role of ACE2.[67] The classical pathway consists of the aforementioned linear pathway wherein angiotensinogen is converted into angiotensin I by renin, and angiotensin I is converted to angiotensin II by ACE.[64,66,67] The alternative pathway was discovered more recently; its key enzyme ACE2 was discovered in 2000.[33] In this pathway, angiotensin I and angiotensin II are converted to angiotensin (1-9) and angiotensin (1-7), respectively, by ACE2.[33,67] The effects of these two pathways are opposite: The classical pathway promotes vasoconstriction, fibrosis, inflammation, coagulation, edema, oxidative stress, and the water reabsorption hormones aldosterone and antidiuretic hormone (ADH).[33,67,68] The alternative pathway, on the other hand, has vasodilative, antifibrotic, anti-inflammatory, anticoagulative, anti-edemic, antioxidant, and natriuretic effects.[33,67,68]

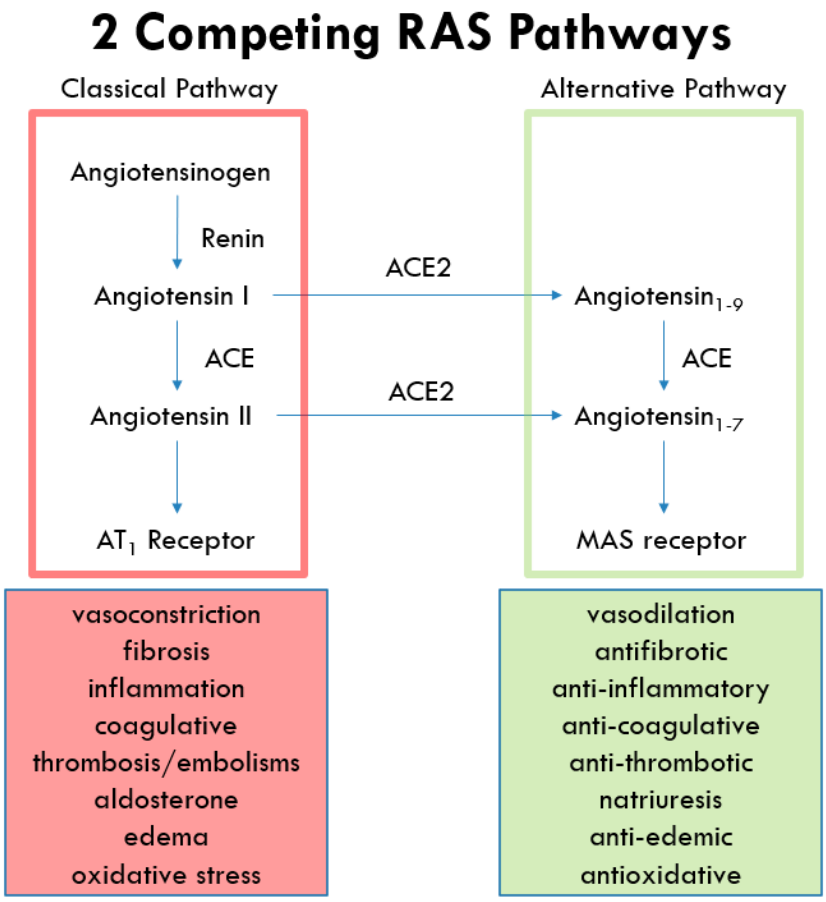

Figure 4: The dual pathway model of the RAS. Made with information from Ocaranza et al., 2020, Verdecchia et al., 2020, and Te Riet et al., 2015.[33,67,68]

RAS enzymes have often been associated with certain organs and assumed to be irrelevant in others.[64] Although there are expression differences for RAS enzymes for each organ, there is enough expression to generate a "local RAS" for each organ and blood vessel.[64,69] When the whole body's RAS or the local RAS for a tissue are unbalanced, pathologies result.[67–70]

The RAS begins with the liver, which produces and secretes most of the body's angiotensinogen.[71] Interestingly, angiotensinogen is also produced in various other organs, including the kidneys, heart, and even the brain.[71–73] Angiotensinogen is the precursor to the angiotensin peptides, and thus both RAS pathways depend on it to elicit changes.[67,72]

Renin is an aspartate protease that converts angiotensinogen into usable angiotensin I.[74] The major source of renin in the body are the juxtaglomerular cells of the kidney.[74] However, prorenin, the renin precursor, is expressed throughout the body.[74] Prorenin is also present in the circulation in levels much higher than renin.[74] This prorenin is inactive, but can give rise to functional renin.[74]

Angiotensin I is a short peptide involved in both the classical and the alternative RAS pathway.[67] Angiotensin I is not known to bind receptors directly, but it is a precursor for multiple receptor ligands.[67] The levels of ACE and ACE2 determine what it becomes.[33]

Angiotensin converting enzyme (ACE) is an integral component of both the classical and alternative RAS pathways.[67] It facilitates the conversion of angiotensin I to angiotensin II as well as the conversion of angiotensin (1-9) to angiotensin (1-7).[67] ACE is originally expressed on cell membranes, but can be cleaved to a soluble form that is still enzymatically active.[75] Many tissues express ACE, including but not limited to the lungs, heart, kidneys, and even certain immune cells like macrophages.[70]

Angiotensin converting enzyme 2 (ACE2) is the gateway for commitment to the alternative RAS pathway.[67] It converts angiotensin I into angiotensin (1-9) and angiotensin II into angiotensin (1-7).[67] This causes a decrease in the effects of angiotensin II and an increase in the effects of angiotensin (1-7), both of which will be discussed shortly. ACE2 shares about a 42% sequence identity to ACE.[75] ACE2 also is initially membrane bound, with the potential to be cleaved into a soluble but still active form.[75,76] A disintegrin and metalloprotease 17 (ADAM17) is one membrane enzyme capable of performing this cleavage.[33,76] ACE2 is expressed throughout the body, especially in the lungs, heart, kidneys, liver, colon, testes, and blood vessels.[33,75,76] Several polymorphisms of ACE2 exist in populations, with catalytic efficiency being reduced in some of them.[77] ACE2 expression is often increased in damaged organs as an attempt to facilitate healing.[78] Often a higher soluble ACE2 level is associated with a lower membrane-bound ACE2 level in organ tissues, and thus indicates a RAS imbalance for those organs in favor of the classical RAS pathway.[79,80] The local ratios of ACE and ACE2 are important in determining which RAS pathway that tissues favor.[76,81]

Angiotensin II is the main facilitator of the classical RAS pathway. It acts on the angiotensin II type 1 receptor (AT_1R) to elicit the vasoconstrictive, fibrotic,

inflammatory, coagulative, oxidative, and water-reabsorbing effects of the classical RAS.[33,67] Alternatively, it can bind to other receptors such as the angiotensin II type 2 receptor (AT_2R) and the angiotensin II type 4 receptor (AT_4R) to cause similar effects to those of the alternative RAS.[33,67,68] However, the effects of AT_2R and AT_4R are not typically pronounced enough to balance the effects of angiotensin II has when binding the AT_1R, making high levels of angiotensin II damaging.[33]

Angiotensin (1-9) is formed from angiotensin I by ACE2.[33,67] It is capable of binding to the AT_2R, countering the effects of the classical RAS.[67] It is also the precursor to angiotensin (1-7), the main facilitator of the alternative pathway.[33,67] This conversion is done by ACE.[33,67]

Angiotensin (1-7) is the final product in the alternative RAS.[33,67] It binds to the Mas receptor (MasR) to elicit vasodilative, antifibrotic, anti-inflammatory, anticoagulative, anti-edemic, antioxidant, and natriuretic effects.[33,67–69] Angiotensin (1-7) is also associated with slowing down aging and has healing effects on organs.[69,82]

In the context of COVID-19, it is easy to perceive ACE2 as the receptor for SARS-CoV-2 without considering the function of ACE2 in the RAS. Several disease states are associated with RAS imbalances, and severe COVID-19 matches the symptoms of these imbalances. Although the direct relationship between severe COVID-19 and RAS imbalances are largely understudied, viewing COVID-19 from the perspective of the RAS elucidates why certain symptoms are experienced in ways that a traditional viral infection perspective fails.

ACE2 Downregulation

Most ACE2 in the body is in the membrane-bound form, but some soluble ACE2 is also detectable in healthy individuals. ACE2 downregulation occurs when subnormal levels of membrane bound ACE2 are present in a tissue. These downregulations can be local, affecting only one tissue through a local RAS, or systemic, affecting the whole body by pervasive downregulation. Recent studies have indicated ACE2 downregulation to be a major factor in several pathologies, and new research is consistently broadening the list of disorders that ACE2 downregulation plays a role in.

