Immune Netw. 2021 Feb;21(1):e3
https://doi.org/10.4110/in.2021.21.e3
pISSN 1598-2629·eISSN 2092-6685

IMMUNE NETWORK

Review Article

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SARS-CoV-2 Infection of Airway Epithelial Cells

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Received: Jan 9, 2021
Revised: Feb 17, 2021
Accepted: Feb 17, 2021

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
ACE2, Angiotensin-converting enzyme 2; ALI, air-liquid interface; BEAS-2B, bronchial epithelium transformed with Ad12-SV40 2B; CatB/L, cathepsin B and cathepsin L; COPD,

ABSTRACT

Coronavirus disease 2019 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been spreading worldwide since its outbreak in December 2019, and World Health Organization declared it as a pandemic on March 11, 2020. SARS-CoV-2 is highly contagious and is transmitted through airway epithelial cells as the first gateway. SARS-CoV-2 is detected by nasopharyngeal or oropharyngeal swab samples, and the viral load is significantly high in the upper respiratory tract. The host cellular receptors in airway epithelial cells, including angiotensin-converting enzyme 2 and transmembrane serine protease 2, have been identified by single-cell RNA sequencing or immunostaining. The expression levels of these molecules vary by type, function, and location of airway epithelial cells, such as ciliated cells, secretory cells, olfactory epithelial cells, and alveolar epithelial cells, as well as differ from host to host depending on age, sex, or comorbid diseases. Infected airway epithelial cells by SARS-CoV-2 in *ex vivo* experiments produce chemokines and cytokines to recruit inflammatory cells to target organs. Same as other viral infections, IFN signaling is a critical pathway for host defense. Various studies are underway to confirm the pathophysiological mechanisms of SARS-CoV-2 infection. Herein, we review cellular entry, host-viral interactions, immune responses to SARS-CoV-2 in airway epithelial cells. We also discuss therapeutic options related to epithelial immune reactions to SARS-CoV-2.

Keywords: COVID-19; Coronavirus; SARS-CoV-2; Respiratory system; Epithelial cells

INTRODUCTION

In the ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), almost all countries are struggling with the global economic, social, and health crisis. The respiratory virus SARS-CoV-2 was first recognized as an outbreak of pneumonia in December 2019 (1). It is transmitted through droplets to the airways and then mainly attacks the respiratory system (2-4). COVID-19 patients present various clinical symptoms, including fever, cough, sore throat, myalgia, headache, loss of smell or taste, nausea or vomiting, and diarrhea (5). Fever and respiratory

chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; GRP78, 78-kDa glucose-regulated protein; HBEC, human bronchial epithelial cell; HNEC, human nasal epithelial cell; hrsACE2, human recombinant soluble ACE2; icSARS-CoV-2-mNG, infectious-clone-derived SARS-CoV-2 mNeonGreen; ISG, IFN-stimulated gene; MERS, Middle East respiratory syndrome; NRP1, neuropilin-1; PRR, pattern recognition receptors; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; scRNA-seq, single-cell RNA-sequencing; S, spike; TMPRSS2, transmembrane serine protease 2.

Author Contributions

Conceptualization: Ryu G, Shin HW; Data curation: Ryu G, Shin HW; Formal analysis: Ryu G, Shin HW; Funding acquisition: Ryu G, Shin HW; Visualization: Ryu G, Shin HW; Writing - original draft: Ryu G, Shin HW; Writing - review & editing: Ryu G, Shin HW.

symptoms are the main symptoms, and clinical manifestations of COVID-19 patients range from asymptomatic to systemic cytokine storm (5,6).

