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OPINION ARTICLE

A Pathophysiological Model for COVID-19: Critical Importance of Transepithelial Sodium Transport upon Airway Infection

Martina Gentzsch[1,2] and Bernard C. Rossier[3*]

[1]Department of Cell Biology and Physiology, Marsico Lung Institute, University of North Carolina, Chapel Hill, USA; [2]Department of Pediatric Pulmonology, Marsico Lung Institute, University of North Carolina, Chapel Hill, USA; [3]Department of Pharmacology and Toxicology, University of Lausanne, Lausanne, Switzerland

*Address correspondence to B.C.R. (e-mail: bernard.rossier@unil.ch)

Abstract

The Coronavirus Disease 2019 (COVID-19) pandemic remains a serious public health problem and will continue to be until effective drugs and/or vaccines are available. The rational development of drugs critically depends on our understanding of disease mechanisms, that is, the physiology and pathophysiology underlying the function of the organ targeted by the virus. Since the beginning of the pandemic, tireless efforts around the globe have led to numerous publications on the virus, its receptor, its entry into the cell, its cytopathic effects, and how it triggers innate and native immunity but the role of apical sodium transport mediated by the epithelial sodium channel (ENaC) during the early phases of the infection in the airways has received little attention. We propose a pathophysiological model that defines the possible role of ENaC in this process.

Key words: SARS-CoV-2; ACE2; ENaC; alveolar fluid clearance; mucociliary clearance; COVID-19

Introduction

Unlike former epidemics caused by coronaviruses such as Severe Acute Respiratory Syndrome caused by corona virus 1 (SARS-CoV-1) or Middle East Respiratory Syndrome (MERS) that could be contained by simple public health measures, the present COVID-19 pandemic caused by SARS-CoV-2 is unlikely to be controlled by such simple measures. The respiratory tract is the main target for most coronaviruses and the main clinical features of SARS-CoV-1 and MERS are largely restricted to the upper and lower airways. For SARS-CoV-2, the situation is different since the circulating virus may infect endothelial cells of vessels irrigating lung, kidney, heart, muscle, and brain causing a systemic disease that may affect multiple organs in the organism resulting in their failure. COVID-19 entails high lethality especially in the elderly with comorbidities such as hypertension, Type 2 diabetes, or obesity, while the disease may be mild or even totally asymptomatic in the young. Another aspect of the disease is the fact that the incubation time may be as short as 2 days and as long as 14 days raising the possibility that asymptomatic patients may transmit the virus to many people for a long time without being aware that they harbor the virus.

Rational treatments are based on our understanding of disease mechanisms. Recently, Garvin et al.[1] proposed a mechanistic model involving a RAS-mediated "bradykinin storm"

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based on a comprehensive analysis of gene expression data from Broncho Alveolar Lavage Fluid obtained from severely ill patients. Convincingly, the authors proposed therapeutic interventions using drugs approved by the US Food and Drug Administration (FDA) to target the bradykinin pathways. Disease mechanisms during the early phase of infection are, however, not yet well defined. Two recent articles have drawn our attention to the potential role of transepithelial sodium transport during the infection of the respiratory tract. The first article[2] proposes a gradient of infectivity that is high in the nasal cavity and low in the alveoli (Figure 1). Moreover, not all cells of the respiratory tract are sensitive to the infection with consequences on the progression of the infection in airways. The second article[4] shows that SARS-CoV-2 has acquired an eight amino acid furin cleavage sequence that is identical to that of the α subunit of epithelial sodium channel (EnaC) suggesting that it could play a role in the infectivity of the virus and manifestation of disease (Figure 2). We propose a disease mechanism model for the early nonsystemic phases of COVID-19 focusing on the entry pathways of SARS-CoV-2 in the respiratory tract and its consequences on mucociliary and alveolar fluid clearance (MCC and AFC, respectively) which critically depend on an amiloride-sensitive ENaC-dependent sodium transport expressed along the entire respiratory tract.

