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Review Article

Corona Virus (SARS-COV-2) Induced Inflammatory Lung Disease a Review on the Role of Renin–Angiotensin System and the Angiotensin Converting Enzyme-2

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Abstract: In December 2019, a novel infectious disease, caused by Severe Acute Respiratory Syndrome-coronavirus 2 (SARS-CoV-2), was identified in Wuhan, China, now declared as a pandemic. The Renin–Angiotensin system (RAS) and angiotensin-converting enzyme 2 (ACE2) have drawn special attention, as ACE2 acts as a receptor for SARS-CoV2. No specific therapy against SARS-COV2 has been invented yet. There is a constant need towards understanding of the underlying pathophysiology to aid the pharmacological research. We have looked at the current evidence on the relationship between SARS-COV-2 and RAS and ACE2. Articles in English language published between 1st January 2003 and 15th June 2020 were searched using keywords and MeSH (Medical Subject Heading). We used Google Scholar and Pubmed as search engines. Conclusion: Bats serve as primary reservoir for SARS-CoV2, but its intermediate host has not been identified yet. 'Hot spots' on ACE2, serve as receptors for SARS-COV2. Imbalance in the activity of ACE/ACE2 is an important contribution towards the pathogenesis of coronavirus related diseases. ACE2 also has protective role and recombinant ACE2 has been shown to improve lung injury and it is a potential therapeutic agent.

Keywords: Renin-Angiotensin System, Angiotensin Converting Enzyme-2, Lung Inflammation, SARS-COV, Angiotensin-II

1. Introduction

Inflammatory response to the SARS-COV-2 leads to disease with mild respiratory symptoms, on one end, and Acute Respiratory Response Syndrome (ARDS) on the other end of the disease spectrum.

SARS-CoV-2 attacks the lower respiratory system to cause viral pneumonia, but it may also affect the gastrointestinal

system, heart, kidney, liver, and central nervous system leading to multiple organ failure. [1]

Various studies, specially conducted over the last decade, have paid special attention towards the Renin–Angiotensin System (RAS) and its member Angiotensin-Converting Enzyme 2 (ACE2). These studied have demonstrated the role of RAS and ACE2 in pathophysiology of SARS-COV-2 infection associated symptoms, specially acute inflammation of the lungs.

In 2003, for the first time, ACE2 was implicated as a receptor for SARS Corona virus. It was also found that maintenance of normal ACE2 levels in the lung helps the host against inflammation of the lungs, a finding supported by a few studies which demonstrated the therapeutic role of ACE2 in the healing process after the lung injury.

However, the mechanism through which RAS and its member ACE2 play a role in lung disease is not clearly understood and there remains a lack of consensus about it. To meet this objective, we have done a literature search to find evidence which explains the pathophysiology of SARS-COV-2 associated lung inflammation, in relation with, RAS and ACE2.

2. Materials and Methods

The clinical evidence was searched in the form of peer-reviewed journal articles using the search engines, which included, Google Scholar and the Pubmed. Studies in English language published between 1st January 2003 and 15th June 2020 which addressed the relationship between SARS-COV-2, Inflammatory lung disease, RAS and ACE2 were included. Our search included the keywords: "Angiotensin Converting Enzyme 2", "Renin Angiotensin System", "Angiotensin-II", "Lung Inflammation", "SARS-COV-2". In addition to this, search using Medical Subject Headings (MeSH) was applied while using Pubmed. Book reviews, book chapters, newspaper and newsletter articles, theses or dissertations were not included in the search. Titles were screened first, followed by the available abstracts. Abstract was read in full and judged for eligibility. A lot of studies were evaluated which met the objective. Relevant studies meeting the criteria were then analyzed and extracted. Irrelevant and duplicate literature was excluded from the review.

3. Discussion

3.1. The Coronaviridae Family

Coronaviruses belongs to Coronaviridae family. They are positive RNA- viruses enclosed in an envelope. The Coronaviridae family is further classified into Alpha, Beta, Gamma and Delta subclasses. Alpha and beta-coronaviruses mainly infect mammals, while gamma and delta-coronaviruses mostly infect bird. Alpha coronaviruses include HCoV-NL63 and HCoV-229E and Beta coronaviruses includes HCoV-HKU1, HCoV-OC43, MERS-HCoV, and

SARS-HCoV.

3.2. Structure of SARS-CoV2

After sequencing the genome of SARS-COV2, it has been found that this virus is closely related to other coronaviruses, such as SARS-CoV and MERS-CoV. SARS-CoV-2 virion is approximately 50–200 nanometers in diameter and its genome encodes four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins and approximately 16 non-structural proteins (nsp1–16), and five to eight accessory proteins. The N protein contains the RNA genome, and the S, E, and M proteins create the viral envelope. The spike protein is responsible for allowing the virus to attach to and fuse with the membrane of a host cell.

SARS-CoV-2 and SARS-CoV bind with angiotensin-converting enzyme 2 (ACE2) while MERS-CoV binds with dipeptidyl peptidase 4 (DPP4) receptors on the host cell. Out of these receptors, ACE2 are expressed in pneumocytes and enterocytes, whereas DPP4 are abundant in liver and lung cells.