Several pathologies are associated with less membrane bound ACE2 presence. The most notable examples are hypertension, type 2 diabetes mellitus, and cardiovascular disease.[83,84] Some others include organ fibrosis, thromboembolisms, acute respiratory distress syndrome (ARDS), and even certain cancers.[33,84–86] Patients with each of these conditions tend to have a higher morbidity during COVID-19.[30,33,87,88] Often, the downregulation of membrane ACE2 in tissues is accompanied by an increase in serum ACE2.[79,84] This is also often seen in severe cases of COVID-19.[89] This is likely due to increased ADAM17 activity, which facilitates this change.[79,84]

In addition to the association of lower membrane bound ACE2 levels with these disorders, ACE2 levels also vary in healthy individuals according to several controllable and uncontrollable factors.[84] Smoking increases ACE2 expression in the lungs, likely as an attempt to heal the damages done by the inhaled toxins.[84] Diets high in salt also increase ACE2 expression and activity to decrease the amount of sodium in the blood by

reducing the aldosterone production and secretion that is caused by angiotensin II, thus promoting natriuresis.[84] Glucose and fat-rich diets reduce ACE2 levels.[33,84] ACE2 levels decrease with age, which is likely due to increased ADAM17 activity, as serum ACE2 levels increase with age.[33,79,84] This age effect is sex-dependent, with males losing membrane ACE2 sooner in life than females.[79,84] One theory explains this sex-effect as a being independent to the amount of sex chromosomes present, despite ACE2 being present on the X chromosome; however, one study found the level of ACE2 expression in mouse kidneys to be dependent on estrogen levels, which are higher in females.[90]

Several mechanisms have been proposed for the downregulation of ACE2 in COVID-19, but this downregulation is largely understudied. This is perhaps due to the overshadowing of ACE2's role in the RAS by its role in the entry of SARS-CoV-2.[33] Other mechanisms that may play a part are barely being considered, if at all. **Table 1** summarizes four mechanisms that will be discussed shortly. It is most likely true that a combination of the following mechanisms, rather than just one, explain the downregulation of ACE2 seen in severe COVID-19.

Mechanism of ACE2 Downregulation in Severe COVID-19	**Effect on ACE2**
Entry of SARS-CoV-2	Removal from cell surface; internalization
Increased ADAM17 activity	Cleavage; less on cell surface, more in serum
HE activity?	Destruction
Genetic Downregulation by COVID-19?	Less production

Table 1: Proposed mechanisms of ACE2 downregulation in severe COVID-19. Each of these mechanisms would reduce the amount of ACE2 on the tissues dealing with infection. The first two mechanisms are most likely correct. The last two mechanisms are largely unproven and understudied in the case of SARS-CoV-2 and COVID-19, which is why there is a question mark after them.

The two roles of ACE2 in COVID-19 as the receptor for SARS-CoV-2 and a healing component for the organs damaged are not mutually exclusive. When SARS-CoV-2 enters the cell, it internalizes ACE2 in a way that prevents its return to the cell surface.[31,78] When lots of viruses are internalized, as in severe cases of COVID-19, enough ACE2 internalization occurs to effectively downregulate local ACE2 levels, whichever tissue the extensive infection is occurring in.[31,78] Higher TMPRSS2 expression allows for more SARS-CoV-2 and ACE2 to be internalized, exacerbating this mechanism of downregulation.[84]

Another way that severe COVID-19 likely downregulates ACE2 is by increasing the activity of ADAM17, which sheds the membrane bound ACE2.[31] Although this leads to higher soluble ACE2 levels in the serum, it is important to realize that serum ACE2 is no longer exclusively present at its source tissue, and thus excessive ADAM17 activity can deprive an organ of its ACE2.[84] ADAM17 activity is increased by the presence of inflammatory cytokines.[84] Severe COVID-19 patients often experience cytokine storms, ubiquitous inflammatory cytokine presence that likely increase ADAM17 activity.[84,91] The high levels of soluble ACE2 often found in severe COVID-19 patient serum are consistent with this mechanism.[92]

COVID-19 may involve other mechanisms of ACE2 downregulation as well. Hemagglutinin-esterase (HE) is present on SARS-CoV-2, as described in the previous section.[43,48] HE has been known to degrade entry receptors in other viruses such as influenza.[43,48] The effects of the HE of SARS-CoV-2 on ACE2 are largely unknown, but perhaps degradation occurs that could contribute to ACE2 downregulation.

Another understudied mechanism is genetic downregulation, wherein less ACE2 mRNA is transcribed. Studies showed that infection with SARS-CoV, which also infects via ACE2, led to less ACE2 mRNA transcription.[93] If the same phenomenon occurs with SARS-CoV-2, this would reduce the amount of ACE2 present on organs experiencing this effect.

Different ACE2 polymorphisms also have differing efficiencies.[77] Although this is not downregulation, mutant ACE2 can have the same effects as ACE2 downregulation. Some less efficient varieties of ACE2 are likely associated with risks for more severe COVID-19.[77] Another consideration with ACE2 polymorphisms is that some likely have a higher affinity for the SARS-CoV-2 spike than others.[94]

Regardless of the circumstances behind ACE2 downregulation, it presents with a clear pattern of symptoms. Organs deficient in ACE2 experience exaggerated classical RAS effects from angiotensin II, leading to inflammation, thromboembolisms, and suboptimal healing that often includes fibrosis.[31,33,78,84] These effects are well documented in ACE2 knockout models, ACE2 deficient disease states, and severe COVID-19. The following meta-analysis illustrates this correlation.

Inflammation

Inflammation is the process wherein white blood cells (WBCs) infiltrate organ tissues to counter infectious agents that may be present. Once these WBCs enter the tissues, they secrete harmful chemicals to kill the pathogen.[95] Inflammation is initiated when an organ's resident macrophages secrete cytokines and chemokines that attract circulating cells of innate immunity, such as monocytes and neutrophils.[95,96] Inflammation continues until the infection is destroyed. If the innate immune system is insufficient for several days, the adaptive immune system attempts to stop the infection. The adaptive immune system, especially its T cells, can also promote inflammation, and can deal collateral damage to healthy tissue cells.[96] In the effort to contain an infection, inflammation also generates cell-damaging reactive oxygen species (ROS).[97] The RAS plays a major role in inflammation both for innate and adaptive immunity.[98] When angiotensin II binds the AT_1R on immune cells, a transcription factor called nuclear factor κB (NF-κB) is activated and causes the transcription of pro-inflammatory genes encoding cytokines and chemokines (**Figure 5**).[95,98,99]

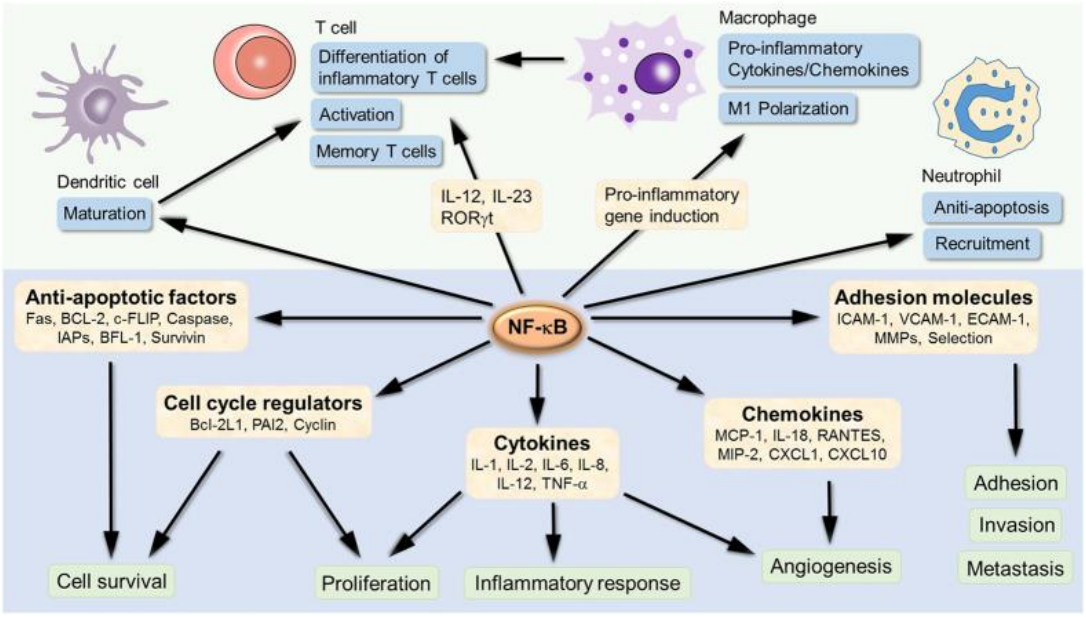

Figure 5: NF-κB expression in immune cells causes inflammation. The main cells that facilitate this inflammation are macrophages, neutrophils, and, later during the infection, T cells. Diagram made by Liu et al., 2017.[95]

