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Why COVID-19 transmission is more efficient and aggressive than viral transmission in previous coronavirus epidemics?

Running title: Reasons for the high COVID-19 transmission efficiency

Fatma Elrashdy[1], Elrashdy M. Redwan[2,*], Vladimir N Uversky[2,3,4,*]

[1]Department of Endemic Medicine and Hepatogastroenterology, Kasr Alainy School of Medicine, Cairo University, Cairo, Egypt.

[2]Biological Science Department, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah, 21589, Saudi Arabia;

[3]Department of Molecular Medicine and USF Health Byrd Alzheimer's Research Institute, Morsani College of Medicine, University of South Florida, Tampa, FL, USA.

[4]Institute for Biological Instrumentation of the Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences", Pushchino, Moscow region, 142290, Russia;

Corresponding authors: Uversky V.N.: vuversky@usf.edu; Redwan E.M.: lradwan@kau.edu.sa

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is causing pandemic of coronavirus disease 2019 (COVID-19). The worldwide transmission of COVID-19 from human to human is spreading like wildfire, affecting almost every country in the world. In the past 100 years, the globe did not face microbial pandemic similar in scale to COVID-19. Taken together, both previous outbreaks of other members of the coronavirus family (SARS-CoV and MERS-CoV) did not produce even 1% of the global harm already inflicted by COVID-19. There are also four other CoVs capable of infecting humans (HCoVs), which circulate continuously in the human population, but their phenotypes are generally mild, and these HCoVs received relatively little attention. These dramatic differences between infection with HCoVs, SARS-CoV, MERS-CoV, and SARS-CoV-2 raise many questions, such as: Why is COVID-19 transmitted so quickly? Is it due to the some specific features of the viral structure? Are there some specific human (host) factors? Are there some environmental factors? The aim of this review is to collect and concisely summaries the possible and logic answers to these questions.

Keywords: Severe acute respiratory syndrome coronavirus 2; SARS-CoV-2; coronavirus disease 2019; COVID-19; viral infection; virus-host interaction;

Introduction

In addition to the seasonal flu that annually infects 9% of the world population and causes 291,000-600,000 deaths each year (death rate around 0.1%), the past 100 years witnessed several outbreaks of viral infections, such as the 1918 influenza pandemic (500 million infected; 50 million died; mortality rate 10%), 2002-2004 severe acute respiratory syndrome (SARS) outbreak (8,098 cases; 774 deaths; mortality rate 9.5%), 2009-2010 H1N1 influenza pandemic (1.649 billion infected; i.e., 24% of global population (~61 million cases in USA); 284,000 died (~12,500 deaths in USA); mortality rate 0.02%), 2012-2020 middle east respiratory syndrome (MERS) outbreak (2,519 cases; 866 deaths; mortality rate 34.4%), 2014-2016 Ebola outbreak (~28,650 cases across 10 countries; 11,325 deaths; mortality rate 39.5%), and currently developing coronavirus disease 2019 (COVID-19) pandemic. It is difficult to make a projection of the final outcomes of COVID-19 pandemic, which is still developing, but the currently available data are staggering: there are almost 4.5 million COVID-19 cases in almost 220 countries, with almost 300,000 patients died). Although current statistics indicates that 7% of the SARS-CoV-2 infected have died word-wide, the COVID-19 mortality rates are not equal in all affected territories and vary in a wide range in different countries (from 0.56% in Iceland to >18% in France). Of these six global outbreaks of viral infections, three were caused by coronaviruses (SARS, MERS, and COVID-19), of which COVID-19 is

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characterized by the most efficient and aggressive transmission. In fact, COVID-19, which is caused by the infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, also known as 2019 new CoV, 2019-nCoV), is spreading like a wildfire worldwide, affecting almost every country in the world. Taken together, both previous outbreaks of other members of the coronavirus family (SARS-CoV and MERS-CoV) did not produce even 1% of the global harm already inflicted by COVID-19. Furthermore, in addition to SARS-CoV, MERS-CoV, and SARS-CoV-2 (all are β-CoVs of the B and C lineage), there are four other coronaviruses (CoVs) capable of infecting humans (HCoVs), which circulate continuously in the human population. These are HCoV-OC43 [1,2] and HCoV-HKU1 [3] (β-CoVs of the A lineage or β1CoVs), and HCoV-229E [4,5] and HCoV-NL63 [6,7] (α-CoVs). Being identified in the late 1960s (HCoV-229E and the HCoV-OC43) [8-12] and in 2004-2005 (HCoV-NL63 [6,7,13] and HCoV-HKU1 [3]), these HCoVs are known to be responsible for 3% – 10% cases of common cold and short-term upper respiratory infections that occur mainly in winter, with a short incubation time [14,15], with about 2% of the human population being healthy carriers of an HCoV [16,17]. Although these HCoV strains can also cause more serious diseases of the lower respiratory tract, such as bronchitis, bronchiolitis, and pneumonia, especially in newborns or infants, elderly people, and immunocompromised patients [16,17], their phenotypes are generally mild and as a result, these four HCoVs received relatively little attention.

These dramatic differences between infection with HCoVs, SARS-CoV, MERS-CoV, and SARS-CoV-2 raise many questions, such as: Why is COVID-19 transmitted so quickly? Is it due to the some specific features of the viral structure? Are there some specific human (host) factors? Are there some environmental factors? The aim of this study is to collect and concisely summaries the possible and logic answers to these questions.

Intrinsic viral factors

CoVs belong to the subfamily *Coronavirinae* of the *Coronaviridae* family (which also includes the *Torovirinae* subfamily) in the order *Nidovirales* (http://ictvonline.org/virusTaxonomy.asp?version=2012). They are divided into four genera, namely α-, β-, γ-, and δ-CoVs, with β-CoVs being further separated into A, B, C, and D lineages or clades [18]. Of four CoV genera, α- and β-CoV are able to infect mammals (including humans and domestic animals), while γ- and δ-CoV tend to infect birds. The emergence of human-infecting CoVs is likely associated with the cross-species transmission events [19]. For example, SARS-CoV-2 shows close genetic similarity to bat coronaviruses [20-23]. SARS-CoV and MERS-CoV are zoonotic viruses that crossed the species barrier using bats/palm civets [24] and dromedary camels [25], respectively. Similarly, HCoV-OC43 originated from a zoonotic

transmission event of a bovine coronavirus (BCoV) [26,27], HCoV-HKU1 from a bat coronavirus [28], and HCoV-NL63 originated from ARCoV.2 (Appalachian Ridge CoV) detected in North American tricolored bat (*Perimyotis subflavus*) [29]. Finally, HCoV-229E originated in hipposiderid bats, with camelids serving as potential intermediate hosts [30].

The single-stranded RNA genome of SARS-CoV-2 includes 29,903 nucleotides and encodes three structural proteins, such as spike glycoprotein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N), 6 accessory proteins, encoded by *ORF3a*, *ORF6*, *ORF7a*, *ORF7b*, and *ORF8* genes, and several non-structural proteins (NSPs) in a form of a polyprotein encoded by a large, 5′-located *ORF1ab* replicase gene that covers more than two-thirds of the viral genome [31-33]. This *ORF1ab* replicase gene encodes a set of NSPs that play a number of important roles in the viral replication. This replicase gene encodes the overlapping polyproteins named pp1a and pp1ab, which are necessary for viral replication and transcription. The longer pp1ab is a 7,073 amino acid-long polypeptide containing 15 non-structural proteins. NSP1, NSP2, and NSP3 are released from polyprotein via proteolytic processing using a viral papain-like proteinase (NSP3/PL^{Pro}), whereas the rest of NSPs are cleaved by another viral 3C-like proteinase, NSP5/3CL^{Pro} or main protease M^{pro}, that utilizes 11 or more conserved sites to digest the polyprotein. This digestion starts with an autocatalytic cleavage of this enzyme itself from pp1a and pp1ab.