Structure of proteins of SARS-CoV, MERS-CoV, and other coronaviruses, along with their interactions with other viral and host proteins is being investigated. Various experiments are being conducted to test the anti-viral properties of small-molecule inhibitors. However, experimental investigation for SARS-CoV-2 may take the research community years to obtain. Srinivasan et al used an integrated bioinformatics approach and provided the first comprehensive structural genomics and interactomics analysis of the SARS-CoV-2. Their study displayed the first three-dimensional roadmap of SARS-CoV-2 viral proteins and their functional complexes, thereby, immensely helping the searchers in finding new antiviral agents and vaccines. [2]

3.3. Chronology of Viral Infections and Its Spread

The human coronaviruses, HCoV-229E and HCoV-OC43, were discovered in the year 1960. They are known cause common cold. After a gap of almost 4 decades, the epidemic of severe acute respiratory syndrome human coronavirus (SARS-CoV) reintroduced the Coronaviridae family in the community. In 2002, SARS-CoV emerged in the Guangdong province of China and it spread to five continents causing 774 deaths. It caused atypical pneumonia characterized by high fever and dyspnea. Thereafter, two more coronaviruses were identified namely NL63 in 2004 and HKU1 in 2005. HCoV-NL63 infection causes mostly respiratory tract infection and community-acquired pneumonia, uncommon though. [3]

In 2012, another coronavirus-related epidemic occurred, this time in the Middle East, which led to the identification of the middle east respiratory syndrome (MERS-HCoV). It spread to 27 countries, infecting a total of approximately 2,500 individuals, and claimed 860 lives. In 2019, a similar disease, but, with more rapid and higher virulence emerged in Wuhan, Hubei province of China. It

was identified as coronavirus 2 (SARS-CoV2) and it resulted in the outbreak of coronavirus disease 2019 (COVID-19), which has been declared as a pandemic. SARS-CoV2 was sequenced and isolated in January 2020. The detection of SARS-CoV-2 infection relies on a polymerase chain reaction (PCR) analysis of samples from throat and nasal swabs. [4-6]

3.4. Viral Reservoir and Intermediate Host

Various studies have suggested that MERS-CoV originated from bats, but the intermediate host transmitting infection to humans was proved to be Arabian camel (Camelus dromedarius). [7, 8]

In year 2010, Hou et al, reported about the discovery of SARS-like coronavirus in bats and suggested that bats could be the natural reservoir of SARS-CoV. However, a few studies indicated the ACE2 protein from a horseshoe bat species was unable to act as a functional receptor for SARS-CoV. Thereafter, Hou et al extended their previous study to ACE2 molecules from seven additional bat species. They tested interactions between the ACE2 molecules and S protein of SARS-CoV. Result of their study showed that ACE2 molecules from bat species, namely *Myotis daubentoni* and *Rhinolophus sinicus*, fused with S protein of SARS-CoV. The results suggest that these two species are likely to be susceptible to SARS-CoV and these bats may act as natural host of the SARS-CoV viruses. Their study also demonstrated that the genetic diversity of ACE2 among bats was greater, as compared to, that of mammals. This highlights the possibility that there could exist many more bat species that can act as a reservoir of SARS-CoV viruses. They strongly advocated for continuation surveillance studies among different bat populations. [9]

Similarly, a few fact-finding research studies have found that SARS-CoV-2 too has originated in bats, moreover, SARS-CoV-2 shares 96.2% identity at the nucleotide level with the coronavirus RaTG13, which was detected in horseshoe bats in China. [10]

Few researchers have demonstrated that Palm civets, Pangolins and Racoon dogs acts as intermediate hosts for transmission of SARS-CoV between bats and humans but the intermediate host for SARS-CoV-2 has not been identified yet. [11, 12]

To find an intermediate host for SARS-COV-2, Lam et al discovered multiple lineages of coronaviruses in pangolins and their similarity to SARS-CoV-2. They suggested that the pangolins should be considered as possible intermediate hosts for SARS-CoV2, and therefore, they should be removed from wet markets to control zoonotic transmission. Yet another study by Liu et al, demonstrated the interaction between the key amino acids of S protein 'Receptor binding domain (RBD) and ACE2. They also indicated that, pangolins may act as the potential intermediate hosts for transmitting SARS-CoV-2 to humans. [13, 14]

In a recently published article, Xuhua X explored how the RNA viral genome of SARS-CoV-2 escapes the viral defenses and that this information can be used to determine the intermediate host of the virus. The zinc finger antiviral protein (ZAP) is a key component of the human immune response that specifically binds to CpG dinucleotides (*5′-C-phosphate-G-3′ which has* cytosine and guanine separated by only one phosphate group) in viral genomes via an RNA-binding domain, resulting in viral degradation. Canine coronaviruses, that cause highly contagious intestinal disease in dogs, were found to have similar CpG values as observed in SARS-CoV-2 and RaTG13. Shil et al on the other hand, investigated the propensity of ferrets and animals who are in close contact with humans to SARS-CoV-2. They found that SARS-CoV-2 replicates poorly in dogs, pigs, chickens, and ducks, but ferrets and cats are permissive to infection. [15, 16]

In any case, the recurrences and spill overs of coronaviruses in humans, along with persistent finding of virus belonging to Coronaviridae, suggest that future zoonotic transmission may continue. [17]

3.5. SARS-CoV-2 S-Protein

The entry of coronavirus into host cells is mediated by the transmembrane spike (S) glycoprotein which protrudes from the viral surface. This viral entry into the host cells requires receptor-binding and processing of the S protein. S protein comprises of a S1 subunit, which is responsible for virus–receptor binding and a S2 subunit, responsible for virus–cell membrane fusion. S1 is further divided into an N-terminal domain (NTD) and a receptor-binding domain (RBD). During infection, Corona virus first binds the host cell through interaction between its S1-RBD and the cell membrane receptor, triggering irreversible conformational changes in the S2 subunit that result in virus fusion and entry into the target cell. [18-20]