Though inflammation is important for defending tissues from being compromised by foreign invaders, it can also occur when the foreign object is harmless, thus causing more damage than no response at all would have.[100] Allergies are a good example of inflammation in response to a nonharmful stimulus.[100] Inflammation can also be damaging when it occurs at a much higher level than an infection requires to be quelled.[100] In these ironic instances, efforts to save an organ from an infection sacrifice the organ or even the organism to do so.[100] With allergies, this overreaction is called anaphylaxis and is mediated by cytokines.[100] The RAS can be a major player in this aspect of inflammation as well.[101] When more angiotensin II is present, inflammation occurs to a larger degree, because of its pro-inflammatory effects on leukocytes,

endothelial cells, and vascular smooth muscle.[101] Since ACE2 downregulation leads to an accumulation of angiotensin II, it also causes increased inflammation.[97]

Severe cases of COVID-19 often exhibit excessive inflammation.[102,103] This inflammation can be localized or ubiquitous.[103] Potential causes of ubiquitous inflammation in COVID-19 include macrophage activation syndrome (MAS, also called "cytokine storm") and T cell lymphopenia, among others.[103] With MAS, inflammatory cytokines reach a high enough level to cause monocytes to systemically enter tissues and promote more inflammation, severely harming tissues all over the body.[103] In T-cell lymphopenia, severe infection by SARS-CoV-2 exhausts and depletes T cells, likely leading to an imbalance in adaptive and innate immunity that exacerbates the cytokine storm.[103,104] The high level of inflammation can cause acute respiratory distress syndrome (ARDS), a potentially fatal complication in the lungs (**Figure 6** and **Figure 7**).[102,103,105] They can also contribute to life-threatening kidney injuries in the renal corpuscle and tubules (**Figure 8**).[106]

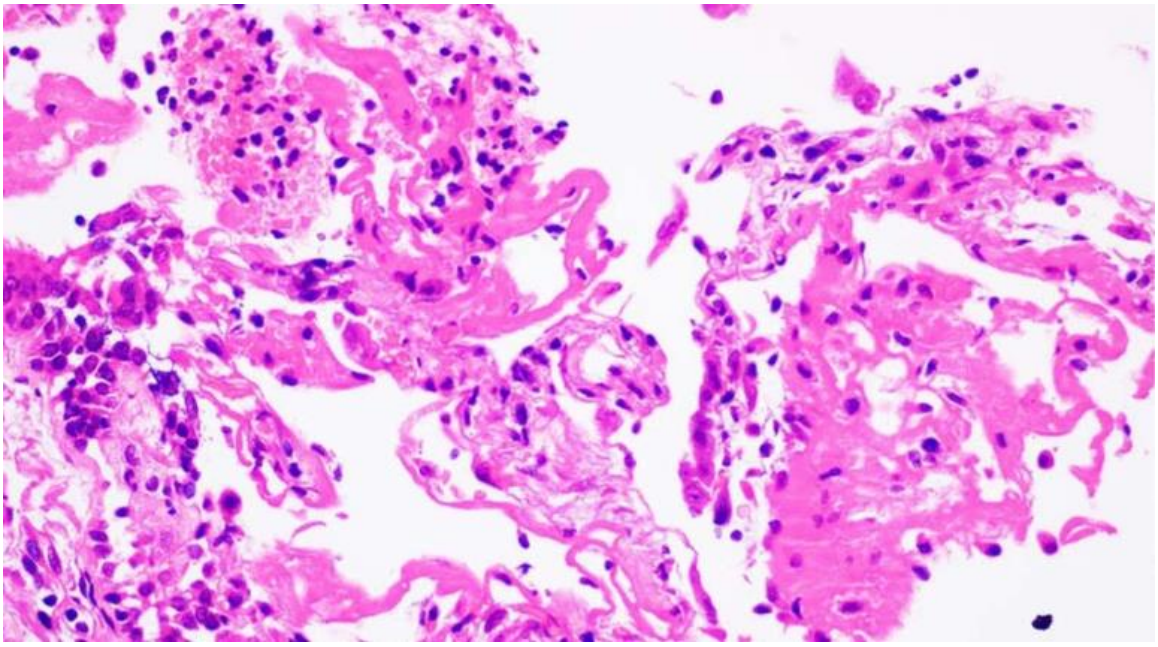

Figure 6: Inflammation of the lungs in severe COVID-19. The alveoli lose their squamous features and macrophages are present in the tissue and the alveolar space. Image from Tian et al., 2020.[102]

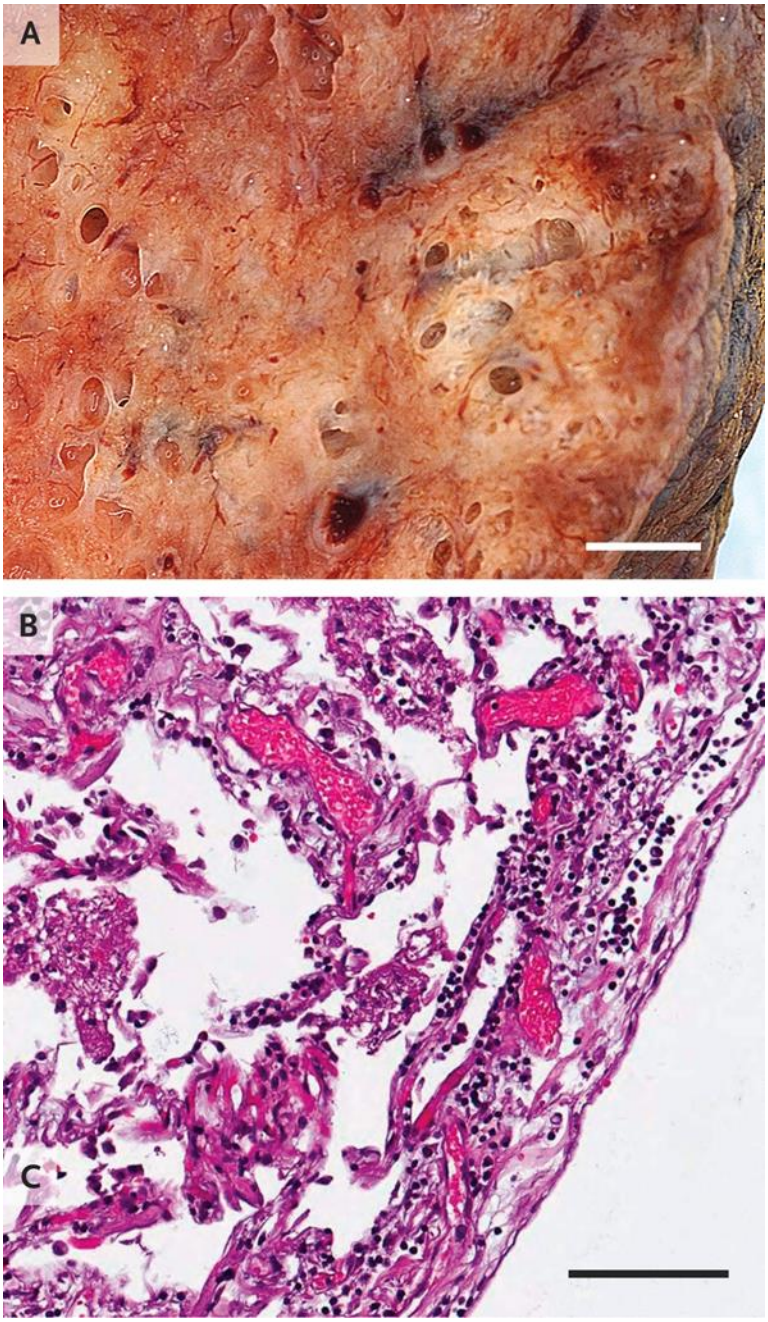

Figure 7: Lungs from a patient who died of COVID-19. A is the macroscopic appearance. B shows the histopathology: the endothelium is inflamed at several points. Image from Ackermann et al., 2020.[105]