SARS-CoV-2 is highly contagious and transmitted through direct contact, respiratory droplets, or possibly aerosols (7,8). Diagnostic test using polymerase chain reaction detects the nucleic acids of SARS-CoV-2 with nasopharyngeal or oropharyngeal swab samples (9). Nasopharyngeal or nasal swabs have shown higher viral loads compared with oropharyngeal or oral swabs (10,11). This finding may suggest that the nasal or nasopharyngeal epithelium is the first gateway for viral invasion and transmission (12). Some cellular interactions between host cells and coronavirus have been discovered through the previous pandemic of SARS-CoV in 2003 and the epidemic of Middle East respiratory syndrome (MERS)-CoV in 2013 (13,14). The viral envelop spike (S) protein trimer of SARS-CoV and SARS-CoV-2 binds to the human angiotensin-converting enzyme 2 (ACE2), and S protein is subsequently primed by host cellular protease, transmembrane serine protease 2 (TMPRSS2) (15,16). Clinical manifestations and complications in specific organs have been associated with the cellular expression of ACE2 and TMPRSS2 (17,18). S protein of SARS-CoV-2 binds human ACE2 with 10 to 20-fold higher affinity than SARS-CoV, which may potentiate the infectivity of the virus (19). The nasal epithelium is the gateway and reservoir for SARS-CoV-2, but the epithelial barrier function and subsequent immune reaction can play a key role in protecting the host from infection (20). It is necessary to understand the host's immune reaction to SARS-CoV-2 and to develop therapeutic strategies to control COVID-19.

Global researches on COVID-19 has been proceeding very rapidly and urgently, and much has been revealed within a short period of time. However, until now, extensive studies on the pathophysiological mechanism of SARS-CoV-2 and host epithelial cells are warranted, and further studies on downstream of immune reaction are also needed. Many experts and researchers have emphasized the importance of therapeutics and vaccination from the beginning of the pandemic (21). Unfortunately, there are no definite curable antiviral drugs for SARS-CoV-2, and various clinical trials are underway (22). In this review, we focused on the cell tropism, host-viral interaction, and immune response of SARS-CoV-2 in airway epithelial cells, based on the latest findings published so far. Also, we reviewed therapeutic targets for the epithelial immune response.

CELLULAR ENTRY OF SARS-COV-2 VIA AIRWAY EPITHELIAL CELLS

The primary role of ACE2 is the maturation of angiotensin of the renin-angiotensin system, which controls blood pressure and vasoconstriction (23). ACE2 is expressed in the heart, blood vessels, kidney, esophagus, ileum, colon, upper and lower airways, cornea, liver, gallbladder, and testis (12). However, compared to other organs, the amount of gene or protein expression of ACE2 in the airways is low (12,24). Still, entry of SARS-CoV-2 depends on the expression of receptors (ACE2, TMPRSS2, or cathepsin B and cathepsin L [CatB/L]) of the airways as the first gateway for the respiratory virus to initiate infection, and the distribution of receptors in the upper airway increases the infectivity of the virus (12). Originally, ACE2 plays a protective role in the acute lung injury in respiratory viral infections, such as SARS-CoV and influenza virus (25-27).