Initially the spike protein is primed by a transmembrane protease, serine 2 (TMPRSS2), which is essential for entry of SARS-CoV-2. In addition, the viral genome also encodes several non-structural proteins, which includes, RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and papain-like protease (PLpro). Once SARS-CoV-2 virion is attached to the target cell, protease TMPRSS2 slashes the spike protein of the virus, exposing a fusion peptide, the virion then releases RNA into the cell. Thereafter, the virus produces and disseminate copies, infecting more cells. [21, 22]

Different coronaviruses use distinctly different domains within the S_1 subunit to recognize a variety of attachments and receptors. SARS-CoV2 interact directly with angiotensin-converting enzyme 2 (ACE2) via S_1 to enter the host cells. As the coronavirus S glycoprotein is surface-exposed, it is a target of neutralizing antibodies (Abs), additionally, it is a potential target for research and development for pharmacological agents and vaccines. S trimers are studded with N-linked glycans. These glycans are important for proper folding and for controlling the approachability to proteases and neutralizing Abs. [23, 24] Figure 1.
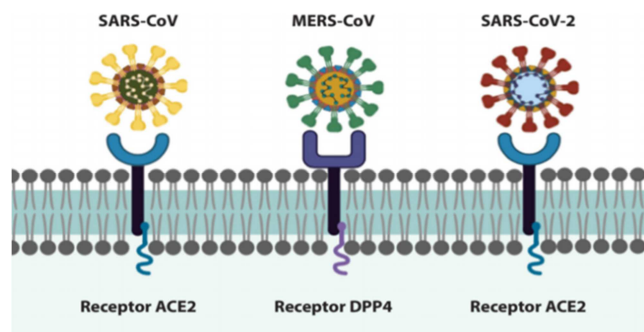
Figure 1. Graphical representation of SARS-CoV, MERS-CoV, SARS-CoV-2 and its cellular receptor. The schematic representation shows the envelope spike proteins of SARS-CoV and MERS-CoV that binds to host receptor angiotensin-converting enzyme 2 (ACE2) and dipeptidyl peptidase 4 (DPP4), respectively. Similar like SARS-CoV, novel coronavirus SARS-CoV-2 uses ACE2 as its receptor for host entry. Binding between receptor binding domain in spike protein and the cellular receptor mediates membrane fusion and initiate the virus life cycle.

In a study, Walls et al found that the human-neutralizing antibodies from rare memory B cells of individuals, infected with SARS-CoV, formed a complex with S protein. These complexes triggered competitive inhibition of S2 attachment to the host cell receptors. As it has been shown that the SARS-CoV-2-S protein shares nearly 76-80% amino acid identity with the SARS-CoV-S protein, therefore, a similar immune reaction is expected against SARS-CoV-2 also. [25, 26]

Wu K et al demonstrated the mechanism by which the virus gains entry in the host cell by using the crystal structures of NL63 coronavirus (NL63-CoV) and SARS coronavirus (SARS-CoV) receptor-binding domains (RBDs). They found that each RBD combined with their common receptor, the ACE2. They also proposed the existence of a virus-binding 'hot spot' on ACE2. To prove the hypothetical hot spot, they used structure-guided biochemical and functional assays. It was shown that the 'hot spot' consists of a salt bridge surrounded by hydrophobic tunnel walls. They demonstrated that the mutations with disrupt the hot spot's structure have significant effects on virus-receptor interactions. They also

found that the tunnel structure at the NL63-CoV/hACE2 interface is more compact than that at the SARS-CoV/hACE2 interface, and hence RBD/hACE2 binding affinities are decreased either by NL63-CoV mutations or by SARS-CoV mutations, as they decreases the tunnel space. It was also demonstrated that SARS-COV spike protein induced ACE2 transduction is inhibited by NL63-CoV RBD. The 'hot spot' theory can be utilised in developing new antiviral medicines against SARS-CoV infections. [27]

4. RAS and ACE2

The renin–angiotensin system (RAS) is essential in regulating function of various organ systems, which includes, the cardiovascular system, lung, kidney, and liver. It plays an important role in maintaining blood pressure, as well as, fluid and salt balance.

It is well known that RAS has important role in maintaining the physiological homeostasis. Studies conducted in the last decade, have uncovered the fact that the RAS also plays an important role locally in various organs and tissues. This hypothesis has been supported by studies on ACE2 mRNA done in rodents, which demonstrated that distribution and enzymatic activity of ACE2 occurs at tissues level. [28, 29]