Entry Points of the SARS-CoV-2 via its Host Receptor Angiotensin-Converting Enzyme 2

Viruses have adopted subtle strategies to deceive and infect their host using the normal cellular machinery of the cell. Due to a molecular mimicry strategy, SARS-CoV-2 can penetrate the epithelial host cell using the host receptor, angiotensin-converting enzyme 2 (ACE2)[5,6] responsible for the conversion of Angiotensin I (Angiotensin 1–10) into Angiotensin 1–9 and Angiotensin 1–7 leading to the production of bradykinin. This hypotensive pathway is counterbalancing the hypertensive

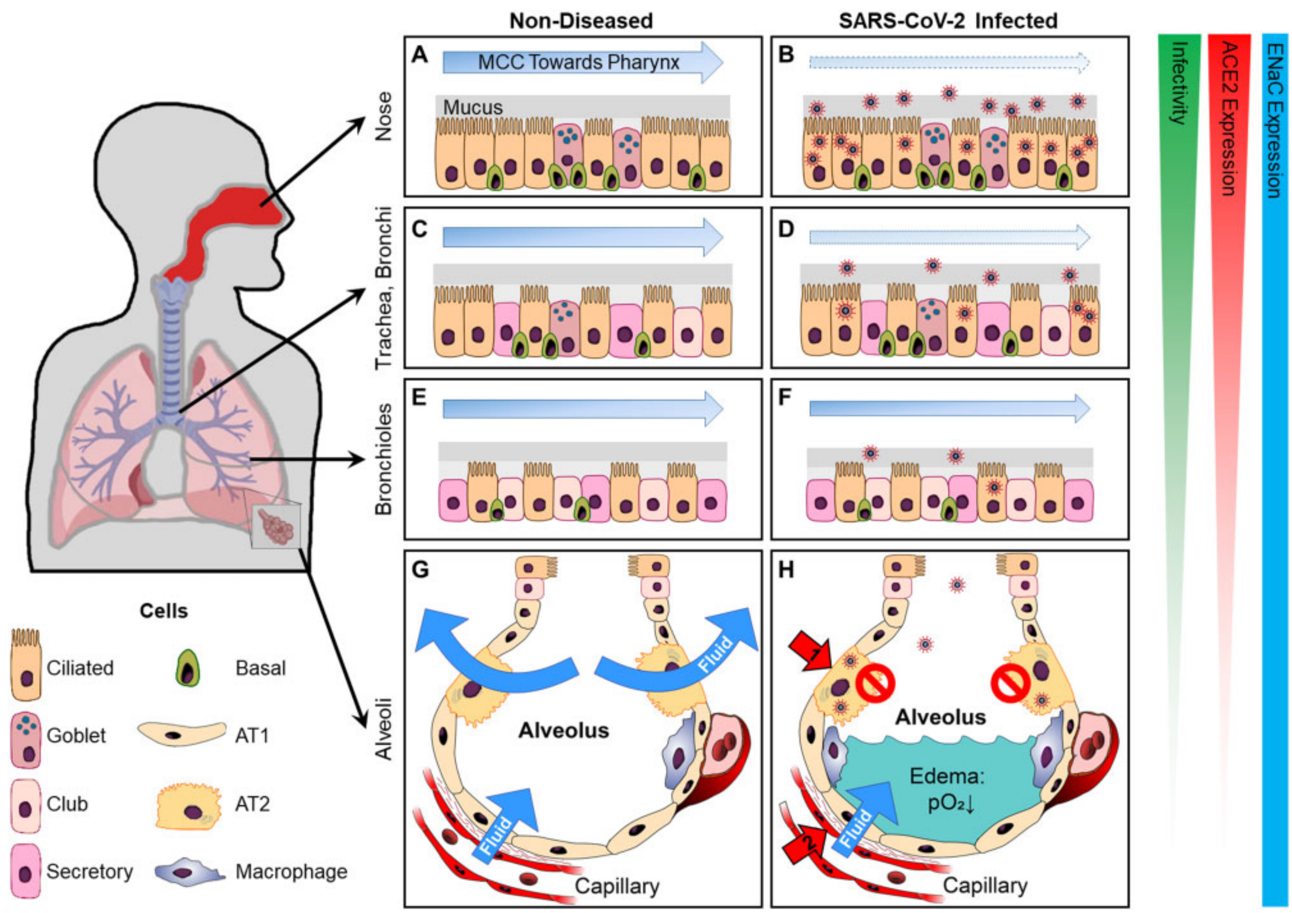

Figure 1. Model of impact of SARS-CoV-2 infection on MCC from proximal to distal airways. During inhalation of SARS-CoV-2, the virus selectively infects ciliated cells by binding to ACE2 and cleavage by TMPRSS2, leaving the function of other cells largely intact. The degree of ciliation decreases toward distal compartments, while the amount of club cells and other secretory cells increases. Aspiration may support transfer of virus from proximal to distal airways. **(A)** Nasal respiratory epithelial cells are highly ciliated and organized in a pseudostratified epithelia. Goblet cells produce mucus that is transported on a periciliary mucus gel toward the pharynx, where it is removed by coughing or swallowing. The