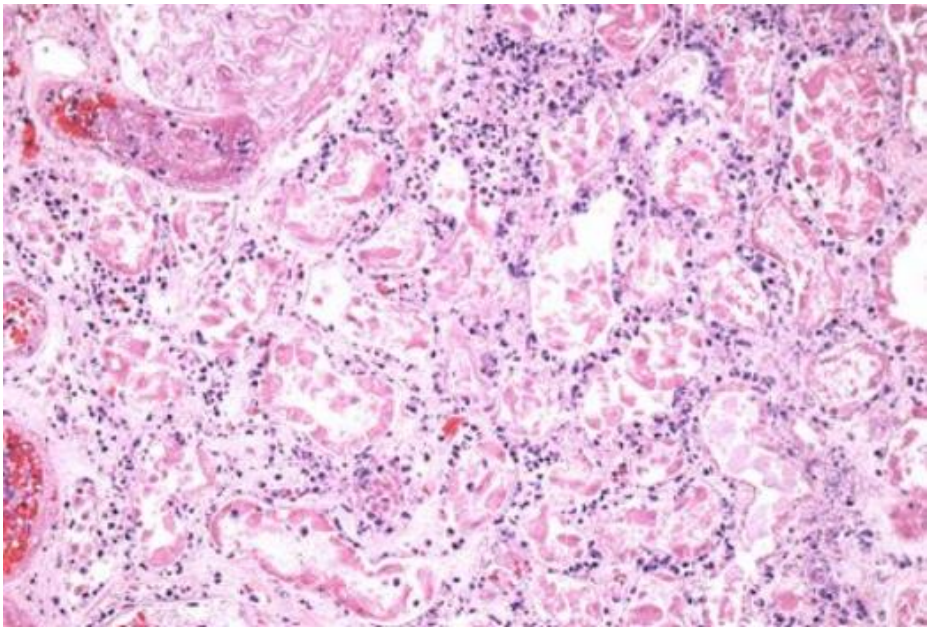

Figure 8: Autopsy of a severe COVID-19 patient reveals necrosis and neutrophil infiltration in the tubules of the kidney. Image from Santoriello et al., 2020.[106]

Similar levels of inflammation are also seen in ACE2 knockout models and other ACE2 deficient disease states. Research on the relationship between ACE2 and inflammation has surged since the start of the pandemic (**Figure 9**). The following meta-analysis sorts a sample of sources reporting inflammation by their organ in these three pathological states (**Table 2**). Sources were obtained from PubMed and Google Scholar by searching "ACE2 knockout inflammation [organ]," "ACE2 inflammation [organ]," or "COVID inflammation [organ]." The results were then screened according to their useful insights, experimental results, and images. Sources that had no input relevant to this study were not included. Three organs, the lungs, heart, and kidneys, were considered, but the

effects of ACE2 downregulation are felt by any tissue with this downregulation. Each bold number indicates the number of sources found that indicated an increase in inflammation. No sources associated ACE2 deficiency with decreasing inflammation.

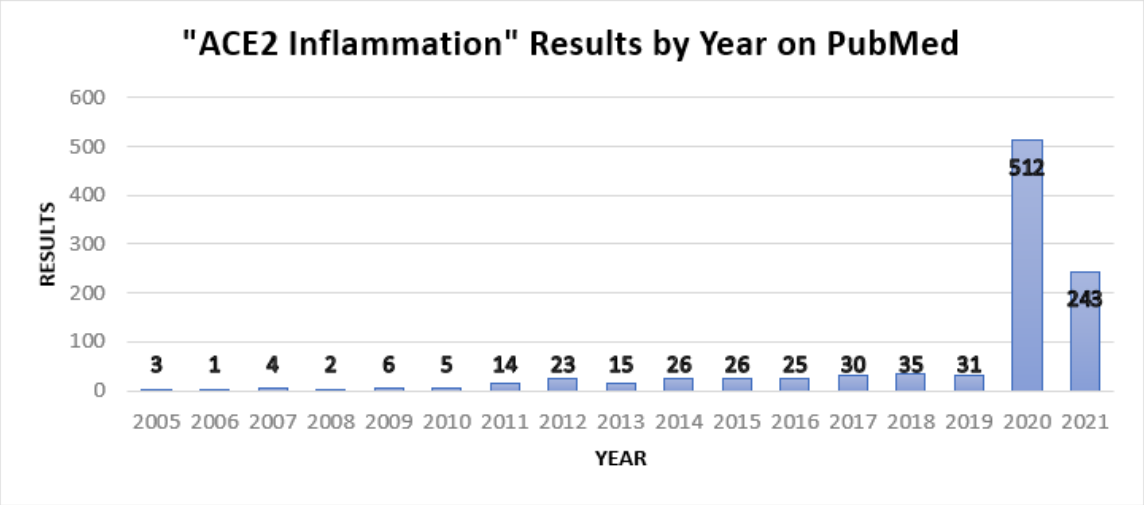

Figure 9: PubMed results for "ACE2 Inflammation" by year. Research in this area was slowly increasing until 2019, which saw a large increase in the amount of research in this area due to COVID-19. Data collected on May 3, 2021.

Organ	ACE2 Knockout	Low ACE2	Severe COVID-19
Lungs	**5** 107–111*	**6** 33,63,112–115*	**11** 88,105,112,113,115–121*
Kidneys	**3** 122–124*	**9** 63,115,125–131*	**9** 113,115,121,127,128,130,132–134*
Cardiovascular System	**2** 135,136*	**10**	**9**

		63,76,113–115,130,137–140*	31,113,115,121,130,138,141–143*

Table 2: Meta-analysis data on the increase in inflammation reported for ACE2 knockout models, low ACE2 presence in tissues, and severe COVID-19. Each of these pathological conditions promote inflammation in each of the organs examined. *The smaller numbers correspond to the sources.

Coagulopathy

Coagulopathies are pathologies regarding excessive or insufficient blood clotting. In healthy individuals, coagulation occurs to seal wounds, preserve fluids, and prevent any microbes present from entering circulation. The formation of a blood clot occurs through the actions of the coagulation cascade (**Figure 10**).[144–146] An injury exposes tissue factor and collagen, which bind to clotting factors and platelets.[147,148] Factor X is converted to factor Xa by one of two pathways, depending on whether tissue factor or collagen was detected.[144] Factor Xa then converts prothrombin to thrombin.[144–146] The thrombin subsequently converts fibrinogen into fibrin, which acts like a net, covering the wound and trapping all cells present in a scab, including red blood cells, platelets, and any bacteria that is present.[144,145] Coagulation can also occur for internal bleeding, in which endothelial tissue integrity is compromised, through the same coagulation cascade; this internal coagulation creates a thrombus.[138,144] Blood clots, specifically the fibrin nets holding them together, are eventually broken down by plasmin, the active form of plasminogen.[149] Angiotensin II activates platelets to coagulate and discourages anticoagulative actions from endothelial cells by causing the secretion of plasminogen activator-inhibitor type 1.[150] ACE2 is highly expressed in endothelial cells and plays a protective role for them thus countering the formation of thrombi.[150] Also, platelets have a Mas receptor for Angiotensin (1-7), which upon binding it promotes production of nitric oxide (NO), which has anticoagulative properties.[150]