Renin is a protease that is generated primarily in the kidneys and it cleaves angiotensinogen to angiotensin I (Ang I). Another protease, known as angiotensin-converting enzyme (ACE), cleaves Ang I to produce Angiotensin II (Ang II). Ang II exerts its functions through two Ang II receptors, namely, Ang II receptor type 1 (AT1) and Ang II receptor type 2 (AT2). Ang II is hydrolysed by angiotensin-converting enzyme 2 (ACE2) to generate Ang1–7, Ang 1-9, Ang III, Ang IV and Ang3-7. These in turn bind with their respective receptors to act either agonist or an antagonist for ANG II receptors. Angiotensin II causes vasoconstriction and exerts multiple biological functions. Capillary blood vessels and the adjoining pneumocytes in the lung are one of the major sites of ACE and angiotensin II production [30] Figure 2.
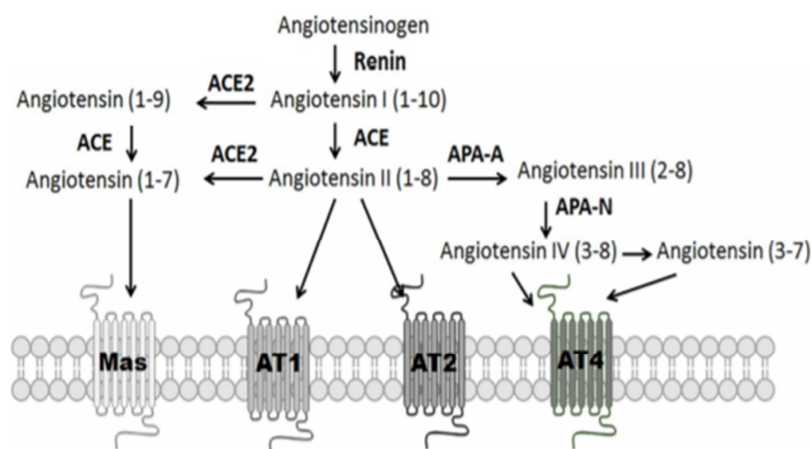

Figure 2. Metabolic Pathway of angiotensinogen. This figure shows metabolites of angiotensinogen and their respective receptors and the associated enzymes. AT4 is an insulin regulated membrane aminopeptidase (IRAP).

The RAS has been proved to be associated with the

pathogenesis of pulmonary hypertension and pulmonary

fibrosis, as found in chronic lung diseases. Recent studies have suggested that the RAS also plays a role in acute lung diseases, for example, acute respiratory distress syndrome (ARDS). Altered functioning of RAS is contributes to the pathogenesis of many diseases such as hypertension, heart failure, myocardial infarction, diabetes mellitus and inflammatory lung disease. In an experiment, it was demonstrated that the injection of SARS-CoV Spike (S) protein into mice worsens acute lung failure that can be mitigated by blocking the RAS pathway. [31, 32]

ACE2 is a metallopeptidase, is a homologue of ACE. It was discovered in the year 2000. Although ACE2 functions similarly to ACE but ACE2 it acts on different substrate. Genetic analysis has revealed that the ACE2 gene contains 18 exons which are located at the chromosomal site of Xp22. [33-35]

Whereas, ACE cleaves dipeptides from the C-terminus of peptide chain and produces an octapeptide known as ANG II from the decapeptide ANG I. ACE2 cleaves one residue from ANG I and produces angiotensin-(1–9), and another single residue from ANG II, to generate angiotensin-(1–7) and hence, ACE2 reduces angiotensin II levels. [36, 37]

It has been suggested that ACE2 might limit the vasoconstrictor action of Ang II through its inactivation. In addition to that, ACE2 counters the actions of Ang II through Ang 1–7. [38]

Studies have identified the G-protein-coupled receptor for Ang 1–7, termed as Mas. In an experiment on mouse kidney, when the Mas receptor was genetically deleted, it abolished the binding of Ang 1–7. [39]

A study by Nehme et al showed that affinity of ACE2 for Ang II is 400-fold greater than for Ang I, which points towards the role ACE2 plays in the conversion of Ang II to Ang 1–7. Ang 1–7 acts as a vasodilator and participates in anti-growth and anti-proliferative activities. In the lungs, the ACE2 protein amalgamate with cholesterol and sphingolipid, which are the microconstituents of the plasma membrane, thereby, expressing itself which is proportional to airway epithelial differentiation. [40, 41]

In early 2013, avian-origin influenza A (H7N9) virus emerged in China. Most infections caused mild respiratory symptoms, but a few resulted in acute respiratory distress syndrome (ARDS). A study conducted in mice reported that ACE2 could be involved the lung disease caused by influenza A (H7N9) virus infection. Yet another study, during influenza A (H5N1) outbreak, showed that the infected patients exhibited a significant increased serum levels of angiotensin II. High serum levels of angiotensin II were proportional to the severity and of infection. It was also found that the downregulation of angiotensin-converting enzyme 2 (ACE2) expression in the lung, was in parallel with, increased serum angiotensin II levels. [42]

Genetic inactivation of ACE2 causes severe lung injury in H5N1 infected mice, confirming a role of ACE2 in H5N1-induced lung pathologies. A study demonstrated that administration of recombinant human ACE2 improved the avian influenza H5N1 virus-induced lung injury in mice. This finding provided a potential treatment strategy treat and limit flu pandemics. [43] Yang et al. also found that ACE2 deficiency markedly worsened the disease, mainly by targeting the angiotensin II type 1 receptor (AT1). Their finding supports the hypothesis that ACE2 plays a critical role in influenza A (H7N9) virus-induced acute lung injury. They also advocated using these findings towards a potential therapeutic target for treating influenza A (H7N9) outbreaks. [44]

Therapeutic proteins can trigger immune responses in animals and humans. Liao et al. in their article, demonstrated that an enzyme, known as NAb assay, can detect neutralizing antibodies to both endogenous and recombinant ACE2 and other enzymes. They advocated monitoring of anti-drug antibody (ADA) and neutralizing antibody (NAb) responses to both the recombinant therapeutic enzyme and endogenous enzyme during various phases of clinical studies and enzyme therapeutic trials. [45, 46]