Based on the evaluation of the levels of intrinsic disorder in the nucleocapsid (N) and membrane (M) proteins of SARS-CoV-2 it was proposed that this virus is characterized by high resilience to the conditions outside the body and in body fluids, suggesting that SARS-CoV-2 belongs to viruses with intermediate levels of both respiratory and fecal-oral transmission potentials [34,35], which favor alternative ways for the COVID-19 transmission vertical and horizontally.

An important feature that differentiates β-CoVs of the B and C lineages (SARS-CoV, MERS-CoV, and SARS-CoV-2) from β-CoVs of the A lineage (β1CoVs) is the lack of hemagglutinin-esterase (HE) protein, which is present in toroviruses, influenza C and D viruses, and in β1CoVs [36-40]. HE is a receptor-binding/receptor-destroying viral protein interacting with the 9-*O*-acetylated sialic acids (9-*O*-Ac-Sias) [38], which are the glycan components commonly present in mammals and birds [41]. Therefore, in β1CoVs, both spike and HE proteins bind 9-*O*-Ac-Sias, whereas virus elution is promoted by receptor destruction via the action of the HE esterase domain.

These opposing activities of receptor binding and receptor destruction define dynamic and reversible attachment of β1CoV to sialoglycans. The sialate-*O*-acetyl-esterase activity promotes escape from attachment to non-permissive host cells or decoy and facilitates the release of viral progeny from infected cells [42]. Curisously, it was shown that the HE lectin function is progressively lost during the in-host evolution of the human β1CoVs, HCoV-OC43 and HCoV-HKU1 [43]. Spike proteins of MERS-CoV interact with a specific receptor, dipeptidyl peptidase-4 (DPP4), which is a key factor in the signaling and activation of the acquired and innate immune responses in infected patients [44]. On the other hand, the host cell entry of SARS-CoV, HCoV-NL63, and SARS-CoV-2 are mediated by interaction with the angiotensin converting enzyme-2 (ACE2) receptors, which are expressed in the brain, gut, heart, kidney, lung (particularly in type 2 pneumocytes and macrophages), vessels, and testis [45]. However, besides this protein membrane receptor, the host cell entry of HCoVs, including SARS-CoV-2, also depends on the sialic-acid-containing glycoproteins and gangliosides, which might act as primary attachment factors for viruses along the respiratory tract [38]. In fact, the N-terminal domain (NTD) of the spike (S) glycoprotein of SARS-CoV-2 was shown to contain a ganglioside-binding site that can be efficiently blocked by chloroquine (CLQ) and its more active derivative, hydroxychloroquine (CLQ-OH) [46]. Therefore, the SARS-CoV-2 S protein acts on both protein and 9-*O*-acetylated sialic acid-containing receptors, with the receptor-bind domain (RBD) being involved in ACE2 receptor recognition, and the NTD being responsible for finding a ganglioside-rich landing area (lipid raft) at the cell surface [46]. It was hypothesized that the interaction of S protein with the lipid rafts defines an adequate positioning of the viral S protein at the first step of the infection process [46]. Importantly, evolutionary analysis revealed that the ganglioside-binding subdomain (residues 111–162) of the NTD is completely conserved in 11 clinical isolates of SARS-CoV-2 of various geographic origins. Furthermore, this subdomain is also completely conserved in the bat coronavirus the bat RaTG13, but noticeable variability is detected in other bat SARS-like and human SARS-CoVs, suggesting that higher levels of SARS-CoV-2 contagiousness in comparison with previously characterized HCoVs can be attributed to recent evolution [46].

Just few weeks after the first reports on COVID-19 infection it was revealed that virus entries to the lung alveolar type II (AT2) via the angiotensin converting enzyme 2 (ACE2), which is expressed on the surfaces of the heart, kidneys, intestine, and lung alveolar epithelial cells. Here, a specific role is played by the spike glycoprotein S. In fact, the S glycoproteins of coronaviruses have two subunits, S1, and S2. The S1 subunit binds to the ACE2 enzyme, via its receptor-binding domain (RBD), on the cell membrane [47,48], and S2 fuses with the cell membrane [49]. Although genome of SARS-CoV-2 shares 79.6% sequence identity to SARS-CoV, and although SARS-CoV-

2 is capable of using the same cell entry receptor (ACE2) as SARS-CoV to infect humans [21,50], the affinity of SARS-CoV-2 spike protein to the human ACE2 is ~10-20 fold higher than that of the SARS-CoV spike, protein [51,52]. This is because of the presence of the distinctive structural differences between the receptor-binding domains (RBDs) of the spike proteins from SARS-CoV and SARS-CoV-2 that represent energetically favorable changes in the amino acid sequence for the more efficient interaction of the SARS-CoV-2 spike protein with the ACE2 receptor. In fact, the local environment within the ACE2 receptor allows SARS-CoV-2-specific residues in the RBD of the spike protein to make a significant number of electrostatic stabilizing interactions. Furthermore, the presence of the two capping loops in the RBD of the SARS-CoV-2 spike protein is likely to produce a higer stabilization effect over the interaction with the cellular receptor. These two loops around the RBD of SARS-CoV-2 might promote interaction with the ACE2 receptor, improving the binding to the ACE2 by increasing the number of groups involved. Therefore, these amino acid substitutions and the longer capping loops could explain the increase in the binding affinities in SARS-CoV-2 compared to SARS-CoV. These higher values of affinity might be related to the higher dynamics of the infection and the rapid spread observed for this virus [53]. This is in line with the outputs of the computational analysis showing that when all the residues favoring interaction of the CoV S protein with human ACE2 would be combined into one RBD, this RBD would bind to ACE2 with super affinity, and the corresponding spike protein would mediate viral entry into human cells with super efficiency [54].

Furthermore, SARS-CoV-2 uses the transmembrane protease, serine 2 (TMPRSS2, also known as serine protease 10) for the viral spike glycoprotein priming, a process crucial for the viral entry [55]. In fact, host TMPRSS2 priming of the S glycoprotein causes irreversible conformational changes and activation of the S2 subunit, thereby facilitating fusion of the virus to the cell membrane. The virus with the processed S protein then enters the cell [56,57]. Importantly, S protein of SARS-CoV-2 contains a polybasic cleavage site (RRAR) at the junction of S1 and S2 [51,52,58,59], which defines the effective cleavage by furin and other proteases and has a role in determining viral infectivity and host range [60]. The presence of this unique furin cleavage site within the SARS-CoV-2 spike protein, which is a novel feature setting this virus apart from SARS-CoV, and the almost ubiquitous expression of furin-like proteases could participate in expanding cell and tissue tropism of SARS-CoV-2 and increasing transmissibility and/or altering pathogenicity of this virus [51,52,58,59].

While S2 facilitated the fusion step after proteolysis by TMPRSS2 and furin proteases in a sequential pattern [51,52], there is also evidence suggesting that these enzymes are not the exclusive players in priming S protein for the

efficient COVID-19 entry. It is known that airway and alveolar type I and II epithelial cells are expressing other proteases, such as trypsin, kallikrein, and plasminogen, which are also expressed in endothelial cells and which might contribute to the priming of S glycoprotein. The possibility for non-furin proteases to cleave viral envelope proteins is supported by the evidence that the plasmin cleaves the S proteins of SARS-CoV *in vitro* [61]. Furthermore, S protein of HCoV-HKU1 is cleaved by kallikrein within the S1/S2 region and mediate the entry of HCoV-HKU1 to non-permissive rhabdomyosarcoma cells [62]. Altogether, the S protein of coronaviruses may be cleaved by plasmin, trypsin, cathepsins, elastase, and TMPRSS family members, with such cleavage of S protein mediating the enhancement of the virus entry into the bronchial epithelial cells [61].