MCC allows for the effective elimination of pathogens (bacteria, viruses). **(B)** Ciliated cells in the nasal epithelium are heavily infected with SARS-CoV-2, which disrupts their MCC activity. **(C)** Airway epithelium in trachea and bronchi is composed of various cell types with decreasing amounts of ciliated cells in more distal compartments. **(D)** Similar to nasal cells, MCC is disrupted by infection of ciliated cells by SARS-CoV-2, albeit at a lower degree than in nasal cells. **(E)** In bronchioles, the club cells and other secretory cells are responsible for maintaining homeostasis of the PCL layer. **(F)** As the infection progresses, MCC slows down, allowing more and more distal cells from small bronchi and bronchioles to be infected. **(G)** Alveolar clearance requires alveolar fluid reabsorption that occurs in specialized cells, the AT2 cell, which express ENaC for sodium reabsorption and secrete surfactant. **(H)** SARS-CoV-2 infects AT2 cells and rapidly inhibits sodium reabsorption (red arrow 1). Infection of the capillary endothelium (red arrow 2), causes inflammation and secretion of cytokines and chemokines, which increases fluid secretion in the alveoli, and finally, alveolar fluid increases (edema) causing a decrease in pO_2 and ARDS. (Panels G and H are adapted with permission from B.C.R.[3]).