5. ACE2, Lung Inflammation and Healing

Pathophysiology of inflammatory lung disease is multifactorial. These factors include bacterial infection, viral infection (SARS-CoV, H1N1, H5N1 influenza viruses), sepsis, lung injury from pancreatitis, trauma, gastrointestinal inflammations, and gastric aspiration. Pulmonary involvement is the leading clinical feature of SARS-COV2 infection. [47, 48]

ACE2, as identified as the functional cellular receptor for SARS-CoV, is expressed at high level in pneumocytes and surface enterocytes of the small intestine. However, few studies have demonstrated that human airway epithelia are susceptible to SARS-CoV infection depending on stages of cell differentiation and ACE2 expression [49]. On the other hand, few studies have indicated that the cells without detectable ACE2 expression may also be infected by the virus, this includes studies done on human cell culture model, which have shown that that the presence of ACE2 alone is not responsible for maintaining viral infection. Therefore, presence of other virus receptors or co-receptors cannot be ruled out and needs to be investigated [50].

ACE2, its substrate Angiotensin II (Ang II) and its catalytic product Ang1–7 have been implicated in triggering inflammatory changes in the lungs giving rise to acute lung injury (ALI) or more established changes in the form of ARDS. Studies have also shown that Ang II stimulates release of proinflammatory mediators such as interleukin-8/Cytokine-induced Neutrophil Chemoattractant -3 and interleukin-6. [51]

Another gap in our knowledge is the interaction between SARS-CoV and the immunological or lymphoid systems. To understand the pathogenesis of inflammation, the interaction between RAS and the Kinin–Kallikrein system (KKS) becomes important. The KKS–RAS systems have been implicated to be interacting in many pathological processes at various levels. In addition to KKS, bradykinin and its active metabolite also acts as substrate of ACE2 [52] Figure 3.

It been shown that an imbalance in the activity of ACE/ACE2 is an important contributor towards the pathogeneses of many diseases including inflammatory lung disease. [53, 54]

Several studies have demonstrated that ACE2 is expressed

within nuclei. The role of nuclear ACE2 is not fully understood though. Few studies have demonstrated that exogenous ACE2 could stimulate the Medicinal signaling cells (MSCs) and cause its proliferation and differentiation. They then participate in healing process of the inflamed lung.
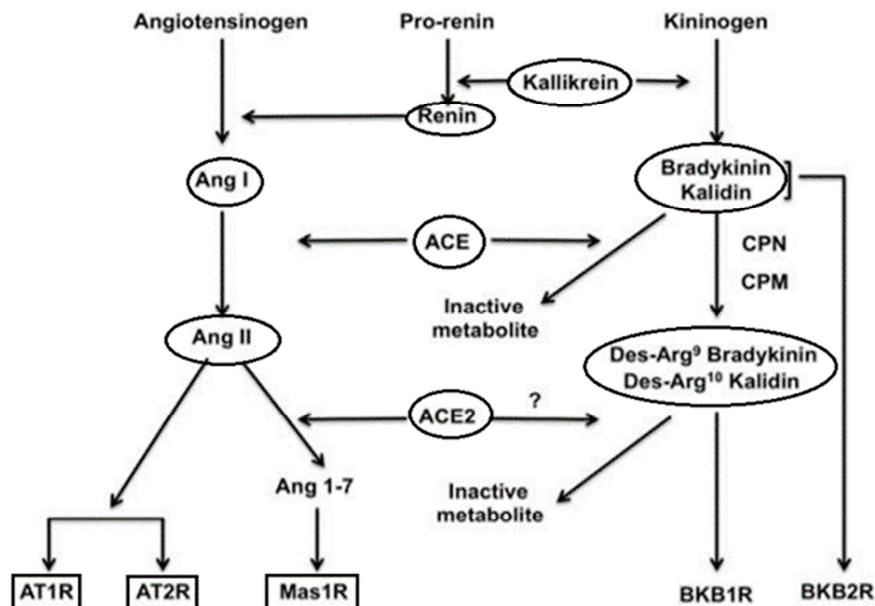

Figure 3. *Interaction between Renin-angiotensin system and Kinogen-Kallikrein system, showing action sites of ACE and ACE 2.*

Therefore, it is rational to hypothesize that nuclear ACE2 triggers gene transcription and thereafter cause cell proliferation and differentiation. Together with other factors, these cells participate in the healing of the lung inflammation. Based on this theory, recombinant ACE2 can be used as therapeutic agent to treat inflammatory lung diseases. [55, 56]

6. ACE2 and Lung Protection

Tumour necrosis factor-α-converting enzyme (TACE) and other sheddases, cleave the transmembrane proteins and soluble ectodomains from the cell surface. In this process, ACE2 and its homolog ACE are released from the surface of epithelia into airway surface liquid (ASL) in the soluble form [57]. Jia et al, in an experiment, demonstrated that a single gene mutation in human ACE2 prevented its shedding from the cell membrane, in addition to this, the shed ACE2 had abnormal or attenuated enzymatic activity. This implies that attenuation of ACE2 shedding might contribute to disease pathogenesis [58]

Imai et al. in an experiment on murine ARDS models, found that the lack of ACE2 expression in the lung reduced vascular permeability, increased lung edema, neutrophil infiltration, and together, they further deterioration in respiratory functions. Curiously, catalytically inactive recombinant ACE2 protein alleviated the symptoms of acute lung injury. They concluded that functional ACE2 protects the lung from acute injury. [59] This finding was supported by another study by Treml et al. on endotoxin induced ARDS model in pigs. They demonstrated that active ACE2 protein, which was infused intravenously,

significantly improved the outcome of respiratory failure. [60]