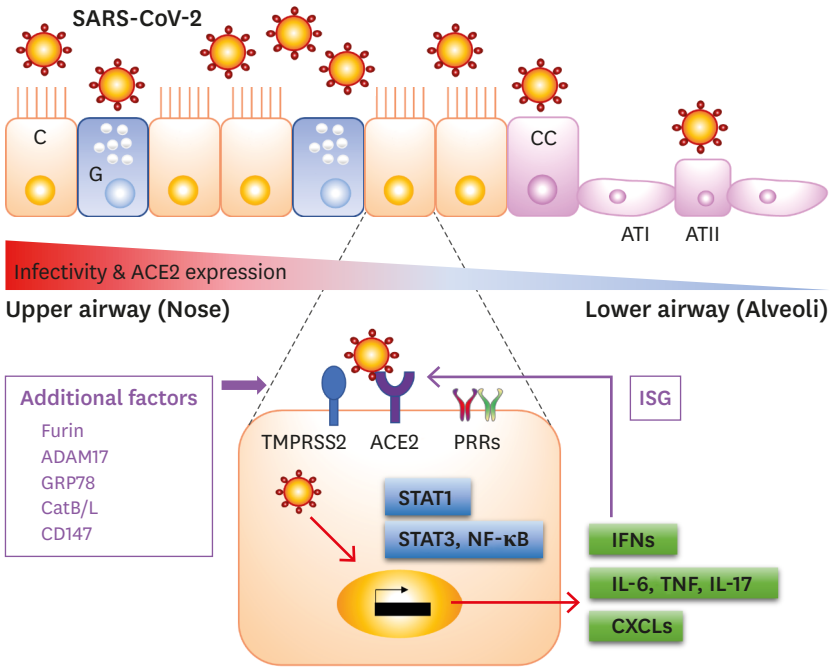

Figure 1. An overview of SARS-CoV-2 infection via airway epithelial cells. ACE2 is expressed in various types of airway epithelial cells, such as C, G, CC, and type II pneumocytes (ATII). The infectivity and ACE2 expression are gradually decreased from the upper airway (red color) to lower airway (blue color). Cellular protease, TMPRSS2, is promoting the cellular entry of the virus with other co-factors. SARS-CoV-2 can activate transcription factors and can stimulate to produce proinflammatory cytokines, chemokines, and IFNs. ACE2 is upregulated by IFNs, so it acts as an ISG.
C, ciliated cells; G, goblet cells; CC, club cells; ATI, alveolar type I cell; ATII, alveolar type II cell; ADAM17, a disintegrin and metalloprotease domain metallopeptidase domain 17.

The trimeric S protein of SARS-CoV-2 is cleaved into S1 and S2, and S1 directly binds to the ACE2 (28). Another cleavage site on S2 is subsequently cleaved by host proteases. To understand the host infection of SARS-CoV-2, we reviewed recent studies of the processes involved in the epithelial cell entry (**Fig. 1**).

Single-cell RNA-sequencing (scRNA-seq) and immunostaining analyses

ACE2 is a well-known cellular receptor of SARS-CoV and SARS-CoV-2 in humans, while MERS-CoV binds dipeptidyl peptidase 4 (29). Many researchers have identified the cellular expression of ACE2 using scRNA-seq data from non-SARS-CoV-2-infected samples. In the upper airway, 1.3% of all secretory cells and 4% of goblet cells expressing *ACE2* in the ethmoid sinus and inferior turbinate from healthy human donors (30). Another study confirmed the SARS-CoV-2 tropism of host cells and genetic expression of viral entry-related genes in the nasal epithelium (12). The authors found that *ACE2* was highly expressed in nasal ciliated and secretory cells, and *ACE2* was also detected in nasal epithelial cells cultured *in vitro* under the air-liquid interface (ALI) condition, a method of culturing cells that expose the apical surface in the air to resemble the airway (31). ACE2 was identified using immunofluorescence staining on the motile cilia of the nasal turbinate, ethmoid sinus, uncinate process, trachea, and bronchus (24). However, another study using immunofluorescence staining showed that ACE2 abundantly observed in the apical surface of sustentacular cells in the olfactory mucosa harvested from patients with chronic rhinosinusitis, and it was expressed at low levels in the respiratory epithelium (32). The

authors suggested that SARS-CoV-2 replicates actively in the nasal and olfactory mucosa, and the expression of ACE2 at specific sites is associate with nasal symptoms, such as anosmia.

TMPRSS2, a host cellular protease promoting the cellular entry of the virus, was broadly expressed in the nasal epithelial cells (4% of goblet cells express *ACE2* and 28% express *TMPRSS2*) (30). This finding suggests that ACE2 is a limiting factor of the transmissibility of SARS-CoV-2 to host epithelial cells (12). The expression of *ACE2* on nasopharyngeal swab was higher in patients with COVID-19 than healthy control, and *ACE2*-positive cells were not increased after SARS-CoV-2 infection, suggesting that ACE2 expression in the upper airway is related to susceptibility for infection (33). In COVID-19 patients, secretory cells were $ACE2^+$/$TMPRSS2^+$/$Furin^+$ cells on scRNA-seq data (33). Furin is a pro-protein convertase and can cleave S protein of SARS-CoV-2, but not SARS-CoV (34). In particular, *Furin* was abundant in the glandular cells of the olfactory epithelium (30).