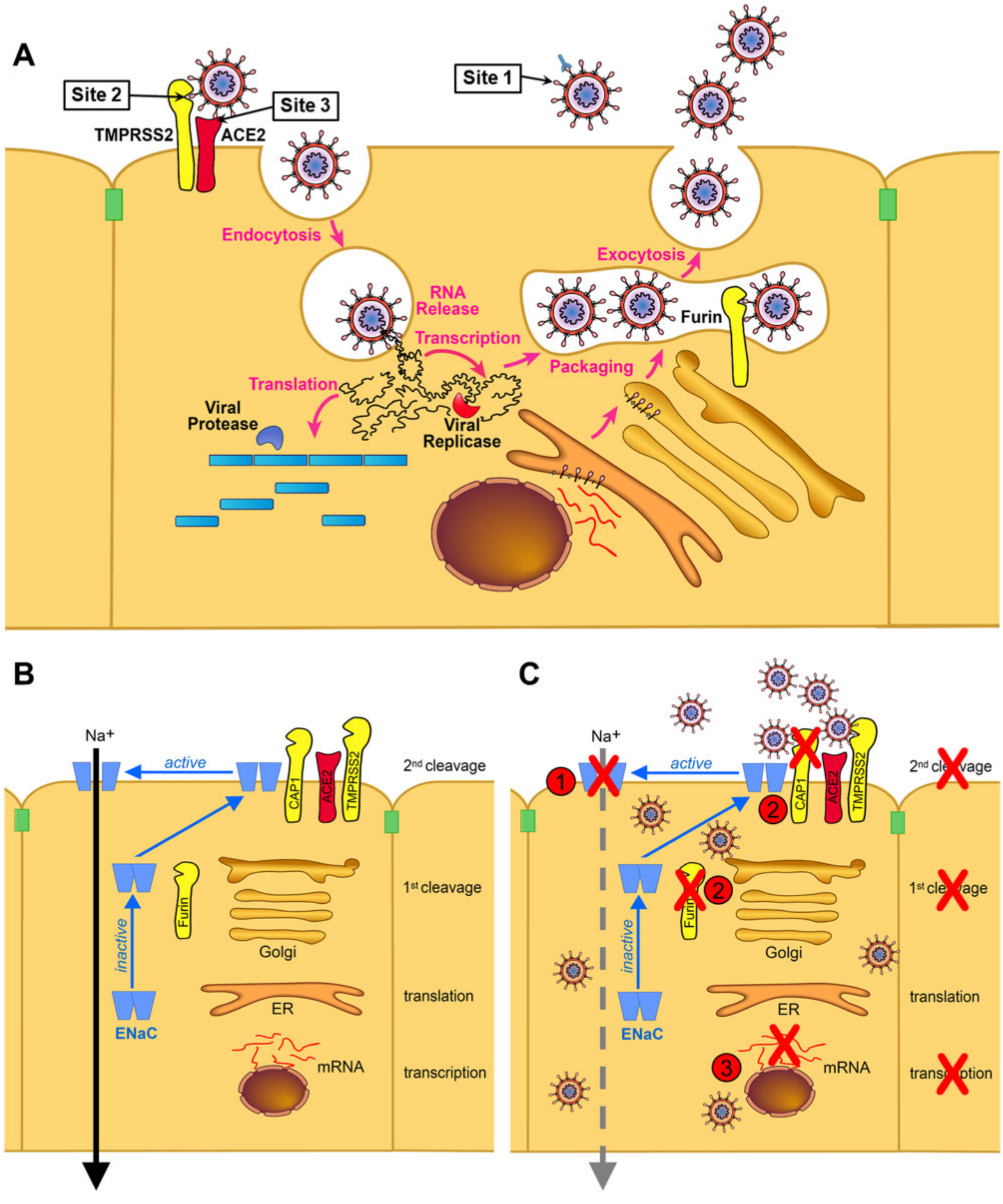

Figure 2. The life cycle and cleavage of SARS-CoV-2 and ENaC in airway cells. (**A**). In AT2 and ciliated cells, spike proteins of the SARS-CoV-2 virion bind to ACE2. After subsequent cleavage of the spike protein by TMPRSS2, SARS-CoV-2 enters the cells through an endosomal pathway. Following the entry of the virus into the host cell, the viral RNA is released into the cytoplasm. A viral replicase assists with RNA transcription. After translation, polyproteins are cleaved by a viral protease. Following the production of SARS-CoV-2 viral proteins, nucleocapsids are assembled and packaged together with structural proteins to assemble a virus particle in the Golgi that is then released by exocytosis. Furin in the Golgi compartment may participate in proteolytic processing of the spike protein. Main therapeutic strategies aimed at preventing virus entry into host cells: Site 1: neutralizing antibodies, Site 2: inhibiting TMPRSS2 activity (nafamostat and camostat), Site 3: peptide-blocking spike protein interaction with ACE2. (**B**) ENaC activation in airway cells. ENaC is first cleaved in the Golgi by furin, which allows its transport to the apical membrane (inactive) where it undergoes a second cleavage (activation) by a second serine protease (channel activating protease [CAP1]) to produce an active ENaC channel. (**C**) In AT2 cells after SARS-CoV-2 infection, three synergistic mechanisms contribute to complete inactivation of ENaC: (1) endothelialitis of the alveolar capillaries produces a massive secretion of cytokines ("cytokine storm") that inhibits ENaC activity at the apical membrane; (2) the multiplication of viral particles and subsequent increased concentration of viral spike proteins will compete for the cleavage of ENaC (competitive antagonism); ENaC will no longer be cleaved and can no longer be exported to the membrane; (3) the virus blocks endogenous gene transcription. (Panels B and C are adapted with permission from B.C.R.[3]).

pathway that is promoted by Angiotensin II (Angiotensin 1–8, which results from cleavage of Angiotensin I by ACE1).