Kuhn JH et al, in their study showed that the entry of enveloped viruses into the cells is often dependent on receptors (attachment proteins) present on the cell surface. They showed that viral envelope's proteins bind with these receptors leading to the fusion of cellular and viral membranes, thereby, introducing the viral genome into the cytoplasm. The fusion protein gene of SARS coronavirus (SARS-CoV) has been cloned and, subsequently, ACE2 was shown to be its receptor. The discovery that ACE2 is a receptor for SARS-CoV will help in developing anti-viral medicines and vaccines. In addition to that, it would help in the identification of the animal reservoir for SARS-CoV. [61]

7. Role of RAS, ACE2 in ARDS

Acute respiratory distress syndrome (ARDS) is an inflammatory disease which shows disruption of the alveolar-capillary barrier at a microscopic level. ARDS is characterized by pulmonary edema, accumulation of inflammatory cells and severe hypoxia. ARDS carries high mortality rates which range from 38.5–46.1 percent. Several animal studies have implicated ACE2 in the pathogenesis of ARDS. ARDS leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1–7) or an angiotensin II receptor antagonist (ARB). [62-67]

In cases of inflammatory diseases, various biomarkers, which includes, plasma angiopoietin-2, intracellular adhesion molecule 1 (ICAM-1), interleukin (IL-6), IL-8, protein C, Von-Willebrand factor and plasminogen activator inhibitor 1

(PAI-1) are already known but they are not specific to any particular disease process. Therefore, it becomes important to identify the specific biomarkers, expressed in ARDS. This will help in prognosticating, and, in using them in therapeutics-based researches. [68, 69]

ACE2, an enzyme that counters RAS activation but also functions as a receptor for both SARS viruses. The interaction between the SARS viruses and ACE2 has been proposed as a mechanism for their infectivity. Therefore, there are concerns about the use of RAS inhibitors that may alter or reduce the level ACE2. A few of the media sources and health systems have published advisory to discontinue ACE inhibitors and angiotensin-receptor blockers (ARBs). On the other hand, based on the available evidence, Aronson et al [70] opine that concerns are more theoretical, as there prevails lot of uncertainty regarding the effect of RAAS inhibitors on ACE2. They advocated that RAAS inhibitors should be continued in patients, in otherwise stable condition, who are at risk for OR being evaluated for Covid-19 infection. They have advised more studies in this field. Their position has been supported by multiple specialty societies. [71, 72]

Reddy et al [73] did a scientific study about chemical processes involving metabolites, small molecule substrates, intermediates and products of metabolism (metabolomics). They worked on the hypothesis that RAS peptides serve as biomarkers in prognosticating the patients with ARDS. They found that the accumulation of Ang-l and reduction in the level of Ang (1-9) was found in non-survivors suffering from ARDS. This suggested that ACE2 was reduced in patients with ARDS. They concluded that plasma levels of Ang l and Ang (1-9) and their ratios (Ang 1/Ang 1-9) can be used as biomarkers for the prognosis purpose in ARDS patients. This outcome differs from the findings by Chappel et al and Yingchuan et al, which showed that an imbalance in the activities of ACE and ACE2 are important contributor towards the pathogeneses of many diseases, including inflammatory lung diseases, and they advocated the monitoring of ACE/ACE2 ratio. [74-76]

8. ACE2 as a Receptor for SARS-CoV and SARS-CoV-2

Kuba et al in their study on a mouse with acute lung injury, induced by SARS-CoV2 spike (S) protein, showed that the spike protein binds to ACE2. In doing so, it down regulates ACE2 protein expression, which resulted in worsening of pneumonia. They also demonstrated that there was deficiency in ACE2 level in the lung and that the Ang II levels were elevated, resulting in increased vascular permeability. [77] This finding was supported by an article by Zhang et al, who proposed that ACE2 and few other components of the RAS might play a key role in the pathogenesis of acute lung injury. [78]

A few recent studies have shown that ACE2 protects murine lungs from SARS-COV S protein mediated lung injury, suggesting that ACE2 plays a dual role, i.e limiting SARS infections and protection from ARDS. [80]

Studies have demonstrated that the S protein from SARS-CoV, once binds to ACE2, triggers ACE2 shedding into the lung airways by further activating cellular Adam17 (TACE). The same study also indicated that the bacterial endotoxin, Lipo-polysaccharides (LPS), also triggers ACE2 shedding. [81, 82]

Li at al. also demonstrated that the S proteins of coronaviruses, combine with ACE2, to mediate infection of their target cells. They found that the S protein which was isolated from SARS-CoV cultured on Vero E6 cells (Cells used for research purposes isolated from African green monkey kidney) binds with ACE2. They also found that it is the soluble form of ACE2 which blocked association of the S1 domain with Vero E6 cells. They showed that HEK 293T cells (a human cell line which expresses a mutant version of the SV40 large T antigen) which were transfected with ACE2, formed syncytia with cells expressing S protein. Furthermore, SARS-CoV replicated efficiently on ACE2-transfected but not mock-transfected HEK 293T cells. In addition to this, they found that anti-ACE2, but not anti-ACE1 antibody, blocked viral replication on Vero E6 cells. Analysis the data put together indicates that ACE2 is a functional receptor for SARS-CoV. [83]