The clinical relevance of non-furin cleavage remains unknown due to the paucity of *in vivo* evidence for the roles of non-furin proteases (e.g., plasmin) in cleavage of SARS-CoV. Also, it remains to be demonstrated that the envelope proteins of SARS-CoV-2 can be cleaved by plasmin [63]. Meanwhile, there is evidence indicating the presence of at least some interplay between SARS-CoV-2 and plasmin. In fact, the enhanced plasmin(ogen) levels and resulting alterations in the fibrin D-dimer levels are the common features observed in the COVID-19 patients [64]. Plasmin proteolytically breaks down excess fibrin and elevates levels of D-dimer (which is a cross-linked dimer of the two smallest fibrin degradation products, with increased D-dimer levels indicating increased fibrinolysis or inability to clear the products from the circulation, and with D-dimer assays being commonly used in clinical practice [65]) and other fibrin degradation products in both bronchoalveolar lavage fluid and plasma, which decreases platelets and results in hemorrhage [64]. Clinical data showed that in the COVID-19 patients, the lungs are the most injured organs, followed by the moderate injury in the heart, liver, kidney, and brain. Systemic microthrombi in the circulatory system and hemorrhage in the affected organs result from the miscoordinated responses between the coagulation and fibrinolysis systems [64]. Coagulation and hemorrhage ranks among the top three leading causes of COVID-19-associated death [58].

In addition, elevated levels of plasmin can be related to some other pathological conditions. For example, the epithelial sodium channel (ENaC) subunits, located at the apical membranes of epithelial cells in the airway, lung, and kidney, are also cleaved by plasmin. This increases the ability of Na^+ ions to enter epithelial cells resulting in the hypertension and dehydration of the fluid lining lung airways and alveolar cells [64]. Plasmin is a potent protease that cleaves human γ ENaC subunit at 16 sites, including the cleavage sites of trypsin, chymotrypsin, prostasin, and elastases [66]. Significant harm is induced by the uncontrolled proteolysis of these proteins, which are highly

expressed on epithelial cells, considered as the major pathways for Na^+ entry, and play important roles in maintaining the proper depth of airway and alveolar lining fluids, the reabsorption of edema fluid in injured lungs, and the regulation of salt retention in the collecting tubules [64,67-74]. Of note, the renin angiotensin system (RAS) is mainly known to regulate blood pressure and Na^+ reabsorption via its roles in the maintaining blood pressure homeostasis [75] and salt and fluid balance [76].

The role of plasmin in the pathogenesis of other viruses is rather well established. For example, it is known that plasmin cleaves the influenza virus HA proteins to enable fusion with the target host endosome [77-82]. Also, the plasminogen (fibrinolytic zymogen, precursor of plasmin) has been shown to cleave the influenza HA proteins [82-84]. The cleavage of HA from the A/WSN/1933 H1N1 influenza virus governs virus spread in a plasmin-dependent manner [83]. In addition, the plasmin fragment (Mini-plasmin) is distributed predominantly in the epithelial cells of the bronchioles and potentiates the replication of both plasmin-sensitive and plasmin-insensitive influenza A virus strains, suggesting a pivotal role of plasmin in the spread and pathogenicity of the influenza virus [80].

Furthermore, there is a place for other non-furin proteases in viral pathogenesis too. For example, HA proteins from the H1, H2, and H3 subtypes of the influenza virus are sensitive to kallikreins cleavage and can be activated by this protease [85]. Similar to CoVs and influenza viruses, plasmin, trypsin, thrombin, and furin were shown to enhance cytopathology induced by respiratory syncytial virus (RSV) [86]. Curiously, the cleavage of a target protein by different proteases may enhance or decrease its activities. For example, prostasin (which is a serine protease with trypsin-like substrate specificity that is found in prostate gland, kidney, bronchi, colon, liver, lung, pancreas, and salivary glands) increases the activity (60–80%) of human ENaC, whereas TMPRSS2 markedly decreases ENaC function and protein levels [87]. Similarly, plasmin is capable of cleaving the subunit of human ENaC at the furin sites [64,88], which may increase the patient complications and subsequently promote viral vertical (and maybe horizontal) tissue tropism and transmissibility [64,89].

TMPRSS2, TMPRSS4, TMPRSS11A, and HAT (human airway tryptase) belong to the type II transmembrane serine proteases (TTSP) family, which included 19 members, most of them expressed in the human respiratory tract [90]. These TTSP can cleave and activate influenza A virus hemagglutinin as well as S proteins of CoVs for host cell entry [91,92]. A comprehensive study detected extensive coexpression of ACE2, TMPRSS2, and HAT in the epithelia of the aerodigestive tract, although exceptions were noted, including the epithelia of trachea, vocal

folds and epiglottis [92]. Therefore, TMPRSS2 and HAT are present in major viral target cells and could promote viral spread in infected humans [93]. Both enzymes were shown to cleave and activate the HCoV-229E S-protein for cathepsin L-independent virus-cell fusion [93]. Furthermore, TMPRSS2 and HAT were shown to activate all influenza virus subtypes previously pandemic in humans [94,95], and TMPRSS4 was found to activate the HA protein of the 1918 influenza virus [96].

These observations on the roles of various non-furin proteases in pathogenesis of different viruses raise important questions, such as: Can the plasmin increase the pathogenicity of COVID-19 by cleaving the SARS-CoV-2 S glycoprotein extracellularly, and thereby modulating the ability of this protein to interact with ACE2 receptors of host cells and probably facilitating virus entry and fusion? Can the elevated plasmin(ogen) levels in patients with some pre-existing conditions be considered as one of the avenues for the enhanced susceptibility to SARS-CoV-2 infection and fatality?

There are also some other players from the host protease realm that can contribute to the COVID-19 pathogenesis. In fact, an additional layer of complexity has been added to the interplay between the CoV S protein and host proteases by the observations that not only S of SARS-CoV-2 but also its receptor, ACE2, is proteolytically processed. ACE2 is known to be shed into the extracellular space upon cleavage by the sheddase ADAM17/TACE (disintegrin and metalloproteinase domain-containing protein 17 or TNF-alpha-converting enzyme) [93,97,98]. ADAM17 is a 610-rsidue-long protein that was initially described in 1997 by Black *et al*. to specifically cleave the precursor of the tumour necrosis factor a (pro-TNF-a) [99,100]. ACE2 shedding by ADAM17 was first described by Lambert *et al*., when they studied human HEK293 cells (embryonic kidney cells) expressing human ACE2 (HEK-ACE2) in 2005 [98,99]. In 2008, Haga *et al*. demonstrated that binding of S protein from SARS-CoV also induced ACE2 shedding by ADAM17 and provided evidence that the ACE2 shedding is important for the uptake of SARS-CoV into the target cells [101]. The up-regulation of ACE2 shedding by ADM17 may inhibit the infectivity of the SARS-CoV [98,99]. Furthermore, it was demonstrated that an ADAM17 inhibitor displays modest antiviral activity in SARS-CoV infected mice [102]. Furthermore, it was found that TMPRSS2 competes with the metalloprotease ADAM17 for ACE2 processing, but only cleavage by TMPRSS2 resulted in the augmented SARS-S-driven entry [93].

Since the ACE2 expression levels within the main COVID-19 target, lungs, is relatively low, some researchers suggested that there could be some co-receptors needed for the SARS-CoV-2 entry [103]. Using single-cell RNA sequencing of 13 human tissues it was established that *ANPEP* (alanyl aminopeptidase), *ENPEP* (glutamyl aminopeptidase) and *DPP4* (dipeptidyl peptidase-4) are the top three genes correlated with *ACE2* [103]. It is known that both ANPEP (which is a membrane-bound broad specificity aminopeptidase) and DPP4 (which is a cell surface glycoprotein receptor) can serve as receptors for HCoVs [104], whereas involvement of the membrane-bound peptidase ENPEP in virus infection is unclear [103]. One should also keep in mind that human coronaviruses regularly use peptidases as their receptors [48]. ANPEP is the targeted receptor for many viruses belonging to the *Coronaviridae* family, such as porcine epidemic diarrhoea virus, HCoV-229E, feline coronavirus, canine coronavirus, transmissible gastroenteritis virus, and infectious bronchitis virus. It is mainly expressing in colon, ileum, rectum, kidney, liver, and skin [103], demonstrating that receptor of coronavirus may have similar expression profiles in human body. Are these data consistent with the fact that CoVs infect similar types of cells and CoV-infected patients share similar clinical symptoms [103]?