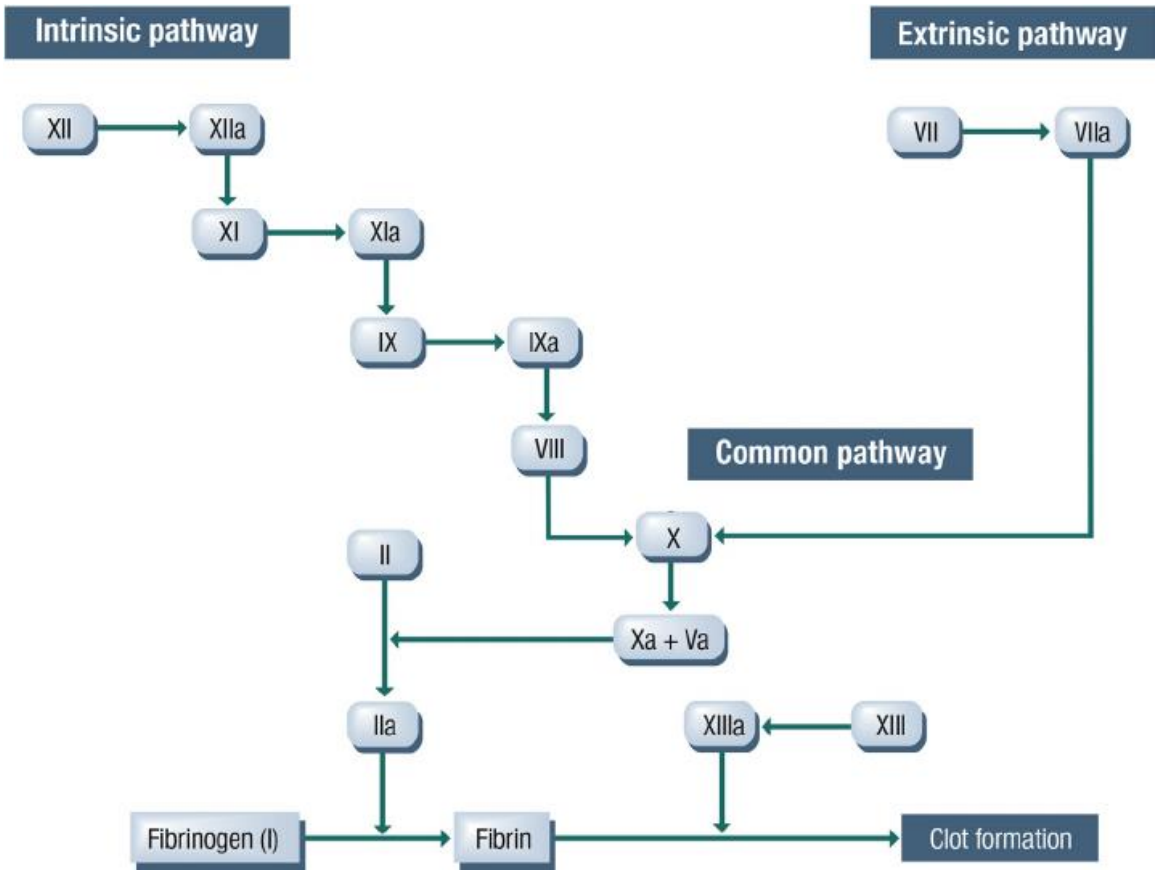

Figure 10: The coagulation cascade. Angiotensin II promotes coagulation by encouraging platelet activation and causing endothelial cells to inhibit circulating plasminogen. ACE2 decreases coagulation by reducing angiotensin II levels and inactivating platelets by increasing angiotensin (1-7) levels, which stimulates the platelets to produce NO. Diagram from Overbey and Jones, 2014.[146]

Although blood clotting is crucial to survival, coagulopathies can reduce the fitness advantage it provides. Thrombosis describes when blood clots form in the absence of injury, blocking blood flow to the cells that need the nutrients and oxygen from the blood.[151,152] The blockage of oxygen access leads to ischemia and parenchymal death, and potentially fibrosis.[152] Another potential complication of thromboses are embolisms, which occur when a thrombosis is formed in one tissue, often leg veins, then dislodges

and travels through the blood stream to another tissue, similarly blocking blood flow to the new tissue.[151] The word thromboembolism describes both phenomena.[151] Thromboembolism can result in tissue death, such as that seen in acute lung injury (ALI).[145]

Tiny thromboembolisms are extremely common in severe cases of COVID-19.[105,116,117] Microthrombi can also contribute to respiratory failure and ARDS, which can occur with or be independent of severe COVID-19.[105] Microthrombi in the lungs of a casualty of severe COVID-19 are shown in **Figure 11**.[105] Several autopsies of severe COVID-19 patients have also found excessive coagulation in the kidneys (**Figure 12**).[106]

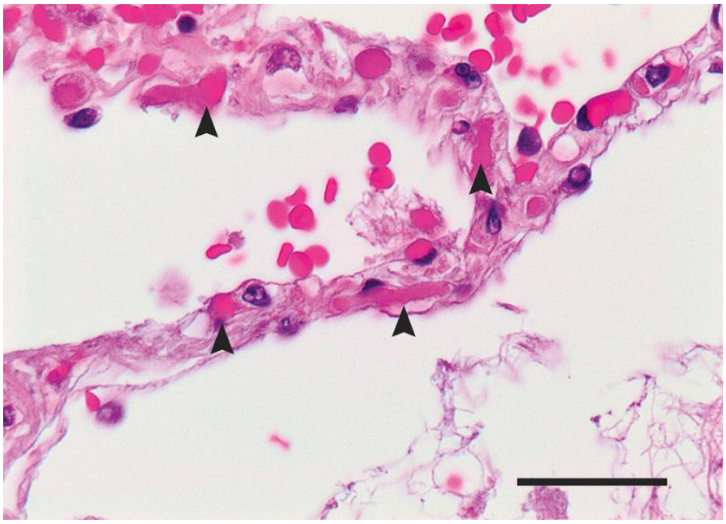

Figure 11: Inflammation with microthrombi in the lung tissue of a patient who died of severe COVID-19. Blood is also visible in the alveolar space. The arrowheads indicate the clots. Image from Ackermann et al., 2020.[105]

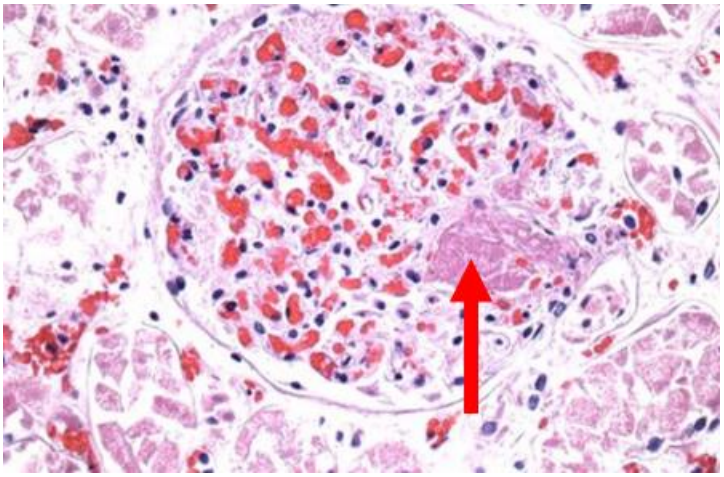

Figure 12: Autopsy of a severe COVID-19 patient reveals capillary congestion and a large thrombus in the glomerulus of a kidney. The arrow indicates the large thrombus. Image modified from Santoriello et al., 2020.[106]

A high frequency of thromboembolisms also manifests in ACE2 knockouts and other ACE2 deficient disease states.[150] Research in this area has recently surged (**Figure 13**). The following meta-analysis sorts a sample of sources reporting thrombosis by their organ in these three pathological states (**Table 3**). Each bold number indicates the number of sources found that mentioned an increase in thrombus formation. Sources were obtained from PubMed and Google Scholar by searching "ACE2 knockout thrombosis [organ]," "ACE2 thrombosis [organ]," or "COVID thrombosis [organ]." The results were then screened according to their useful insights, experimental results, and images. Sources that had no input relevant to this study were not included. Three organs, the lungs, heart, and kidneys, were considered, but the effects of ACE2 downregulation are felt by any tissue with this downregulation, especially considering the potential mobility of thrombi. No sources associated ACE2 deficiency with less thrombosis. No ACE2 knockout studies were found to examine the relationship between ACE2 and

thrombosis; however, this area of ACE2 is largely understudied compared to inflammation and fibrosis. The low ACE2 studies and COVID-19 studies examined both indicated more thrombosis.

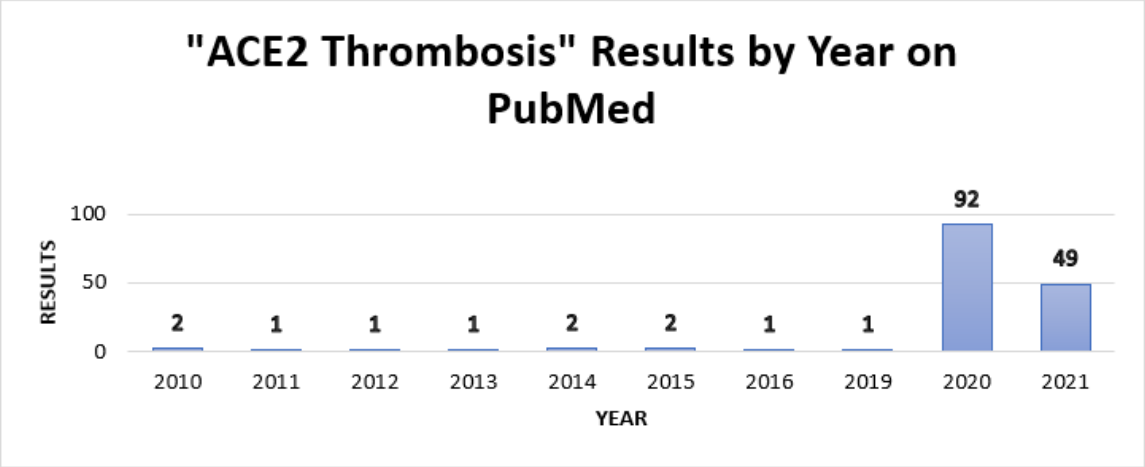

Figure 13: PubMed results for "ACE2 Thrombosis" by year. Research in this area was low until 2019, which saw a large increase in the amount of research due to COVID-19. Data collected on May 3, 2021.