Hoffmann et al. showed that ACE2 expression on cell lines correlates with susceptibility to the S protein associated infection, which again suggested that ACE2 is a major receptor for SARS-CoV. They indicated that the soluble ectodomain (membrane protein that extends into the extracellular space) of ACE2 specifically annulled the S-mediated infection. Therefore, this can be used for the generation of inhibitors. [84]

Ho et al. in an experiment, evaluated the inhibitory effects of small peptides on the binding of S protein to ACE2 and on the S-protein pseudo-typed retrovirus infectivity. They concluded that the small peptides can be designed to disrupt the binding of SARS-CoV S protein to ACE2. They suggested that SP-10 (residues 668-679) can be developed as an anti-SARS-CoV agent for the treatment of SARS-CoV2 infection. [85]

Wong et al. showed in their study that the coronavirus spike (S) protein infects by attaching on receptors of host cells and therefore, it is a crucial target for antiviral neutralizing antibodies. They also demonstrated that a 193-amino acid fragment of the S protein bound ACE2 more efficiently than did the full S1 domain which is a smaller S protein fragment. Another important finding was that a point mutation at aspartic acid 454 abolished association of the full S1 domain and of the 193-residue fragment with ACE2. From Biochemical research point of view, the 193-residue fragment blocked S protein-mediated infection with an IC_{50} of less than 10nano-Meter, whereas the IC_{50} of the S1 domain was ~50 nanometer. This data identifies an independent receptor-binding area of the SARS-CoV S protein. [86]

Mathewson et al, in their experimental study, investigated the ability of SARS-CoV S protein to bind with ACE-2 in solution and on the cell surface. In both assays, they found that

the S protein of NL63 (HCoV-NL63 has been shown to infect mainly children and the immunocompromised) has a weaker interaction with ACE-2 than the SARS-CoV S protein. They also confirmed that the ACE-2-binding site of NL63 S lies between residues 190 and 739, an important finding from research point of view. [87]

It has been demonstrated that the S protein of SARS-CoV stimulated the TNF-α-converting enzyme (TACE) which, in turn, resulted in shedding of the ACE2 ectodomain. The control of TACE activity by SARS-S depended on the cytoplasmic dominion of ACE2. Interestingly, the viral infection, as evaluated by RT-PCR analysis of SARS-CoV mRNA expression, was significantly attenuated by deletion of the cytoplasmic end of ACE2 or demolishing of TACE expression by Small interfering RNA (siRNA). The data suggest that signals triggered by the interaction of SARS-CoV with ACE2 lead to tissue damage. These findings may help in the development of anti-SARS-CoV agents. [88]

In the case of SARS-CoV, the spike glycoprotein (S protein) on the virion surface mediates receptor recognition. During viral infection, the S protein is cleaved into S1 and S2 subunits. S1 contains the receptor binding domain (RBD), which directly binds to the peptidase domain (PD) of angiotensin-converting enzyme 2 (ACE2). S2 is mainly involved in membrane fusion. When S1 binds to the receptor ACE2, another site on S2 is exposed and it is cleaved by host proteases. It again proves that S protein of SARS-CoV-2 use ACE2 for host infection. [89, 90]

It has been shown that the SARS-CoV-2-S protein shares nearly 76-80% amino acid identity with the SARS-CoV-S protein. Additionally, it has been demonstrated that Anti-human ACE2 antibodies can inhibit SARS-CoV-2-S protein-mediated entry into cultured cells in vitro. This similarity with SARS-CoV is crucial because ACE2 is a functional SARS-CoV receptor in vitro and in vivo. [91, 92] Hence, SARS-CoV-2 too utilizes ACE2 for target cell entry. However, it is still not clear whether ACE2 is the sole receptor for SARS-CoV-2 infection, as in an experiment, CD209L (a glycoprotein of C-type lectin, also known as, L-SIGN) is identified as a possible secondary receptor for SARS-CoV in cultured ovary cells of Chinese hamster. [93, 94]

Analysis of the receptor binding motif (RBM), a portion of the receptor binding domain (RBD) which binds with ACE2, showed that most amino acid residues essential for ACE2 binding by SARS-S were also present in SARS-2-S. Additionally, antiserum raised against human ACE2 blocked SARS-S- and SARS-2-S. BHK-21 cells, infected with SARS-CoV-2, suggest that spike (S) protein of both, SARS-CoV2 S and SARS-CoV, use ACE2 for cellular entry. [95]

Similarities between SARS-CoV-2 and SARS-CoV have also been demonstrated by Xu et al by using computer modelling. They showed that the spike proteins of SARS-CoV-2 and SARS-CoV have almost identical 3-D structures in the receptor-binding area.[96] SARS-CoV-2 and SARS-CoV S proteins share 76.5% identity in amino acid sequences and, importantly, the SARSCoV-2 and SARS-CoV spike proteins have a high level of homology.[97] Further

analysis even suggested that SARS-CoV-2 has higher affinity and binding efficiency with ACE2 than SARS-CoV. This increases the ability of SARS-CoV-2 to transmit from person to person. [98]

9. Potential Therapeutic Targets

Small molecules or small peptide inhibitors can be developed to act as anti-viral agents against S protein or ACE2 receptors and against their mutual binding.

Multiple studies have demonstrated the beneficial role of ACE2 in SARS-CoV-2 associated lung injury and advocated more research towards developing recombinant ACE2 molecule for therapeutic uses.

Neutralizing antibodies have proven to be effective against SARS-Cov-2 S protein and N-linked glycans and major research is underway to develop and expand its usage.