Figure 2A illustrates the life cycle of SARS-CoV-2. The spike protein of the virus binds first to the host receptor ACE2. After subsequent spike protein cleavage by the host protease Transmembrane Serine Protease 2 (TMPRSS2), the virus enters the cells through an endosomal pathway, and then viral ribonucleic acid (RNA) is released into the cytoplasm. After production of viral RNA and proteins, viral particles are packaged and assembled in the Golgi and released by exocytosis.

The initial entry of the virus may depend on two main factors; the viral load and the density of host receptors.[7] A number of studies indicate that there is a gradient of ACE2 expression from the nasal epithelium (very high) to the alveoli (low) (Figure 1). It is fair to postulate that the major entry points of the virus are through cells expressing the highest density of receptors.

Nasal Cavity

The nasal cavity has both respiratory and olfactory functions (Figure 1A and B). The main effect of initial infection would be the cytopathic destruction of nasal epithelial cells composing the pseudostratified columnar epithelium, which is responsible for the nasal MCC. ENaC and the cystic fibrosis transmembrane conductance regulator (CFTR) play a key role here in insuring hydration for the proper function of the ciliated cells, which is to expel various pathogens or airborne particles to the pharynx, where the transported mucus is either swallowed or coughed up. A runny nose is the clinical correlate of the destruction of ciliated epithelia that are responsible for MCC and fluid reabsorption mediated by ENaC. Infection and demolition of sustentacular (supporting) olfactory epithelial cells that are critical for the function of the olfactory sensory (receptor) neurons that are not directly infected, may explain the anosmia, which is clinically well documented in COVID-19 patients.[8,9]

The Oropharynx and Laryngopharynx

A second major entry point would be the nonkeratinized stratified squamous epithelium of the oropharynx and laryngopharynx (hypopharynx). The clinical correlate of the infection is laryngopharyngitis that produces inflammation of the larynx (laryngitis) and pharynx (pharyngitis) as an early sign of COVID-19. Of interest is the potential infection of taste bud epithelial cells. It is important to note that ENaC is expressed in taste buds involved in salt tasting. Experimental evidence in a transgenic mouse model clearly indicates that the sensitivity to low salt concentration is critically dependent on α ENaC expression in taste buds.[10] This would explain the ageusia, which is clinically well documented.[8,9]

From here the infection can proceed either along the gastrointestinal (GI) or the respiratory tract (trachea, bronchi, bronchiole, and alveoli). It appears that SARS-CoV-2 is resistant to changes in pH and thus could survive the gastric acidity and then infect different cells of small and large intestines. Early GI symptomatology such as diarrhea and abdominal pain are well documented but will not be discussed further here.