Organ	ACE2 Knockout	Low ACE2	Severe COVID-19
Lungs	**0**	**8** 33,63,112–115,150,153*	**11** 105,112,113,115– 118,120,121,154,155*
Kidneys	**0**	**4** 63,115,127,130*	**9** 113,115,121,127,130,132,134,154,156*

Cardiovascular System	**0**	**8** 33,63,113–115,130,138,150*	**8** 113,115,121,130,138,141,142,157*

Table 3: Meta-analysis data on the increase in thrombosis reported for ACE2 knockout models, low ACE2 presence in tissues, and severe COVID-19. Low ACE2 and severe COVID-19 both exhibit more thrombosis. ACE2 knockout studies did not examine thrombosis; no increase, decrease, or normal thrombosis rates were discussed by these studies. Low ACE2 levels and severe COVID-19 both report thrombosis. *The smaller numbers correspond to the sources.

Fibrosis

Scarring occurs to preserve the integrity of tissues. Fibrosis is simply the pathological scarring of internal organs.[158] Fibrosis and scarring in general are facilitated by cells called myofibroblasts, which differentiate from fibroblasts.[158] Myofibroblasts migrate to sites of tissue injury to secrete extracellular matrix (ECM) proteins, especially collagen, to essentially plug holes in tissue.[158] Angiotensin II increases the fibrotic activity of myofibroblasts and promotes their differentiation.[158] Thus, by reducing the amount of angiotensin II in a tissue, ACE2 has antifibrotic effects.[159] Fibrosis can be connected to inflammation: when leukocytes secreting pro-inflammatory cytokines, the amount of collagen present in wounded tissue is increased.[158] It can also be connected to thrombosis: a thromboembolism blocks blood, resulting in ischemia, followed by necrosis and fibrosis.[152] There are many benefits to maintaining tissue structure by short-term organ scarring, such as the prevention of internal bleeding, the blocking of entry to pathogens, and decreasing the likelihood of a second injury.[158] However, if excessive scarring occurs, as in fibrosis, organs lose functionality.[158] The amount of functional parenchymal tissue decreases as fibrosis increases.[158] Also, fibrosis can cause organs to lose their balance of elasticity that they rely on, as in pulmonary fibrosis, which increases the elastic recoil of the lungs by causing them to make too much elastic fibers in their ECM.[160] Fibrosis can afflict any tissue capable of scarring, and several factors can lead up to it (**Figure 14**).[158]

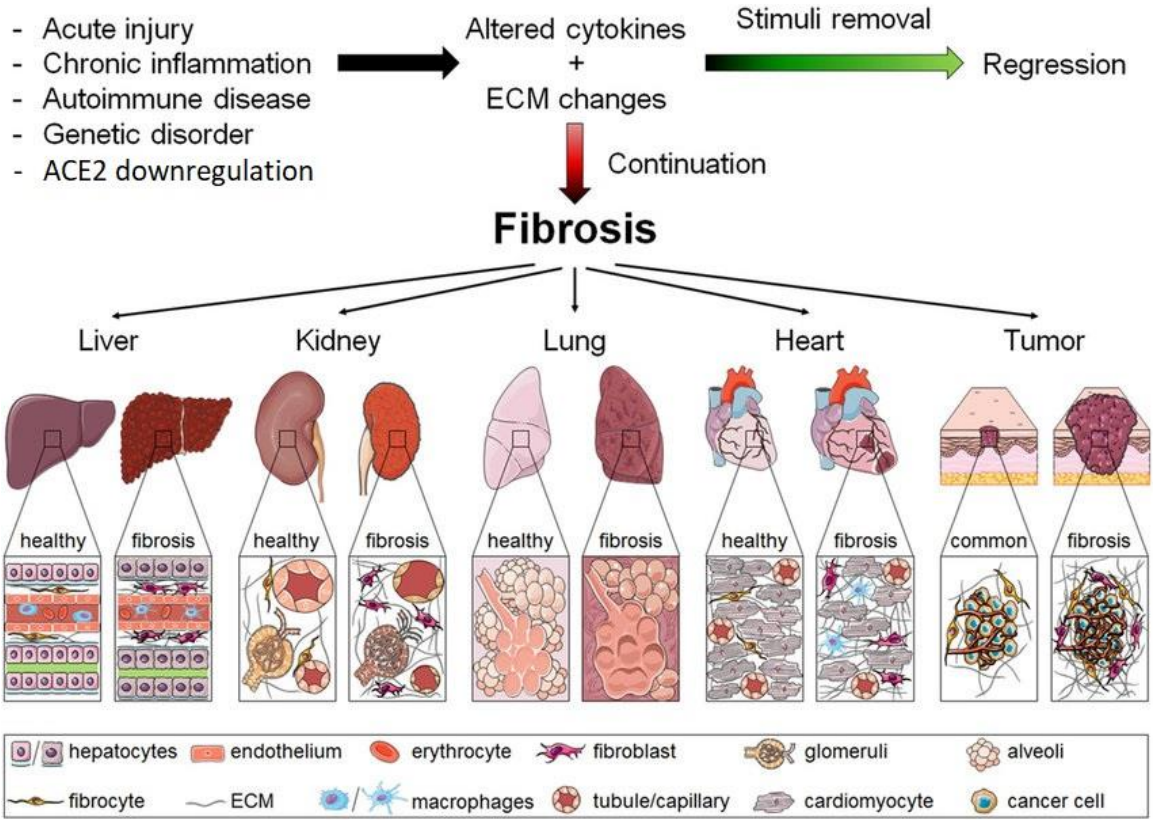

Figure 14: Contributing factors to fibrosis and examples of organs that it can manifest in. Excessive collagen formation without removing the stimuli causing it can lead to fibrosis in the organs. ACE2 downregulation is a causal factor. Modified from Baues et al., 2017.[161]

Pulmonary fibrosis decreases the productivity per breath, because with more fibrotic tissue presence, less functional alveolar tissue is there to capitalize on the inhaled air (**Figure 15**).[158,162,163] Also, the elastic recoil of the lungs is increased due to the higher elastin presence, which makes breathing more difficult as well as decreasing the forced vital capacity, the volume of air that can be forcibly exhaled after a full breath.[160,162] Pulmonary fibrosis is a major threat to survivors of serious lung injuries.[164] The damages to lung tissue caused by regular smoking, acute respiratory distress syndrome (ARDS),

diffuse alveolar damage (DAD), and severe COVID-19 often force the lungs to repair themselves via fibrosis.[119,164–166] Pulmonary fibrosis tends to increase in severity over time.[167] The mean survival time after diagnosis with pulmonary fibrosis is 2 – 5 years, and antifibrotic therapies and lung transplants are the main treatments.[162,163,167,168]

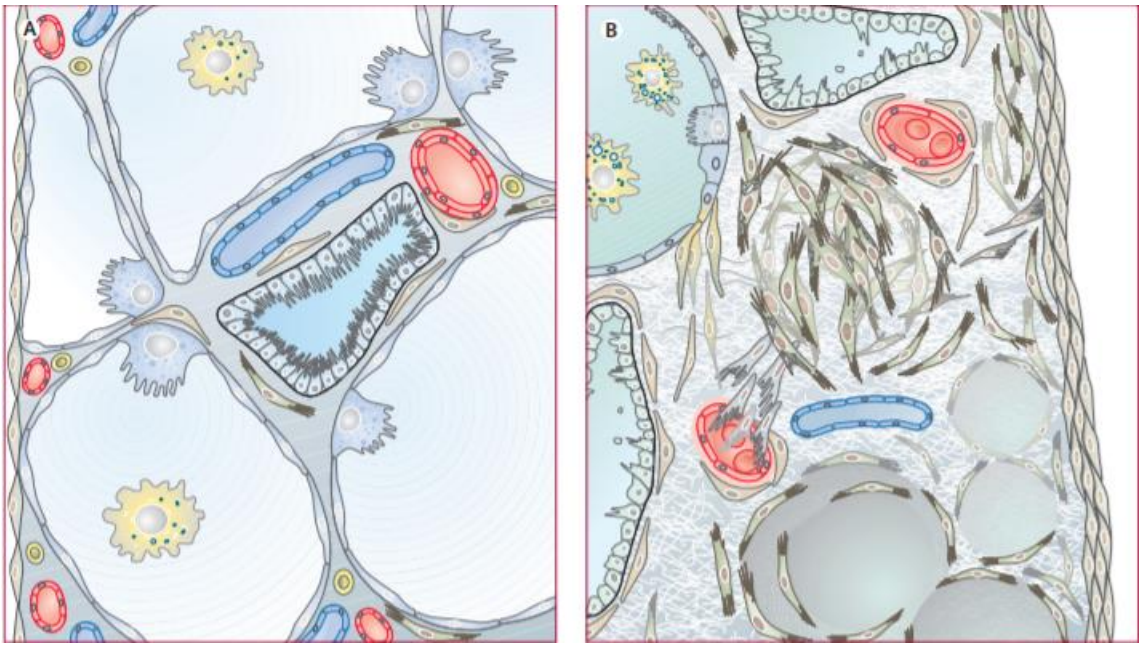

Figure 15: Diagram of normal lung tissue vs. pulmonary fibrosis. In A, healthy lung tissue has a minimal fibroblast presence, healthy alveoli, a thin visceral pleura, and an intact terminal bronchiole. In B, fibrotic lung tissue reveals higher fibroblast presence, alveolar damages, a thicker visceral pleura, and a damaged terminal bronchiole. Image from Fernandez and Eickelberg, 2012.[163]