10. Conclusion

SARS-CoV2 has originated in few species of bats, most likely, they serve as primary reservoir for this virus but the intermediate host for SARS-CoV2- has not been identified yet. The recurrences and spill overs of coronaviruses in humans, on one hand, and persistent finding of virus belonging to Coronaviridae, on the other hand, suggest that future zoonotic transmission may continue.

The entry of coronavirus into host cells is mediated by the transmembrane spike (S) glycoprotein which protrudes from its surface. The S glycoprotein is a target for neutralizing antibodies (Abs), and therefore, it is a potential therapeutic target for pharmacological agents and vaccines.

RAS plays important role, both at systemic and local levels. RAS plays a role in acute lung disease, which includes, Acute Lung Injury and ARDS. ACE2, a key member of RAS, serves as receptor for SARS-COV2 and has a beneficial role in acute lung inflammation. SARS-CoV2 interact directly with angiotensin-converting enzyme 2 (ACE2) via S_1 to enter the host cells. Virus binds to 'hot spots' on ACE2. In doing so, it down regulates ACE2 protein expression and resulted in worsening of pneumonia.

ACE2 is released into airway surface liquid. Treatment with recombinant ACE2 improved virus induced lung injury in various studies and it is a potential treatment strategy. ACE2 might limit the vasoconstrictor action of Ang ll, additionally, it counters the action of Ang ll through Ang 1-7. Ang 1-7 acts as vasodilator and participates in anti-growth and anti-proliferative activities. Imbalance in the activity of ACE/ACE2 is an important contribution towards the pathogenesis of many diseases.

Spike protein of SARS-CoV (SARS-S) stimulates the TNF-α-converting enzyme (TACE) which, in turn, results in shedding of the ACE2 ectodomain. TACE's activity is controlled by S glycoprotein and it depends on the cytoplasmic dominion of ACE2. Interestingly, the viral infection, as evaluated by RT-PCR analysis of SARS-CoV mRNA expression, was significantly attenuated by deletion of

the cytoplasmic end of ACE2 or demolishing of TACE expression by Small interfering RNA (siRNA). This data suggest that cellular signals triggered by the interaction of SARS-CoV with ACE2 are positively involved in viral entry but lead to tissue damage. These findings may lead to the development of anti-SARS-CoV agents.

RAS and KKS interaction play an important role in lung inflammation. Abrupt withdrawal of RAS inhibitors in high-risk patients, including those who have heart failure or have had myocardial infarction, may result in clinical instability and adverse health outcomes. Until further data are available, RAS inhibitors should be continued in patients in otherwise stable condition who are at risk for, being evaluated for, or with Covid-19.

Highlights

1) SARS-CoV2 has originated in few species of bats, most likely, they serve as primary reservoir for this virus but the intermediate host for SARS-CoV2- has not been identified yet.

2) The recurrences and spill overs of coronaviruses in humans, on one hand, and persistent finding of virus belonging to Coronaviridae, on the other hand, suggest that future zoonotic transmission may continue.

3) The entry of coronavirus into host cells is mediated by the transmembrane spike (S) glycoprotein which protrudes from its surface. Neutralizing antibodies (Abs) act against S glycoprotein, and therefore, it is a potential therapeutic target for pharmacological agents and vaccines.

4) RAS acts both at systemic and local levels and it plays a role in acute lung disease, which includes, Acute Lung Injury and ARDS.

5) ACE2, a key member of RAS, serves as receptor for SARS-COV2. SARS-CoV2 interact with ACE2 via S_1 sub-unit of S protein to enter the host cells. Virus binds to 'hot spots' on ACE2.

6) ACE2 is also released into airway surface liquid. Treatment with recombinant ACE2 improved virus induced lung injury in various studies and it is a potential treatment strategy.

7) Imbalance in the activity of ACE/ACE2 is an important contribution towards the pathogenesis of many diseases.

8) ACE2 might limit the vasoconstrictor action of Ang ll, additionally, it counters the action of Ang ll through Ang 1-7. Ang 1-7 acts as vasodilator and participates in anti-growth and anti-proliferative activities.

9) Spike protein of SARS-CoV (SARS-S) stimulates the TNF-α-converting enzyme (TACE) which, in turn, results in shedding of the ACE2 ectodomain.

10) SARS-CoV-2 has higher affinity and binding efficiency with ACE2 than SARS-CoV. This increases the ability of SARS-CoV-2 to transmit from person to person.

11) Human-neutralizing antibodies from rare memory B cells of individuals, infected with SARS-CoV, form a complex with S protein. These complexes trigger competitive inhibition of S2 attachment to the host cell receptors.

12) Presence of ACE2 alone is not responsible for maintaining viral infection. Presence of other virus receptors or co-receptors cannot be ruled out and needs to be investigated.

13) RAS and KKS interaction play an important role in lung inflammation.

14) Abrupt withdrawal of RAS inhibitors in high-risk patients, including those who have heart failure or have had myocardial infarction, may result in clinical instability and adverse health outcomes. Until further data are available, RAS inhibitors should be continued in patients in otherwise stable condition who are at risk for, being evaluated for, or with Covid-19.

Further Research

1) There is an urgent need to find the intermediate hosts for SARS-CoV-2.

2) Recombinant ACE2 is a promising therapeutic target and needs further research and development.

3) Human Neutralising Antibodies is another therapeutic product with potential to treat infection with corona virus, it needs to be developed further, to achieve an effective and economical mass production.

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Conflict of Interest

The authors declare that they have no competing interests.

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