Progression of the Infection Down the Lower Airways

Trachea and Large Bronchi

It is clear that the route of entry through the tracheobronchial tree remains a frequent feature of the disease (Figure 1C and D). While the nose is the initial site of infection, subsequent aspiration of the viruses from the oropharynx may spread the virus to distal airway compartments. Depending on the viral load and the innate or adaptative immune response of the host, the progression through the lower airways may be rapid (1 or 2 days) or slow (7–14 days), and it can be clinically symptomatic (i.e., cough) or not. The tracheobronchial tree is equipped with magnificent MCC machinery to eliminate dust, allergens, and pathogens. It is the ciliated epithelial cells, which, by their rapid synchronized movement toward the trachea, create a veritable "conveyor belt" for the mucus layer, which advances from the bronchi to the pharynx at a rate of 60 microns/s. The largest inhaled particles (bacteria, viruses) are typically eliminated in about 20 min from the end of the large bronchi to the pharynx. Normally, this treadmill does not cause a cough because the secretions are simply swallowed without awareness. One small disadvantage of this mechanism is that swallowed pathogens can then infect the GI tract (see above). For this "treadmill" to work, it is absolutely necessary to maintain a layer of liquid with a fixed height on the surface of the epithelium. There are actually two layers: the periciliary liquid (PCL) and the mucus layer. The PCL is a few microns deep (the width of an eyelash) and is watery and very fluid to allow ciliary mobility (Figure 1C). PCL is topped by a layer of fluid containing mucins secreted by specialized cells (goblet cells). The model has been further refined to a "gel-on-brush model" in which the PCL is occupied by membrane-spanning mucins and mucopolysaccharides densely tethered to the airway surface.[11] This brush prevents mucus penetration into the periciliary space and causes the "gel" (mucus) to form a distinct layer. In our model, MCC in trachea and large bronchi will be affected by the destruction of the epithelium (Figure 1D). It is important to note that the inflammatory response to the infection (innate and native immunity) will induce the release of bradykinin and in turn an increased expression of ACE2[1] favoring the progression of the infection toward small bronchi and bronchioles.

Bronchioles

This region of the respiratory tree is physiologically very important because it represents the last barrier preventing the infection of alveoli (Figure 1E and F). Bronchiole cells control the height of the two-fluid layers of PCL within 1 micron (Figure 1E) via another specialized cell, the golf club-shaped club cell, formerly called Clara cell, making up ∼20% of the cell population. The club cell or bronchiolar exocrine cell is specialized in producing a solution resembling alveolar surfactant. Additionally, it is involved in a chloride channel-dependent fluid secretion and sodium-selective fluid uptake by ENaC, resulting in water reabsorption to the vascular compartment. Since the identification of the chloride channel gene in 1989, CFTR,[12] whose loss-of-function mutations cause cystic fibrosis, and the identification of ENaC in 1994,[13] we have realized the importance of these two channels in the MCC. Thus, the absence of secretion caused by gene inactivation of CFTR is accompanied by an increase in reabsorption by ENaC, which leads to a dramatic decrease in MCC that results in chronic superinfection and inflammation characteristic of cystic fibrosis. The critical importance of club cells in determining this phenotype is provided by a transgenic mouse model overexpressing ENaC in club cells[14] but not in ciliated cells (Richard C. Boucher, personal communication). Conversely, the loss of ENaC function, observed in pseudohypoaldosteronism Type 1 (PHA-1), causes an increase in MCC[15] both at the level of the lower airways and of the nasal mucosa ("runny nose"). MCC is therefore controlled by a perfect balance between secretion (CFTR) and reabsorption (ENaC). Are club cells infected with the virus? If so, MCC could be strongly affected, but if not, there would be a modest effect on MCC. Recent experimental studies[2] using a refined genetic method have clearly demonstrated the entry gate of the virus into the respiratory tree: ciliated epithelial cells are primarily infected while club cells appear to be spared. We, therefore, expect a modest slowdown of MCC (Figure 1F) but not a cessation as seen in cystic fibrosis in humans or in transgenic mouse models overexpressing ENaC specifically in club cells.[14] It seems that the presence of a dry cough without mucus expectoration may be well explained by this pathophysiological mechanism. Although MCC may be minimally affected at the start of the infection, the patient may remain asymptomatic and nevertheless infect a large number of people. Transmission by asymptomatic carriers appears to distinguish this pandemic from SARS-CoV-1 or MERS infections, which were contagious only when symptoms first appeared. The slowing down of the MCC will gradually become more pronounced as bradykinin-induced ACE2 receptor expression increases (inflammatory response) and thus promote infection of the most distal bronchioles. Symptoms can subside after a few days, but sometimes a sudden worsening is observed 6–7 days after the first symptom: a bilateral acute viral pneumonia can be detected with a CT scan showing peripheral "ground glass" infiltrates, suggesting pulmonary edema, which can quickly lead to a drop in partial pressure of oxygen (pO_2) requiring an additional supply of oxygen and sometimes assisted ventilation.