Severe COVID-19 can damage many organs besides the lungs, some of which include the kidneys, heart, digestive system, and liver.[115,121] This means that fibrosis can occur in any of these organs as well as a result of these damages.[115,121] Many reports of such fibroses have been documented in case studies and autopsy reports of severe

COVID-19 patients, such as Spagnolo et al., 2020, as seen in **Figure 16**.[119,121] Additionally, fibroses, especially pulmonary fibrosis, have also been documented in survivors of severe COVID-19.[88] Organ fibroses are also seen in ACE2 knockout models and other ACE2 deficient disease states (**Figure 17**).[121,169–171]

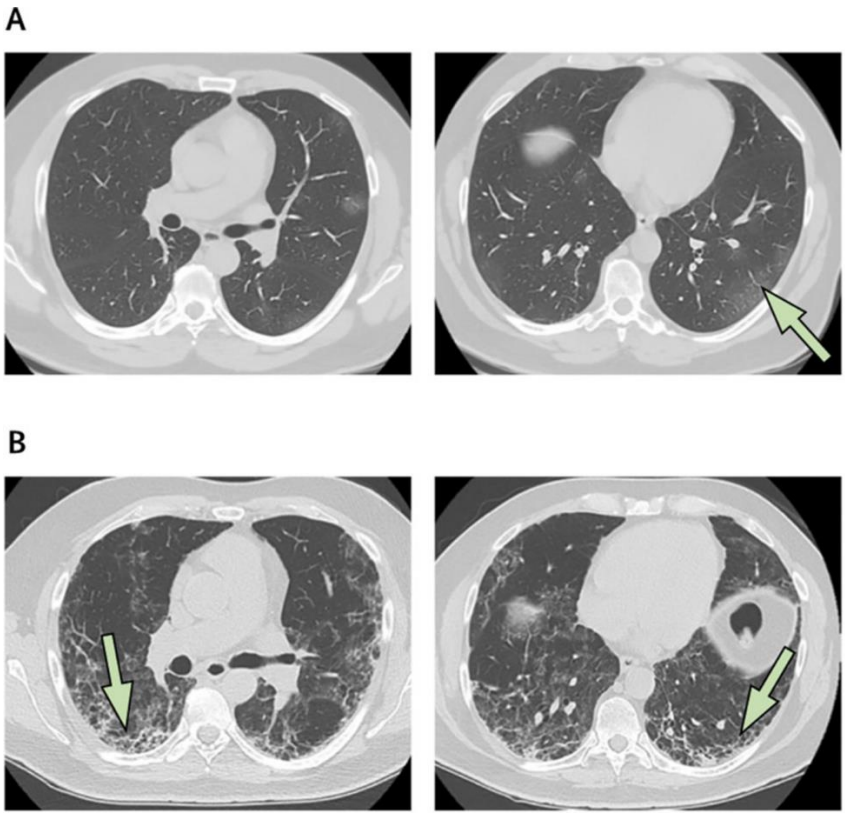

Figure 16: Chest X-ray of a patient's development of pulmonary fibrosis during severe COVID-19. A shows small fibroses (white fibrous masses like the one indicated by the arrow). B was taken 3 weeks later; many more fibroses are present. Image from Spagnolo et al., 2020.[119]

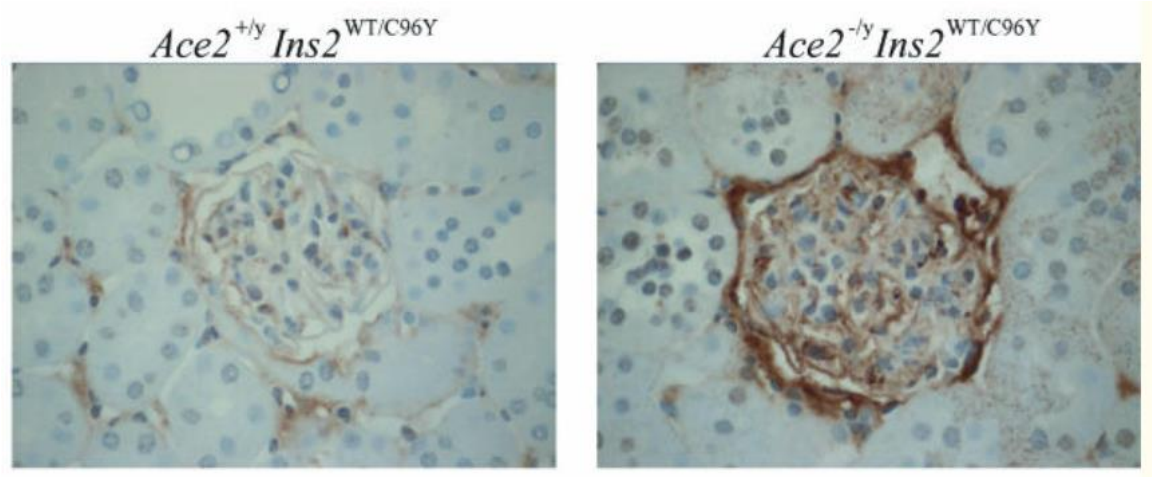

Figure 17: Renal fibrosis in mice with diabetic kidney injury with and without the gene for ACE2. The brown dye indicates fibronectin, a marker of fibroblast activity. The left image is of a mouse with a normal ACE2 gene; minimal fibrosis is present. The right image is of an ACE2 knockout mouse; excessive fibrosis is visible. Image from Wong et al., 2007.[169]

Since the advent of the COVID-19 pandemic, research on the relationship between ACE2 and fibrosis has been amplified (**Figure 18**). The following meta-analysis sorts a sample of sources reporting fibrosis by their organ in these three pathological states (**Table 4**). Each bold number indicates the number of sources found that mentioned an increase in fibrosis. Sources were obtained from PubMed and Google Scholar by searching "ACE2 knockout fibrosis [organ]," "ACE2 fibrosis [organ]," or "COVID fibrosis [organ]." The results were then screened according to their useful insights, experimental results, and images. Sources that had no input relevant to this study were not included. Three organs, the lungs, heart, and kidneys, were considered, but the effects of ACE2 downregulation are felt by any tissue with this downregulation. No sources associated ACE2 deficiency with decreasing fibrosis. One ACE2 knockout study

indicated that no change was observed with regards to fibrosis in the heart.[172] However, 2 other studies did observe fibrosis after knocking out ACE2.[135,136]

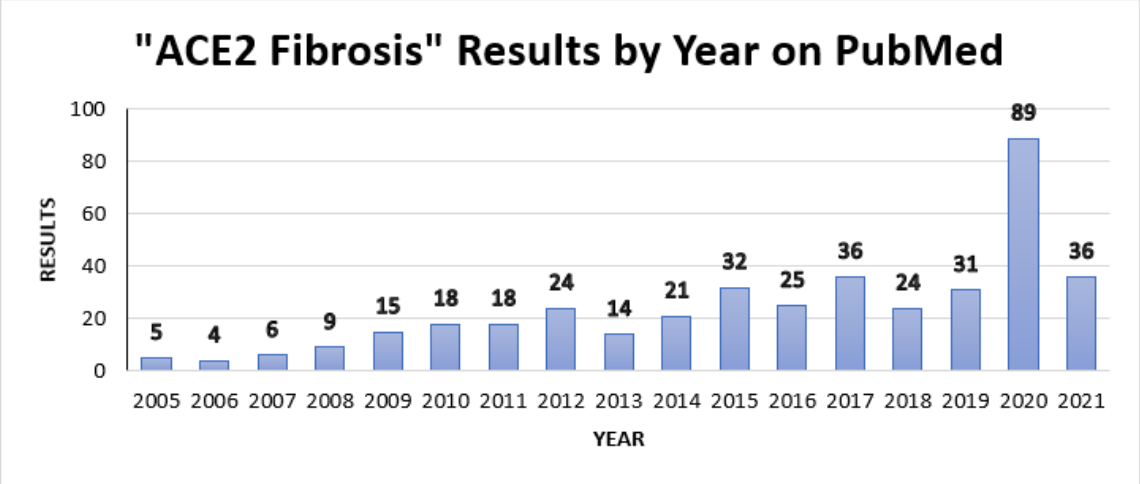

Figure 18: PubMed results for "ACE2 Fibrosis" by year. Research in this area was slowly increasing until 2019, which saw a large increase in the amount of research in this area due to COVID-19. Data taken on May 3, 2021.