Some reports went to discussion of the non-peptidase SARS-CoV receptors as potential avenues for the COVID-19 entry to the host cells. Among such SARS-CoV receptors are DC-SIGN1 (dendritic cell-specific ICAM-3-grabbing non-integrin 1), CLEC4G (C-type lectin domain family 4 member G), and CLEC4M (C-type lectin domain family 4 member M) [103,105,106]. It is known that the RNA enveloped viruses are using extracellular vesicles (exosomes) to translocate into new host cells [107-109]. These vesicles enable the viruses to infect cells in both receptor-dependent and receptor-independent manner and promote viral persistence. They modulate the host immune response, transport populations of viral particles and genomes, increase multiplicities of the ways of viral infection, facilitate cooperative interactions, and enhance the viral replicative fitness [107]. Is SARS-CoV-2 (which is an enveloped RNA virus) follow this pathway to cellular entry and to propagate very quickly? If so, is it dependent or independent on receptor entry? Are there any additional factor that would be increasing the virus entry into the cell?

In search for additional receptor for SARS-CoV cellular entry, SARS pseudovirus or HCoV-NL63 [110,111] were used to explore the possibility of additional routes of the viral entry. It was found that the SARS virus used both of S spike and membrane (M) proteins for interaction with common cellular receptors, heparan sulfate proteoglycans (HSPGs), which are present on most cells [112]. These results demonstrated that HSPGs can serve as

adhesion receptors that provide the binding sites for SARS-CoV invasion at the early attachment phase. HSPG blockage results in the failure of SARS virus entry even in the presence of the internalization factor ACE2 [113]. From this perspective it is important to note lactoferrin/lactotransferrin (LTF) is known to co-localize with the widely distributed cell-surface HSPGs [114-116]. During SARS-CoV infection, a host immune response against the virus is triggered. The innate immune response plays an essential role in the inhibition of viral infection. It has been reported that many genes involved in the innate immune response, such as those encoding LTF, S100A9, and Lipocalin 2, are up-regulated, and their corresponding proteins participate in the SARS-CoV clearance. Among these up-regulated genes, *LTF* expression was elevated by approximately 150 fold in SARS patients compared with healthy controls [117]. Importantly, LTF possesses strong antiviral activity against a broad spectrum of RNA and DNA viruses, such as Sindbis virus, cytomegalovirus, herpes simplex virus, Semliki forest virus, human polyomavirus, human papillomavirus, echovirus, human immunodeficiency virus, hepatitis C Virus and rotavirus [114-116]. These viruses utilize common molecules on the host cell membrane to facilitate their invasion of the cells. These molecules, including HSPGs [114-116] provide the first anchoring sites on the cell surface and help the virus make primary contact with host cells [112]. It has been shown that LTF is able to prevent the internalization of some viruses by binding to HSPGs [118]. Based on these results, it was hypothesized that an underlying mechanism for the anti-SARS-CoV effect of LTF involves the capability of this protein to bind to the broadly distributed HSPGs molecule on host cells [110,111]. Is it possible that the SARS-CoV-2 can use a similar entry pathway?

Finally, there is compelling evidence that CoVs can use multiple pathways to enter the host cell (see **Figure 1**). In one scenario, the entry of SARS-CoV into cells might occur by direct fusion of envelopes with the plasma membrane at the cell surface [119-121]. However, this virus can also take an advantage of the endocytic machinery of the target cell. Here, SARS-CoV enters cells by endosomal pathways, where the S protein is activated for fusion by trypsin-like protease in an acidic endosomal environment [120]. The endocytic pathways used by viruses to get into the host cells include macropinocytosis, clathrin-dependent endocytosis, and caveolae-dependent endocytosis, as well as clathrin- and caveolae-independent endocytosis [122,123]. It was pointed out that in the most cases, only one of these pathways is used by a given virus to enter cells, some viruses might use multiple endocytic pathways to gain entry into host cells [124-127], with one of these viruses being SARS-CoV [128]. Furthermore, there is also a possibility for the non-endosomal entry of a virus into the host cell. Here, proteases, such as trypsin and thermolysin, promote SARS-CoV cell entry directly from the site where this virus is adsorbed onto the cell surface

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[129]. Furthermore, protease-mediated SARS-CoV entry from cell surface was shown to result in 100- to 1,000-fold more efficient infection than entry through endosome [129]. Therefore, SARS-CoV can entry cell via clathrin- and caveolae-independent endocytic pathway or by the non-endosomal pathways that depends on the presence of the proteases [129]. It is known that SARS-CoV-2, which is an enveloped RNA virus, follows this non-endosomal pathway of cellular entry [130].

Human (host) factors

The outcome of SARS-CoV-2 infection is primarily defined by the virus-host interaction, with transmissibility and pathogenicity of SARS-CoV-2 being related to its interplay with host antiviral defense [131]. The first requirements for the successful COVID-19 transmission are the susceptible host with a permissive cell, which carries its receptor. If all these requirements are met, then other factors (such as the receptor orientation, distribution, and structure) will came to play defining the capabilities of viral particles to be distributed vertically (within the host tissues) and horizontally (within the host population). All this could underhandedly help the virus to be more aggressive (virulent).

Often, the viruses emerging from more resistant hosts have lower overall virulence than viruses emerging from more susceptible hosts. There is a correlative evidence supporting the link between the host resistance and virulence evolution [132-134]. For example, since virulent strains can be favored over avirulent pathogen strains as a result of the within-host competition, resistant hosts may limit competitive interactions between co-infecting pathogens, thereby hampering evolution of virulence [135]. The serial passage of virus through resistant *vs.* susceptible host genotypes produces the largest adaptive responses in a viral pathogen, which in turn is also associated with the most dramatic increases in virulence [136]. It is also possible that hosts with intermediate levels of immunity may provide the optimal environment for virus adaptation as both the strength of immune-mediated selection and pathogen population size may be optimized in such individuals [137]. Until now, all the data sets indicate that the COVID-19 may gain some adaptation and virulence factors, which globally contribute to its pathogenicity and transmission.

ACE2 represents the confirmed protein receptor for the SARS-CoV-2 entry into the host cells. The susceptibility of different cohorts of patients to SARS-CoV-2 is correlated with the ACE2 level, and the distribution of target

organs that are susceptible to the SARS-CoV-2 infection and the spread of COVID-19-related complications are similar to that of the ACE2 [138]. In fact, entry of the SARS-CoV-2 into the lung alveolar type 2 (AT2) cells is determined by the presence of this receptor. Although ACE2 is reported to be expressed in lung AT2 cells, liver cholangiocyte, colon colonocytes, esophagus keratinocytes, ileal epithelial cells (ECs), rectum ECs, stomach (ECs), testis, gallbladder cells, and kidney proximal tubules, its expressing levels are rather low, especially in the lung AT2 cells, where the ACE2 expression levels are 4.7-fold lower than the average expression levels of all ACE2 expressing cell types [103,139]. AT2 cells are considered as alveolar stem cells [140]. They comprise only 5% of the alveoli, but produce the surfactant, a factor essential to maintain lung elasticity, and, most importantly, act as progenitors for AT1 cells, the latter covering 95% of the alveoli and responsible for gas exchange. Therefore, SARS-CoV-2 that targets AT2 cells attacks and kills the lung regenerative pool. Depletion in the AT2 cells and corresponding deficit of surfactant have been previously shown to be associated with incomplete repair of injured alveolar epithelium and fibrotic obliteration [141]. Therefore, these mechanisms could also explain the development of lung injury in COVID-19 [142]. The low expression of ACE2 in the lung may also suggest the presence of selected cells with up-regulated ACE2 expression under certain conditions.