Alveoli

What occurs mechanistically in the alveoli is different (Figure 1G and H). The alveolus is lined by extremely thin cells, alveolar type 1 (AT1) cells, whose transport capacities are poorly understood. About 10% of the alveolar cells consist of alveolar type 2 (AT2) cells, expressing ENaC, responsible for the reabsorption of alveolar fluid that has passed through AT1 cells. In addition, AT2 cells secrete surfactant, which is essential for the unfolding of the alveoli.

Proper ENaC function requires many processing steps. The messenger RNAs encoding the three subunits of ENaC are transcribed in the nucleus to be exported into the cytoplasm and to be translated into their respective protein in the endoplasmic reticulum (ER) path which leads ENaC to the apical membrane of the epithelial cell and includes proteolytic cleavage sites by at least two distinct serine proteases, furin[16–18] and CAP1[19], essential for the assembly and activation of the channel.[20] First at the trans-Golgi level, furin cleaves the α subunit which allows export of the (presumably inactive) channel to the apical membrane where a second cleavage of the γ subunit by CAP1 allows complete activation of the channel (Figure 2B).[21,22]

Histology studies clearly indicate that AT2 cells can be infected with SARS-CoV-2[23] (Figure 1). Three synergistic factors can explain the severity of pulmonary edema due to a complete inactivation of ENaC. First, bradykinin or cytokines such as TNF β or TGF α are produced by inflammation of the capillary endothelium and can greatly decrease the expression and activity of ENaC in AT2 cells[24] (Figure 2C, red circle 1). Then, increased copies of the viral spike protein expressing the same furin cleavage sequence as ENaC could interfere with ENaC processing and export to the apical membrane by competitive antagonism (Figure 2C, red circle 2). Furthermore, the gene transcription to produce host mRNA (including that encoding ENaC) is blocked by the virus for its own benefit (Figure 2C, red circle 3). Therefore, unlike the asymptomatic phase, where the virus spares sodium transport, the virus completely compromises the reabsorption of sodium in the alveolar cells during this phase, leading to an often fatal acute respiratory distress syndrome (ARDS).

Systemic Disease Is Due to Infection of Endothelial Cells: Possible Role of ENaC

If ARDS can result in a rapid fatality, it is because the virus entering the bloodstream will infect the endothelium (acute endothelialitis) of virtually any blood vessel, leading to major endothelial dysfunction and the failure of many organs.[25,26] Of note, ENaC is expressed in endothelial cells and plays an important role in endothelial function. A recent article[27] demonstrated the importance of ENaC in endothelial function using a conditional transgenic mouse model that enables the α ENaC subunit to be inactivated only in the endothelium. The phenotype is striking: the activity of endothelial ENaC contributes to endothelial vasodilation under physiological conditions and to the preservation of the integrity of the endothelial barrier in endotoxemia. Disruption of ENaC function may contribute to the endothelialitis described above. As in the alveolar cell, SARS-CoV-2 may completely suppress endothelial ENaC activity and thus contribute to the severity of systemic disease. Note that the achievement of the integrity of the endothelial barrier in the alveolus allows the passage of liquid therein. This fluid cannot be reabsorbed by AT2 cells, and a vicious cycle is set in motion. Garvin et al.[1] identify bradykinin as the main component of a "bradykinin storm" provoking vasodilation, hypotension, and increased capillary permeability. Interestingly, bradykinin is also a strong inhibitor of ENaC activity.[28] They also propose that the upregulation of hyaluronan synthases and downregulation of hyaluronidase combined with the bradykinin-induced hyperpermeability of the lung capillaries leads to the formation of a hyaluronic acid hydrogel and the formation of hyalin membranes, a typical pathological feature of lungs from patients deceased from fatal ARDS.[23] In our disease model, this late stage of alveolar dysfunction would be preceded by the failure to reabsorb fluid due to a total and rapid inactivation of ENaC activity. To further exemplify the importance of ENaC function in human pathology, it is interesting to note that the "wet lung syndrome," which affects premature newborns is due to the late development of ENaC during gestation. In experimental animals, transgenic mouse models with α ENaC gene inactivation die within 48 h after birth from the inability to clear their lungs from fluid, which mimics ARDS.[29]

Possible therapeutic strategies

Three extracellular targets are of large interest for pharmacological intervention to prevent the SARS-CoV-2 entry into host cells in COVID-19 (Figure 2A, Sites 1–3): Spike protein, TMPRSS2, and ACE2.