Organ	ACE2 Knockout	Low ACE2	Severe COVID-19
Lungs	**3** 108–110*	**7** 33,63,112–115,171*	**6** 88,113,115,118–120*
Kidneys	**4** 122–124,169*	**10** 63,115,125– 127,129,131,170,173,174*	**5** 115,121,127,132,134*
Cardiovascular System	**2/3** 135,136,172*	**8** 33,63,76,114,115,137,140,173*	**4** 31,115,121,141*

Table 4: Meta-analysis data on the increase in fibrosis reported for ACE2 knockout models, low ACE2 presence in tissues, and severe COVID-19. Each of these pathological

conditions promotes fibrosis in each of the organs examined. *The smaller numbers indicate the sources.

RAS-based COVID-19 Treatments

The disruption of the RAS balance in severe COVID-19 provides a therapeutic target, and throughout the course of this pandemic, several RAS-based treatments have been proposed (**Table 5**). When something is present in too high amounts, as is angiotensin II in severe COVID-19, it makes sense to reduce its presence. Conversely, when there are insufficient amounts of something, as with ACE2 in severe COVID-19, it makes sense to increase its presence. Almost every RAS-based treatment proposed for severe COVID-19 attempts to either decrease angiotensin II levels or increase ACE2 levels.

Treatment	**Effect**
ACE2 Inhibitors	**-**
ADAM17 Enhancers	**-**
Renin Inhibitors	**0**
ACE Inhibitors	**0**
AT_1R Blockers (ARBs)	**0**
Human Recombinant Soluble ACE2 (hrsACE2)	**+**
ADAM17 Inhibitors	**+**

Table 5: Proposed and developing RAS-based treatments for severe COVID-19. A positive value indicates that the treatment helps reduce the symptoms of severe COVID-19. A value of zero indicates little to no effect. A negative value indicates a harmful

effect. ACE2 inhibitors and ADAM17 enhancers are predicted to be harmful based on the patterns observed in this study, but no experimental data in the case of severe COVID-19 in humans is available, as it would be unethical to treat patients with medication likely to be harmful.

Upon discovering that ACE2 was the receptor for SARS-CoV-2, some scientists quickly assumed that inhibiting ACE2 would save critical patients and slow the spread of the virus.[175] Several scientists have warned against ACE2 inhibitor treatment in COVID-19, indicating that they shift the balance of the RAS in favor of the classical angiotensin II pathway.[33,175] As shown in this paper, reducing ACE2 levels can be catastrophic. ACE2 inhibitors are no longer being considered as a treatment for COVID-19. Most of the other proposed RAS-based treatments work with the alternative RAS, rather than against it.

Inhibitors of the classical RAS are popular prescription drugs used in the treatment of hypertension.[33,176] Renin inhibitors, ACE inhibitors, and AT_1R blockers (ARBs) all work to reduce the influence of angiotensin II.[33,176,177] As such, they have been examined as potential candidates for reducing the severity of severe COVID-19.[33,176,177] Earlier in the crisis, doctors were worried about renewing prescriptions of these drugs, as they have been associated with ACE2 upregulation.[176–178] The doctors did not want to provide SARS-CoV-2 with more receptors to enter the cells of these high-risk patients.[176–178] Another set of doctors opposingly proposed that blocking the classical RAS would weaken severe COVID-19 symptoms.[176,177] Interestingly, many clinical studies, including a large international cohort study by Morales et al., 2020, showed that classical RAS inhibitors neither increased nor decreased risk of severe COVID-19.[177,178]

One study interestingly found that ACE inhibitors and ARBs reduce the risk of COVID-19, but not severe COVID-19.[179] The reason for the widely reported ineffectiveness of classical-RAS inhibitors in treating severe COVID-19 is currently unknown. Perhaps they simply do not occur at the same level as the ACE2 downregulation in severe COVID-19. Doctors currently recommend that these classical-RAS inhibiting drugs be prescribed as normal, neither increasing nor decreasing prescriptions and/or dosages.[176,177]

Human soluble recombinant ACE2 (hrsACE2) has been the most successful RAS-based COVID-19 treatment.[112] The idea behind it is that it both binds the spike protein on SARS-CoV-2, blocking its entry into cells, and also helps to deplete angiotensin II, lessening the effects of the classical RAS.[112] Before the pandemic, hrsACE2 was already in a clinical trial under the name APN01 as a treatment for various hypertensive, pulmonary, cardiovascular, kidney, and cancerous diseases.[180] Upon realization of its potential as a treatment of severe COVID-19, APN01 studies skyrocketed in popularity. It is now in phase 2 of clinical trials as a severe COVID-19 treatment and is showing very promising results, aiding in several recoveries from the threshold of death.[112] hrsACE2 has a half-life of 10 hours and is given intravenously twice daily.[112,181] Several studies on mice and people have revealed ACE2 treatment to reduce inflammation, thrombosis, and fibrosis in treated organs (**Table 6**). Sources were obtained from PubMed and Google Scholar by searching variations of "ACE2 treatment [organ] [symptom]." In addition to sources found this way, certain sources from other sections of the meta-analysis also discussed and/or performed ACE2 treatments, so they were included here as well.

Organ	Less Inflammation	Less Thrombosis	Less Fibrosis
Lungs	**5** 33,112,114,182,183	**3** 33,112,150	**3** 33,112,114
Kidneys	**3** 124,128,129	**0**	**3** 124,129,174
Cardiovascular System	**1** 114	**1** 150	**0**

Table 6: Meta-analysis data on the reduction of inflammation, thrombosis, and fibrosis by ACE2 treatment by organ. Adding ACE2 to the lungs reduced inflammation, thrombosis, and fibrosis. Adding ACE2 to the kidneys reduced inflammation and fibrosis. Adding ACE2 to the cardiovascular system reduced inflammation and thrombosis. None of the analyzed sources reported on whether ACE2 treatment affects kidney thrombosis or cardiac (interstitial or otherwise) fibrosis. Note that ACE2 treatment is not necessarily APN01 or even hrsACE2; often it was the ACE2 from the species being studied.

Another highly promising RAS-based treatment for severe COVID-19 is ADAM17 inhibition.[184] Severe COVID-19 is often associated with high levels of serum ACE2, indicating that high ADAM17 activity is resulting in the cleavage of membrane ACE2 and the disruption of the local RAS.[79,84,184] By inhibiting ADAM17, less membrane ACE2 is lost, and the tissue is less prone to ACE2 downregulation and the harmful effects of excess angiotensin II.[84,184] One paper naively recommended enhancing ADAM17 rather than inhibiting it.[185] The idea was to increase soluble ACE2 that would block the viral spike proteins.[185] However, this would almost certainly be harmful to the organs experiencing the subsequent ACE2 downregulation.[33,84] Though promising,

ADAM17 inhibition is largely understudied compared to hrsACE2 in treating severe COVID-19.[184,185]

Since COVID-19 and several other diseases are becoming more widely understood to have RAS components as major causal factors, RAS-based treatments are skyrocketing in research focus alongside the RAS itself. Typically, RAS imbalances are in favor of the classical pathway, so treatments try to reduce that difference by promoting the alternative RAS. The future is bright for these drugs.

Conclusion

The COVID-19 pandemic has impacted the entire world, and vaccines are becoming more widely available by the day in a valiant attempt to thwart it. SARS-CoV-2, the virus behind the pandemic, targets ACE2, an enzyme present on cell surfaces throughout the body with several critical functions in maintaining the integrity of tissues as well as blood flow. In severe COVID-19, ACE2 is downregulated by viral entry and increased activity of ADAM17, which causes the accumulation of its substrate, angiotensin II. Angiotensin II then facilitates inflammation, thrombosis, and fibrosis in the tissues and organs experiencing this downregulation.

The meta-analysis performed here elucidates the similarities between severe COVID-19 and other ACE2 deficient states. Two promising but understudied treatments of severe COVID-19 are based on countering the downregulation of ACE2. hrsACE2 (APN01) treatment, currently in phase 2 of clinical trials, directly adds soluble ACE2, which both blocks the spike proteins on SARS-CoV-2 and reduces angiotensin II levels. ADAM17 inhibition prevents ADAM17 from cleaving off membrane bound ACE2, thus increasing the local ACE2 levels.

Although the current pandemic has been catastrophic in many regards, one positive is that ACE2 research has become more mainstream. It is an incredible enzyme with a very high therapeutic potential, but it is also complicated and was understudied until recently. This sudden attention directed at ACE2 may herald treatments for numerous pathologies beyond just severe COVID-19. The pandemic will hopefully end soon, but the memories, wisdom, and insight gained from it must never be forgotten.

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