To address the role of SARS-CoV-2 tropism in the efficiency of COVID-19 transmission, Sungnak *et al*. looked at the single-cell transcriptome expression data in scRNA-seq datasets from different tissues, such as the respiratory tree, ileum, colon, liver, placenta/decidua, kidney, testis, pancreas, and prostate gland of healthy donors [143]. This analysis revealed that *TMPRSS2*, the primary protease important for SARS-CoV-2 entry, is highly expressed in different tissues, whereas the SARS-CoV-2 entry receptor *ACE2* is characterized by relatively low expression levels in all the tissues analyzed [143]. These findings indicated that at the initial stage of infection, ACE2, and not TMPRSS2, represents a limiting factor for viral entry [143]. The authors also showed that *ACE2* is more highly expressed (and co-expressed with viral entry-associated protease *TMPRSS2*) in nasal epithelial cells, specifically in goblet and ciliated cells. This important finding explains an apparent contradiction between the rapid spread of the SARS-CoV-2 and dependency of this virus on alveolar epithelial cells as the primary point of entry and viral replication. The fact that the SARS-CoV-2 entry receptor ACE2 is more highly expressed and co-expressed with the viral entry-associated protease TMPRSS2 in nasal epithelial cells indicates that these cells can serve as loci of original SARS-CoV-2 infection and also act as possible reservoirs for virus dissemination within a given patient and from person to person [143]. It was also pointed that reported data describe the peculiarities of

ACE2 expression in various tissues of healthy donors and that the gene expression landscape in the nose and other tissues can be drastically changed in a course of viral infection [143].

Furthermore, since in addition to lung and airways *ACE2* is expressed in ileum, colon, and kidney [143], other modes of COVID-19 transmission, which involve intestine, kidney, testis, and other tissues should be considered. A special attention should be paid to the intestines, which express the highest level of ACE2. Earlier studies have demonstrated that diarrhea was present in up to 70% of patients infected with SARS-CoV [144]. Furthermore, a recent case report demonstrated the presence of SARS-CoV-2 in feces of a COVID-19 patient with an initial diarrhea episode [145]. Similar findings have been reported in other studies, indicating that tests of feces and urine samples for the presence of SARS-CoV-2 are warranted [146].

Another important question is whether the ACE polymorphism can serve as one of the factors promoting high efficiency of the COVID-19 spread? Besides serving as a CoV receptor, ACE2 plays an important role in regulation of the renin-angiotensin-aldosterone system (RAAS), which includes a cascade of vasoactive peptides that coordinates key processes in human physiology and maintains plasma sodium concentration, arterial blood pressure, and extracellular volume [147]. Angiotensin I is a physiologically inactive decapeptide derived from angiotensinogen by action of renin. It serves as a precursor for an octapeptide angiotensin II, which is the main RAAS effector that acts as an agonist for both angiotensin II receptors type 1 and type 2 (AT_1R and AT_2R, respectively). Angiotensin II is generated from angiotensin I by action of ACE1. Angiotensin II is converted, by ACE2, to the heptapeptide angiotensin-(1–7), which is a vasodilator. ACE2 also converts angiotensin I to the nonapeptide angiotensin-(1–9), which is further processed by ACE1 to generate angiotensin-(1–7) that serves as an antagonist for the AT_1R receptors and an agonist for the MAS1 receptor (also known as proto-oncogene Mas). Therefore, in RAAS, ACE2 acts as an inhibitor by cleaving a single residue from an angiotensin I to generate angiotensin-(1-9), and via degrading angiotensin II to the angiotensin-(1-7) [148]. Therefore, down-regulation or depletion of ACE2 results in the distortions of the angiotensin II levels, which are linked to an overwhelming number of chronic and acute diseases [147]. SARS-CoV-2 infection down-regulates ACE2 expression, leading to the subsequent elevation of the plasma angiotensin II levels, which are in turn correlate with the total viral load and deterioration of lung tissues [75,149]. In fact, plasma of the COVID-19 patients was shown to contain significant levels of angiotensin II when compared with the healthy individuals [150]. Importantly, in addition to ACE2, ACE1 may also be related to the efficient spread of COVID-19. In fact, it is known that circulating and tissue

concentrations of ACE1 can be altered by a genetic deletion/insertion (D/I) polymorphism in intron 16 of the *ACE1* gene, with the D allele being associated with a reduced expression of ACE2 [151]. Based on the analysis of the D-allele frequency of the *ACE1* gene in samples from 25 different European countries Delanghe *et al*. concluded that 38% of the variability of the COVID-19 prevalence can be attributed to the relative frequency of the *ACE1* D-allele, and that there is a significant correlation between COVID-19 associated mortality and the prevalence of the *ACE1* D-allele [151]. These data suggest that ACE1 D/I polymorphism may be regarded as a confounder in the spread of COVID-19 [151]. These observations are in agreement with the known role of ACE1 in pulmonary infections caused by coronaviruses [152]. Therefore, the *ACE1* D/I genotype may affect the clinical course of the infection. In contrast to this conclusion, analysis of the *ACE2* genomic structure revealed that some allelic variants of this gene would potentially offer a resistance against SARS-CoV-2 [153].

It was recently indicated that, at least in part, the COVID-19 success in transmission can be attributed to the intra-host genomic diversity and plasticity of SARS-CoV-2 and its ability to form low-frequency polymorphic quasispecies [154,155]. This may mean three things [156]: (i) This presence of sequence diversity within the population of viral quasispecies can generate differences within the population in terms of replication kinetics, translation efficiency, packaging, coping with innate host defenses, and responding to antiviral therapies. (ii) When many viral genomes have entered the cytoplasm, this genetic diversity could provide an environment for genetic cooperation and lead to increase in the replication efficiency of multiple quasispecies. (iii) Under selection pressure, group cooperation among viral quasispecies can greatly contribute to population fitness, with such group cooperation being commonly observed when the multiplicity of infecting viral particles was high between passages [157]. Structure and dynamics of quasispecies of replicating RNA enable virus populations to persist in their hosts and cause disease. In fact, there is a critical interplay between the host and virus mutual influences (including in some cases the quasispecies organization), which represents the main driving force for long-term survival of viruses in nature. The stability of virus particles may also play a relevant role in successful transmission [158]. The presence of quasispecies has previously been reported for SARS-CoV and MERS-CoV [155,159,160]. It is known that the recombination events lead to substantial changes in genetic diversity of RNA viruses [161,162]. In CoVs, discontinuous RNA synthesis is commonly observed, resulting in high frequencies of homologous recombination [163], which can be up to 25% across the entire CoV genome [164]. For pathogenic HCoVs, genomic rearrangements are frequently reported during the course of epidemic outbreaks, such as HCoV-OC43 [44], HCoV-NL63 [27], SARS-CoV [27,165,166], and MERS-CoV [167]. It should be mentioned that S protein of SARS-CoV is

the most divergent viral protein in all strains infecting humans [168,169]. The variations arise quickly in both C- and N-terminal domains of S protein, providing important means for the immunological escape [170]. Furthermore, the N-terminal region of S protein hosts a recombination hot-spot, indicating the genomic instability of SARS-CoV-2 over the poly-A and poly-U regions [154]. Often, the progress of infection is associated with virus adaptation to host environments. Variants of the same virus can differ in disease potential (virulence) [171,172].

The COVID-19 tropism based on the gender is a controversy. In fact, one study linked COVID-19 infection and transmission power to gender [173], whereas other researchers did not find any dependency of *ACE2* expression on gender on a single cell level [130], suggesting that the inter- and intra-gender viral transmission is equally efficient until this moment. However, the situation is completely different when comparing the patient susceptibility and the efficiency of COVID-19 transmission based on age (see **Figure 2**). It has been suggested that differential levels of ACE2 in the cardiac and pulmonary tissues of younger versus older adults may be at least partially responsible for the spectrum of disease virulence observed among patients with COVID-19 [174]. Persons older than 60 years with chronic diseases, such as hypertension, diabetes, chronic obstructive pulmonary disease (COPD), as well as cardiovascular, cerebrovascular, liver, kidney, and gastrointestinal diseases are more susceptible to the infection by SARS-CoV-2 and experience higher mortality when they develop COVID-19 [64,175,176]. In addition, patients older than 65 years generally have higher viral load lasting up to 14 days [177] in comparison to the younger patients, who have a much lower viral load that is undetectable within 1 week after onset [178]. The association between the viral load and the severity of COVID-19 has been reported [179]. Collectively, it seems the older people are more susceptible than younger people to COVID-19 hijacking, which may make them as better hosts for virus passage.