In the lower respiratory tract, *ACE2* was mainly expressed by type II pneumocyte (alveolar epithelial type II cell) and ciliated cells. Lung ACE2 protein expression was detected using immunohistochemistry of human tissue microarrays, and mucin 1-positive type II pneumocytes exhibited double staining with ACE2, consistent with scRNA-seq data (24). Co-expression of *ACE2* and *TMPRSS2* was identified in 3.8% of type II pneumocytes, while 6.7% express *ACE2* and 29.5% express *TMPRSS2*, respectively (30). *TMPRSS2* was abundantly expressed in type II pneumocytes, club cells, ciliated cells, and type I pneumocytes, and $ACE2^+TMPRSS2^+$ cells included type II pneumocytes, nasal secretory cells, and absorptive enterocytes (30). Lukassen et al. (35) reported that a cluster of $ACE2^+$ cells was identified in the subsegmental bronchial branches and characterized between secretory and ciliated cells, which are referred to as transient secretory cells. These cells co-expressed *TMPRSS2* and *Furin* and highly expressed Rho GTPase, associated with viral replication in the host cells (35).

Additional host proteases or cellular receptors have been known to facilitate cellular entry of SARS-CoV-2 into the host cells, including a disintegrin and metalloprotease domain metallopeptidase domain 17, CatB/L, 78-kDa glucose-regulated protein (GRP78), and CD147 (36). Another host factor known to bind furin-cleaved substrates, neuropilin-1 (NRP1), is one that participates in the cellular entry and infectivity of SARS-CoV-2, and $NRP1^+$ cells were identified in the olfactory epithelium from human COVID-19 autopsies (37,38). These molecules have been studied for their potential as therapeutic targets, which will be discussed later in this review (39).

Airway epithelial cell culture

Respiratory epithelial cell culture systems have been utilized for researches in a variety of fields. Compared with immortalized cell lines, such as Vero E6 or Huh7 cells, fully differentiated human bronchial epithelial cells (HBECs) were effectively infected with SARS-CoV-2, reflecting the biological phenomena occurring in humans (2,40). Submerged or ALI culture with airway epithelial cell lines (A549, bronchial epithelium transformed with Ad12-SV40 2B [BEAS-2B], and Calu-3 cells) or primary cells were analyzed using scRNA-seq or immunostaining (**Table 1**). Researchers have uncovered the same patterns of expression of cellular entry-related molecules as in human tissues (41). Normal HBECs in ALI were analyzed scRNA-seq and expressed *ACE2* and *TMPRSS2* (42). The type II pneumocyte cell line, A549 cells, exhibited strong expression of ACE2 in immunohistochemistry (43). Contrarily, some *in vitro* studies using A549 cell lines showed a low rate of infection and low expression of ACE2 (16,44). Therefore, researchers supplemented A549 cells with an exogenous vector expressing ACE2 for SARS-CoV-2 *in vitro* infection (45). Primary HBECs were expressing ACE2

https://doi.org/10.4110/in.2021.21.e3

Table 1. Summary of airway epithelial cell types used in cell culture experiments with SARS-CoV-2

Cell types	Characteristics	Findings
Primary HNEC	Primary cell	Express ACE2 and TMPRSS2 (46) SARS-CoV-2 infection on the apical surface - Secretion of CXCL10 (46)
Primary HBEC	Primary cell	Express ACE2 and TMPRSS2 (35,42) SARS-CoV-2 infection on the apical surface - Downregulation of tight junction molecules and loss of cilia (48) - Production of IL-6, CXCL9, CXCL10, and CXCL11 (50) - Induction of CCL20, CXCL1, IL-1β, IL-6, CXCL3, CXCL5, CXCL6, CXCL2, CXCL16, and TNF (45) Therapeutic effect: remdesivir (42), camostat mesylate and CatB/L inhibitor (16)
A549 cell	Type II pneumocyte cell line	Strong expression (43) or low expression (16,44) of ACE2
BEAS-2B cell	Normal bronchial epithelial cell line	Therapeutic effect: meplazumab (102)
Calu-3 cell	Lung cancer cell line	Therapeutic effect: camostat mesylate and CatB/L inhibitor (16), IFN-α and IFN-λ (106)
Vero E6 cell	Kidney epithelial cell line	Therapeutic effect: meplazumab (102), human soluble ACE2 (99), IFN-α and IFN-λ (106)

and TMPRSS2, while TMPRSS2 was broadly expressed than ACE2 (35). The SARS-CoV-2 infection also occurred in primary human nasal epithelial cells (HNECs) which were obtained from the inferior turbinate of healthy donors (46). Cultured HNECs expressed ACE2 in the apical side and TMPRSS2 throughout the layer, respectively (46).