Neutralizing Antibodies

Antibodies that neutralize SARS-CoV-2 by binding to the spike protein may provide promising reagents to prevent host cell entry of the viruses (Figure 2A, Site1). Monoclonal antibodies from convalescent COVID-19 patients showed strong reactions against the viral spike protein and were able to neutralize the virus.[30] Currently, convalescent plasma is being evaluated in clinical trials as a treatment for patients hospitalized with

COVID-19. Recently, the FDA issued an emergency use authorization for investigational convalescent plasma for the treatment of COVID-19 in hospitalized patients.

Inhibition of TMPRSS2

The serine protease inhibitors nafamostat and camostat prevent cleavage of the spike proteins by TMPRSS2 and thus block cell entry of SARS-CoV-2 (Figure 2A, Site 2).[5] Both drugs are employed for treatment of pancreatitis in Japan and their effectivity for COVID-19 is currently being evaluated in clinical trials.

Blocking the ACE2 Interactions

Another promising approach is to design peptides that bind to the spike protein and block the interaction of the virus with the ACE2 receptor (Figure 2A, Site 3). No FDA-approved drugs that target this mechanism are available yet, but a recent preprint shows that this approach is feasible because hACE2 peptides block SARS-CoV-2 pulmonary cell infection in vitro.[31]

For patients suffering from severe ARDS, two classes of drugs may be used: (1) corticoid therapy such as dexamethasone, which acts by decreasing the inflammatory response[32] or (2) drugs interfering with bradykinin or hyaluronan production, which are currently being tested clinically in prospective randomized studies.

For asymptomatic patients or **with early manifestation of the disease**, the therapeutic strategy would be to prevent the entry of the virus into the epithelial cell layer along the respiratory tract. Ideally, neutralizing antibodies induced by vaccination would prevent the infection but safety and efficacy are not yet demonstrated for vaccines presently under development. Meanwhile, simple preventive measures may be helpful. O'Donnell et al. have proposed that oral rinses could be an effective and simple measure to decrease virus load in the oropharynx.[33] A simple intervention to prevent transmission that has been proven effective is the wearing of a face masks that cover the nose and mouth.

Conclusions and unanswered questions

In our opinion, SARS-CoV-2 has developed a very sophisticated strategy to establish infection. Initially, it preserves the integrity of club cells and other secretory cells during early onset of infection. This allows the virus to multiply undetected, without symptoms, and thus promotes asymptomatic but contagious carriers of the virus. In a later phase, the virus infects AT2 cells, which, produce the surfactant, but also reabsorb the alveolar fluid via ENaC. Eventually, the virus compromises the function of virtually all organs by infecting the endothelia of blood vessels, where ENaC also plays an important role, causing inflammation and release of cytokines ("cytokine storm" and/or "bradykinin storm"). Influence of SARS-CoV-2 infection on ENaC function in different cells of the airways and at different phases of the disease should be examined to strengthen this hypothesis and further elucidate the pathophysiology of COVID-19. What is the selective advantage for the virus of acquiring a furin cleavage site identical to that of α ENaC subunit? One explanation for more severe disease caused by the furin site could be that the viral proteins pass from the ER into the Golgi to be cleaved by furin (competing for ENaC cleavage), allowing the release of a large number of viral particles ready for direct infection or primed for further cleavage by various proteases, spreading the virus rapidly without the need for cleavage by TMPRSS2, which is a very sophisticated strategy to propagate infection. These questions remain largely unanswered and the many proposed hypotheses[34–38] should now be tested.

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Conflict of interest

None declared.

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