Generally, older persons, and especially those with chronic illness, are more susceptible for COVID-19. In fact, while many younger people experience no or mild symptoms on infection, older adults are highly susceptible to life-threatening respiratory and systemic conditions [180]. It seems that there are many factors defining why older people are more susceptible to COVID-19 and experience a higher mortality when they developed COVID-19. In fact, although aging is associated with many changes, one of the most pronounced transformations is the decline of the immune system, affecting both the innate and adoptive immune responses [181,182]. The process of chronological aging is known to affect various components of the immune response, leading to impaired host defense, defective vaccine responses, and a significantly higher risk of elderly persons developing life-threatening

bacterial infections [181,183,184]. Aging affects all immune cells including hematopoietic stem cells (HSCs) that maintain the immune system by producing all blood cells throughout the lifetime of an organism [185]. There are also age-related changes in the T cell compartment that are characterized by three main hallmarks: (i) Decrease in the number of naïve T cells related to the thymic involution [186,187]. (ii) Shrinking of the T-cell receptor (TCR) repertoire that determines antigenic diversity broadness and thus preconditions the successful elimination of pathogens from the system [188]. (iii) Increased proportion of the terminally differentiated oligoclonal effector memory T-cell population, especially those relate to the control of persistent viral infections [189]. In all age, antigen-specific CD4 and CD8 T cell responses decline in number/frequency or delayed in their generation [190], leaving a significant gap between the early innate control and the recruitment of antigen specific T cells to the tissue site to control infection. Furthermore, the memory CD8 T cell population is noticeably changing with age. Despite the increased total percentage of memory CD8 T cells, CD8 T cells in old age have reduced diversity of both the naïve and memory CD8 T cell receptor repertoire [191-193], which has been linked to poor immune responses of aged hosts to vaccines and viral infections [194-196].

Also, in old humans, the number of peripheral B cells decreases, and the antigen-recognition repertoire of B cells and optimal pro-inflammatory cytokines production is altered [197]. As a consequence of the decreased generation of early progenitor B cells, the output of new naïve B cells is reduced [198,199], and consequently the longevity of the antigen-experienced memory B cells is increased [199]. Since class-switch recombination is impaired in memory B cells with aging [199,200], this may also contribute to the decline of the quality of humoral immune response [201]. The production of higher affinity protective antibodies in elderly individuals is impaired [202] due to the age-associated down-regulation of the activation-induced cytidine deaminase (AID), which is the enzyme for class switching, and its transcription factor E47 [203,204]. All these alterations can be related to the increased susceptibility of elderly people to infection with various pathogens [205,206].

Furthermore, as individuals age, they experience an increase in the basal inflammation [207], which is now recognized as a global phenomenon known as inflammaging [208]. Inflammatory cytokines, including TNF and IL-6, are associated with increased risk for many diseases including sarcopenia, osteoarthritis, and many infectious diseases [209-211]. The elderly are more susceptible to many infections, from those that are commonly diagnosed (influenza and Pneumococcal pneumonia) [212,213] to those considered more exotic (such as anthrax and SARS) [210,214], due to their poor response to and control of infectious agents [215].

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There are also some other age-related changes that can contribute to the increased susceptibility to infection. The NLRP3 inflammasome is a multiprotein complex consisting of the nucleotide-binding domain leucine-rich repeat containing (NLR) family member NLRP3 (NACHT, LRR and PYD domains-containing protein 3), the adaptor protein ASC (Apoptosis-associated speck-like protein containing a CARD, also known as PYD and CARD domain-containing protein), and the cysteine protease caspase 1 [216]. The NLRP3 inflammasome can activate caspase 1 in response to cellular danger, resulting in the processing and secretion of proinflammatory cytokines IL1β and IL18 [217-219]. Many studies have reported high IL18 and IL1β levels in SARS, MERS, and COVID-19 patients, not only in the blood, but also in lungs and lymphoid tissues, indicating the increased inflammasome activation. Maturation of IL1β (interleukin-1β) is achieved through the proteolytic cleavage of pro-IL1β by caspase 1, activation of which requires the formation of the NLRP3 inflammasome. When danger signals are sensed in the cells, NLRP3 is activated to recruit ASC and facilitate its oligomerization. For the full activation of inflammasome, two signals are needed. The first of these signals stimulates the pro-IL1β transcription, whereas the second signal leads to the pro-IL1β cleavage [220].

A diverse array of stimuli can activate the NLRP3 inflammasome including both pathogen-associated molecular patterns (PAMPs) and endogenous host-derived molecules indicative of cellular damage [221,222]. NLRP3 inflammasome responses are tightly regulated [223]. Using aged murine models of infection (Influenza A virus (A/PR/8/1934(H1N1)) it was demonstrated that aged mice within 48 h post-secondary *S. pneumoniae* infection possessed increased morbidity and mortality. Increased susceptibility of aged mice was associated with decreased TLR1, TLR6, and TLR9 mRNA expression and diminished IL1β mRNA expression. Examination of NLRP3 inflammasome expression illustrated decreased NLRP3 mRNA expression and decreased IL1β production in aged lung in response to secondary *S. pneumoniae* infection [223]. Hoegen *et al*. used a pneumococcal meningitis model to demonstrate that the NLRP3 inflammasome can contribute to the increased host pathology instead of pathogen protection and clearance [224]. NLRP3 inflammasome is believed to be one of the major pathophysiologic components in the clinical course of patients with COVID-19 [225,226]. It has been shown that the NLRP3 inflammasome serves an important instrument in the development of acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) [227]. It was also demonstrated that SARS-CoV viroporins (i.e., viral proteins with ion channel activity) E protein, ORF3a and ORF8A act as ion-conductive pores in planar lipid bilayers and are required for maximal SARS-CoV replication and virulence [228]. Furthermore, there are data showing that these

three proteins provoke the activation of the NLRP3 inflammasome [225]. For example, it was recently shown that the SARS-CoV ORF3a protein activates the NLRP3 inflammasome in lipopolysaccharide-primed macrophages by affecting K^+ efflux and mitochondrial reactive oxygen species [229]. Another study showed that the SARS-CoV ORF3a accessory protein activates the NLRP3 inflammasome by promoting the TRAF3–mediated ubiquitination of apoptosis associated speck-like protein containing a caspase recruitment domain (ASC) [230]. Although the ORF8 protein of SARS-CoV-2 does not contain known functional domain or motifs, an aggregation motif VLVVL (residues 75–79) has been found in SARS-CoV ORF8B, which was shown to trigger intracellular stress pathways and activate the NLRP3 inflammasomes. However, this motif is apparently absent in ORF8 of the SARS-CoV-2 [226,231].