Primary HBECs differentiated in ALI culture were infected by SARS-CoV-2, and viral particles were found on the apical surface of ciliated and secretory cells on transmission electron microscopy (47). During the long-term period of ALI culture (up to 51 days), the recurrent replication peak of SARS-CoV-2 was 7 to 10 days, and primary HBECs exhibited decreased tight junction molecule, zonula occludens-1, and loss of cilia (48). As SARS-CoV-2 is attached to the cilia of HBECs in a scanning electron microscopy (49), the virus did not infect HBECs on the basolateral side of the ALI culture system (48). In addition, no viral particles of SARS-CoV-2 were detected in the basolateral chamber (46). Longitudinal analysis of scRNA-seq from HBECs cultured in ALI condition showed that cellular tropism of the virus expands from ciliated cells to basal and club cells, suggesting that basal stem cells are secondary targets (50). Accordingly, ACE2 expression was increased during productive infection in both infected and bystander cells (50). Recombinant SARS-CoV-2, the infectious-clone-derived SARS-CoV-2 mNeonGreen (icSARS-CoV-2-mNG) (51), infected 12.5% of normal HBECs at ALI. Most of the infected cell types were ciliated cells, which was confirmed by flow cytometry and scRNA-seq, but basal cells were not infected by icSARS-CoV-2-mNG (42).

Several studies have been reported the data of SARS-CoV-2 infection of proximal and distal lung organoids cultures (52,53). About 10% of type II pneumocytes organoids or *KRT5*$^{+}$ basal cell organoids revealed SARS-CoV-2 nucleocapsid protein expression, and ACE2 was found in the apical cell membranes (54). Human pluripotent stem cell-derived lung organoid also expressed type II pneumocyte-like cells rich in *ACE2*, *TMPRSS2*, and *Furin*, and infected organoids were induced robust chemokines, such as transcripts of TNF and IL-17 signaling (55). Researchers suggest that airway organoids can provide more physiologic responses to the viral infection compared to conventional cell cultures and can be used for drug screening (56).

Upregulation or downregulation of ACE2 in the airway

ACE2 is a transmembrane protein that is highly expressed in vascular endothelial cells of the lung, and the level of ACE2 expression in patients with COVID-19 varies from host to host (57). Viral receptor gene expression of host cells might be influenced by age, sex, smoking, medications, and comorbid diseases (58).

In an analysis using scRNA-seq of lung tissue and HBECs, there was no difference in *ACE2* expression between males and females (35). The expression levels of ACE2 and TMPRSS2 in children were lower than in adults in nasal and bronchial epithelial tissue assessed using transcriptomic datasets (59). RNA analysis using a cytology brush sample of nasal epithelium, the expression of *ACE2* gene in nasal cytology samples increased significantly with age after adjusted for sex and asthma (60). However, ACE2 expression of bronchioalveolar lavage fluid samples or overall scRNA-seq of the lung had no dependency on age (35,61). Protein levels of ACE2 in lung tissue scored using immunohistochemistry did not show significant differences among 29 subjects with different demographics, such as age, sex, and comorbidities (62). However, aging itself is the most decisive factor in serious illness for SARS-CoV-2 infection (63). In aged COVID-19 patients, the ability to eliminate the virus is impaired and proinflammatory response is augmented (64). The weakened epithelial barrier function and excessive reactive oxygen species production might exaggerate host damage by SARS-CoV-2 in the elderly (65,66).

ACE2 plays an important role in the renin-angiotensin system as a negative regulator by inactivating angiotensin II, which mediates vasoconstriction (67,68). Angiotensin II induces bronchoconstriction, vasoconstriction, fibrosis, cytokine release, and eventually, lung tissue injury (69). Of note, the history of taking ACE inhibitor or angiotensin II receptor blockers did not affect ACE2 expression in the upper airways (24). However, further investigation is needed to identify the effect of ACE inhibitor or angiotensin II receptor blockers in COVID-19.

Gene expression of *ACE2* was the highest in male smokers compared to female smokers or non-smokers (70). Immunohistochemistry of TMPRSS2 and CD147 displayed higher positive cell counts in smokers with chronic obstructive pulmonary disease (COPD) than healthy control, whereas those of ACE2 and GRP78 revealed no differences in 98 human lung samples (36). Transcriptomic analysis of adult COPD patients showed that ACE2 and TMPRSS2 were upregulated compared with healthy controls (59). Furthermore, the level of *ACE2* gene expression was negatively correlated with forced expiratory volume in 1 second and was overexpressed in COPD patients and current smokers (71). These results were consistent with clinical risk factors for COVID-19, including older age, smoking, and COPD (1). Purkayastha et al. (72) performed *in vitro* study to determine the direct effects of cigarette smoke exposure on airway basal stem cells cultured in ALI and infecting SARS-CoV-2. Cigarette smoke exposure increased the number of SARS-CoV-2 infected cells and induced apoptosis.

In contrast, allergy and asthma do not seem to increase the risk of COVID-19 (58). Gene expression data from asthma patients were used to evaluate the association with type 2 inflammation. Type 2 cytokines, *IL13*, but not *IL4* and *IL5*, was inversely correlated with *ACE2* expression, while *IL4* expression was positively correlated with *TMPRSS2* (73). In scRNA-seq data from asthmatic children and healthy controls, the expression of *TMPRSS2* was related to mucus goblet cell markers and type 2 inflammation markers, whereas that of *ACE2* was negatively correlated with type 2 inflammation (74). In the children's cohort, *ACE2* expression in the nasal epithelium was lower in allergic sensitized subjects than non-atopic controls, regardless of asthma status (75). ALI cultured primary HBECs treated with 10 ng/ml (48 h) of IL-13 exhibited downregulation of ACE2 and upregulation of TMPRSS2 in both asthmatic and non-asthmatic atopic groups (73). In addition, chronic stimulation with IL-13 for 10 days on tracheal epithelial culture showed the same results that decreased *ACE2* and increased *TMPRSS2* expression (74). *TMPRSS2* was highly expressed in secretory cells by IL-13 stimulation, suggesting that TMPRSS2 is associated with a mucus secretory network.

However, the role and mechanism of ACE2 and TMPRSS2 in type 2 inflammatory disease is not yet clearly elucidated. In chronic rhinosinusitis, ACE2 and TMPRSS2 expressions seem to be regulated by the inflammatory milieu (76). Initially, it was known that the expression level of ACE2 was low in mucosal tissues of patients with chronic rhinosinusitis (30,77). Same as asthma, ACE2 expression was reduced in eosinophilic nasal polyps characterized by strong type 2 inflammation. However, non-eosinophilic nasal polyps with increased IFN-γ showed higher expression of ACE2 than eosinophilic polyps and control tissues (78).

INTERACTION OF EPITHELIAL CELLS AND IMMUNE CELLS IN SARS-COV-2 INFECTION

Generally, viral infection in airway epithelial cells induces virus-linked pyroptosis, epithelial disruption, loss of cilia, vascular leakage, and triggers local immune responses (79-81). Using pattern recognition receptors (PRRs), airway epithelial cells can detect the pathogen-associated molecular patterns. Single-stranded RNA virus such as SARS-CoV-2 was sensed by TLR7 and TLR8. After PRR activation, transcription of antiviral genes and secretion of cytokines/chemokines are subsequently processed (82). However, the role of PRRs and the innate immune response of SARS-CoV-2 are under investigation.