Apart from the cytokine storm observed in patients infected by the highly pathogenic HCoVs, other cell death programs, such as apoptosis and necrosis might also contribute to the pathogenesis. Cell death is a double-edged sword that can play both antiviral and proviral roles during viral infection [232]. For example, ORF8a from the SARS-CoV was shown to trigger cellular apoptosis [233]. It was shown that the largest of the SARS-CoV accessory proteins, ORF3a, shares membrane insertion characteristics and channel functionality with necrotic effector molecules and interacts with Receptor Interacting Protein 3 (Rip3), which augments the oligomerization of ORF3a causing causes necrotic cell death, lysosomal damage, and caspase-1 activation [234]. Apoptosis was detected in various HCoV-infected samples derived from not only the respiratory tract, but also from the extrapulmonary sites [235]. Autopsy studies of SARS-CoV-infected tissues revealed the presence of apoptosis in lung, spleen, and thyroid [236,237]. The apoptosis induced by SARS-CoV is caspase-dependent and could be inhibited by the Bcl2 overexpression or using the caspase inhibitors [238,239]. In of 293/ACE2 cells infected with SARS-CoV, several apoptosis-associated events were activated [240], among which cleavage of caspase-3, caspase-8, and poly(ADP-ribose) polymerase 1 (PARP), phosphorylation and inactivation of the eukaryotic translation initiation factor 2α (eIF2α) leading to the chromatin condensation, as well as activation of protein kinase R (PKR) and PKR-like endoplasmic reticulum kinase (PERK) [240]. Furthermore, HCoV-induced apoptosis was reported for several immune cells, such as macrophages, monocytes, T lymphocytes and dendritic cells [241]. Infection of primary T lymphocytes by MERS-CoV induced DNA fragmentation and caspase 8 and 9 activation, indicating that in this case both extrinsic and intrinsic apoptotic pathways were activated [242]. Furthermore, MERS-CoV infection was shown to induce pyroptosis (which is a lytic and inflammatory mode of regulated cell death catalyzed by the caspase family) and over-activation of complement (which is an ancient molecular cascade that, being a part of

the immune system, enhances the clearance potential of antibodies and phagocytic cells against microbes and damaged cells, as well as promotes inflammation and regulates attack at the membrane of pathogenic cells) in human macrophages [243].

The physical environment of the lung may also contribute to the efficiency of viral transmission. In fact, this environment is known to change with age [244,245] making the elderly more susceptible to many infections. The elderly experience a decreased lung elasticity and strength of respiratory muscles. Combined with lowered vital capacity [245], this can impair the expulsion of infectious agents through cough reflex, sneezing or breathing. Furthermore, increased incidence of fluid and/or solid aspiration into the lung with old age, and age associated inflammatory disease such as chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis [246]. Lung mucosa or alveolar lining fluid (ALF) is generated, secreted, and recycled by alveolar epithelial cells (ATs), and is essential for proper lung maintenance [247]. In the aged individual, senescent ATs lead to a decrease in lung recycling [247] which in turn can drive a low level of inflammation in the lung [248]. With systemic inflammaging [249], it is therefore reasonable to extrapolate that ALF in old age will also have an elevated inflammatory profile. Indeed, it was shown that ALF, in samples isolated from both of aged experimental animal as well as human, a significantly increased levels of TNF and IL-6 and a trend for increased IL-1β, which confirming that inflammatory cytokines were also present in pulmonary fluids of humans in old age [250]. The presence of increased inflammation within the lung mucosa was also highly associated with changes in multiple innate molecular defense mechanisms. Surfactant proteins A and D (SP-A, SP-D) as well as components of the complement system, notably C3b, were found to be increased in aged mice and elderly human subject ALF [250].

We conclude this overview of the pathogenic pathways and transmission potentials of HCoVs by considering the interplay between epigenetics and the coronavirus infection. This short section complements the description of molecular mechanisms regulating pathogenesis of the emerging coronaviruses, which are complex processes that include virus–host interactions associated with entry, egress, innate immune regulation, and control of various types of programmed cell death. Epigenetics studies how the genetic and non-genetic factors can regulate phenotypic variation. Typically, epigenetic effects are caused by external and environmental factors that alter host expression patterns and performance without any change in the underlying genotype. Therefore, epigenetic regulation links genotype and phenotype by promoting changes in the function of the gene locus without affecting the sequence of the underlying DNA. Some of the most common epigenetic modifications includes chromatin

remodeling, DNA methylation, histone modifications, and non-coding RNAs. These factors act as important regulators of the remodeling of host chromatin and alter host expression patterns and networks in a highly flexible manner. It was pointed out that viruses are able to regulate the host epigenome via a set of highly evolved, intricate, and well-coordinated processes aiming at promotion of the robust virus replication and pathogenesis [251]. Some of these viral mechanisms to disturb and antagonize epigenetic regulatory programs of the host include interference with the histone modification enzymes of the host [252], interference with the chromatin remodeling machinery [253], and the presence of viral proteins that directly bind to the modified histones of the host [254,255]. For example, it was shown that the highly pathogenic H3N2 influenza A virus interferes with the epigenetic control of the gene expression to inhibit the initiation of the host innate immune response using histone mimicry (the C-terminal region of viral NS1 protein mimics the H3 histone tail and interacts with the transcription complex) [256,257]. SARS-CoV and MERS-CoV were shown to delay and/or antagonize pathogen recognition by successfully delaying interferon (IFN)-stimulated gene response [258]. This was achieved by modulation of the histone modifications (such as enrichment in H3K27me3 and depletion in H3K4me3) for a subset of genes, favoring a closed chromatin conformation that inhibits interferon-stimulated gene (ISG) expression [251,258]. In patients with systemic lupus erythematous, which already have elevated ACE2 levels due to the hypomethylation and overexpression of *ACE2*, oxidative stress induced by SARS-CoV-2 infection resulted in exacerbation of these lupus-induced DNA methylation defects, leading to further *ACE2* hypomethylation accompanied by the overexpression of ACE2 and enhanced viremia [259].

Concluding remarks

Data collected in this review clearly indicate that SARS-CoV-2 uses multiple ways for efficient transmission. It has virion structure optimized for various environmental conditions, allowing this virus to use both respiratory and fecal-oral transmission modes. Its S protein has amended structure for efficient interaction with ACE2 receptor and is optimized for furin cleavage. Furthermore, S protein can be primed and activated by TMPRSS2, furin, and multiple non-furin proteases (e.g., plasmin). In addition to ACE2, SARS-CoV-2 can interact with other cellular peptidase receptors, such as ANPEP and DPP4, and also can utilize non-peptidase receptors, such as DC-SIGN1, CLEC4G, and CLEC4M. SARS-CoV-2 utilizes multiple ways for cellular entry (both non-endosomal and endosomal) and potentially uses various means of epigenetic control to inhibit the initiation of the host innate

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immune response. During the course of pandemic, this CoV efficiently undergoes genomic rearrangements, thereby developing important means for the immunological escape. SARS-CoV-2 is engaged in intricate interplay with various host systems and pathways. It initiates cytokine storm and promotes various cell death programs, such as pyroptosis, apoptosis, and necrosis that might contribute to the COVID-19 pathogenesis. This remarkably broad spectrum of means for the efficient SARS-CoV-2 transmission indicates that it is very unlikely that COVID-19 can be cured targeting just one segment of this complex mosaic. Better understanding of various molecular mechanisms associated with all stages of SARS-CoV-2 infection is needed for finding the most appropriate approaches for COVID-19 prevention and treatment.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Author biosketches

Fatma Elrashdy, M.Sc., Teaching assistant, graduated from the Faculty of Medicine, Cairo University, Egypt (2012). She received clinical and academic training in Kasralainy hospitals, Faculty of medicine, Cairo University, Egypt (2013-2014). She worked as Resident in the Department of Tropical medicine, Faculty of medicine, Cairo University, Egypt (2014-2017). She obtained her Master degree in infectious disease, hepatogastroenterology and Tropical medicine from Cairo University of Egypt (2017), and in 2019, she was promoted to the current permanent teaching assistant position at the Department of Tropical Medicine, Faculty of Medicine, Cairo University, Egypt.

Elrashdy Moustafa Redwan, Ph.D., Professor, obtained his PhD in biomedicine and immunology from Cairo University, Egypt (1995) and was promoted to a permanent senior researcher position at the Department of Biomedical Research, VACSERA, where he conducted research on viral and bacterial vaccines development and vaccine immuology. In 1998, he was nominated for a permanent professorship at the City for Scientific Research and Technology Applications (New Borg Alrab, Alexandria 21934, Egypt), where he has established his Laboratory of the Protective and Therapeutic Proteins within the Protein Research Department. For two years, Dr. Redwan worked as visitor scientist at the Scripps Research Institute (San Diego, California, USA) on monoclonal antibody humanization and crystallization. Over the past 20 years, Dr. Redwan has focused his scientific research on the topics related to the life-treating infectious diseases, such as enteric pathogens, Haemophilus influenzae type B virus (HIB), hepatitis C virus (HCV), and hepatitis B virus (HBV). He also looks for roles of natural products as potential anti-viral and anti-bacterial alternative medicine. Dr. Redwan has established a cluster of platforms for production of new biopharmaceuticals and/or biosimilar. Over the years, he supervised and trained many Ph.D. and Master Degree students, as well as Postdoctoral Fellows in the fields of immunology and infectious diseases. Dr. Redwan published many scientific articles and book chapters, served as an editor and reviewer for many academic journals, and worked as a Guest Professor in several national and foreign universities and institutes. He served as an expert in national organizations, such as Egyptian Academy of Scientific Research and Technologies. Dr. Redwan received many national academic honors (2008, 2009, 2010). Currently he is a Sabbaticals Professor at the Biological Science Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

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Vladimir N. Uversky, PhD, DCs, Professor, obtained Ph.D. in biophysics from the Moscow Institute of Physics and Technology (1991) and D.Sc. in biophysics from the Institute of Experimental and Theoretical Biophysics, Russian Academy of Sciences (1998). He spent his early career working on protein folding at the Institute of Protein Research and the Institute for Biological Instrumentation (Russian Academy of Sciences). Here, while working on the experimental characterization of protein folding, Dr. Uversky has found that some mostly unstructured proteins can be biologically active. These findings, together with the similar observations of other researchers, eventually forced him to reconsider the generality of the protein structure-function paradigm and to suggest that natively unfolded (or intrinsically disordered) proteins represent a new important realm of the protein kingdom. In 1998, he moved to the University of California Santa Cruz to work on protein folding, misfolding, and protein intrinsic disorder. In 2004, he moved to the Center for Computational Biology and Bioinformatics at the Indiana University−Purdue University Indianapolis to work on the intrinsically disordered proteins. Since 2010, he has been with the Department of Molecular Biology at the University of South Florida, where he is now a Professor. At the University of South Florida, Dr. Uversky has continued his work on various aspects of protein intrinsic disorder phenomenon and on analysis of protein folding and misfolding. He has published over 900 peer-reviewed articles and book chapters in these fields. Dr. Uversky was included by the Thomson Reuters to the list of the world Highly Cited Researchers in 2014, 2015, 2016, 2017, 2018, and 2019. This distinction is given to researchers who wrote the greatest numbers of reports officially designated by Essential Science Indicators℠ as Highly Cited Papers—ranking among the top 1% most cited for the subject field and year of publication, thereby earning these reports the mark of exceptional impact. Dr. Uversky participated in the establishment of the Intrinsically Disordered Proteins Subgroup at the Biophysical Society and the Intrinsically Disordered Proteins Gordon Research Conference.

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Figure legends

Figure 1. Suggested scenarios for SARS-CoV-2 cellular entry pathways and their potential effects on the viral load and transmission capability.

Figure 2. Suggested scenarios for the COVID-19 pathogenicity in old and young patients.

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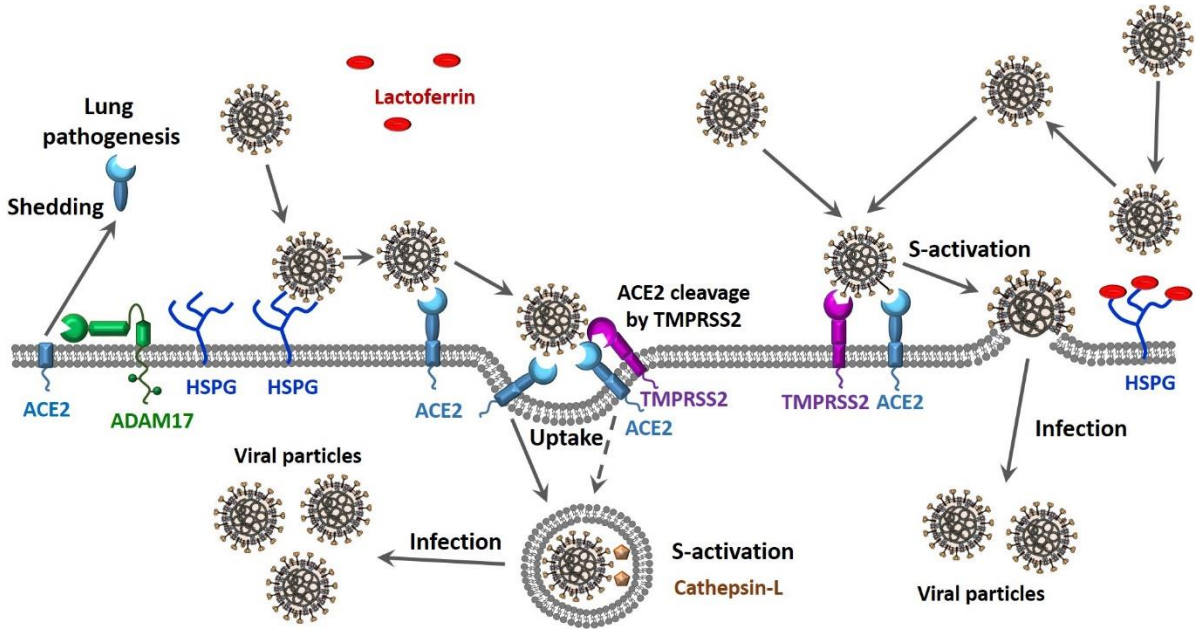

Figure 1

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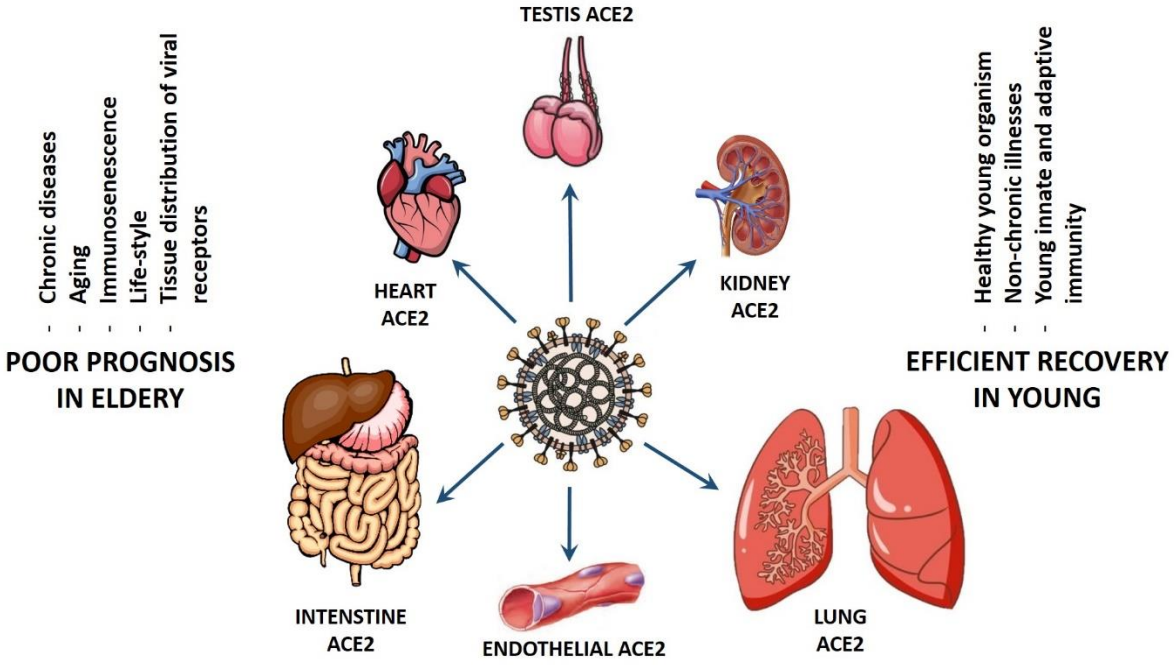

Figure 2