After intracellular PRRs sense viral RNAs, transcription factors are activated to initiate cellular antiviral responses, including induction of type I and III IFNs and secretion of chemokines (83-85). During infection and replication of the SARS-CoV-2, airway epithelial cells secrete inflammatory mediators and play a role in triggering host immunologic reactions (41). Unlike other respiratory viruses, cells infected with SARS-CoV-2 induce a low level of IFNs but drive strong expression of chemokines and cytokines (45). Type I IFN response impairment was associated with severe cases among COVID-19 patients (86). According to the gene set enrichment analysis of peripheral white blood cells from COVID-19 patients, innate and inflammatory pathways were increased in severe and critical patients, whereas type I IFN responses and IFN-stimulated genes (ISGs) were downregulated in those groups (86). Additionally, patients with severe COVID-19 had impaired IFN-α production when stimulated with TLR ligands in dendritic cells (87). Some patients with severe COVID-19 presented multisystemic inflammation due to cytokine storm, with a prolonged elevation of cytokines and chemokines (88,89). These imbalances in the immune reaction conferring low IFNs and high cytokines might cause serious and fatal disease in COVID-19 patients (90). Bronchioalveolar lavage fluids of COVID-19 patients were analyzed using scRNA-seq, and the results showed different immunologic profiles according to the disease severity (91,92). Severe cases had more vital interaction between epithelial cells and immune cells and more potent inflammatory macrophages and cytotoxic T cells (33).

Epithelial cell-derived cytokines and chemokines

Proinflammatory cytokines were elevated in the serum of COVID-19 patients, with higher levels of these cytokines associated with severe cases (93). Especially, serum IL-6 levels correlated with acute respiratory distress syndrome, respiratory failure, and increased mortality (94). Innate immune response in ALI culture with scRNA-seq resulted that infected cells (ciliated, basal, and club cells) produced IL-6, CXCL9, CXCL10, and CXCL11 (50). Bronchial secretory cells infected with SARS-CoV-2 expressed IL-6 at 24 h post-infection, which was blocked by remdesivir pretreatment (42). Another SARS-CoV-2 infection study showed that CXCL10 was detected in the basolateral chamber of ALI culture system of HNECs

(46). Normal HBECs infected by SARS-CoV-2 induced CCL20, CXCL1, IL-1β, IL-6, CXCL3, CXCL5, CXCL6, CXCL2, CXCL16, and TNF (45). In COVID-19 patients, ciliated cells expressed CCL15, and secretory cells highly expressed CXCL1, CXCL3, CXCL6, CXCL16, and CXCL17, resulting in recruitment of monocytes, macrophages, neutrophils, T cells, and mast cells (33). SARS-CoV-2 infection can activate NF-κB via PRRs and angiotensin II can co-activate NF-κB and IL6-STAT3 axis (95). IL-6-mediated proinflammatory responses are associated with cytokine release syndrome in COVID-19 (89).

IFN signaling

Several studies confirmed that ACE2 is behavior as an ISG in human airway epithelial cells (30,33). ACE2 was directly enhanced by IFN-α stimulation in primary HNECs (30), same as ACE2 was upregulated by IFN-β in HBECs (96). Upper and lower airway epithelial cells, especially *ACE2*$^{+}$*TMPRSS2*$^{+}$ cells, have been found to be upregulated with genes involved in IFN signaling, including *ADAR*, *GBP2*, *OAS1*, *JAK1*, and *DUOX2* (12,30). Induction of IFN in normal HBECs was strongly correlated with levels of SARS-CoV-2 replication, and ISGs were robustly induced in ciliated cells (42). SARS-CoV-2 *in vitro* infection did not elicit type I or III IFN expression but exhibit moderate levels of ISGs and proinflammatory cytokines in normal HBECs and unmodified A549 cells (45). Conversely, human parainfluenza virus 3 and respiratory syncytial virus induced high levels of IFNs and ISGs. Recently, Onabajo et al. (97) have identified the role of truncated isotype of *ACE2*, *dACE2*, as an ISG. IFNs and respiratory viruses, including SARS-CoV-2 induced *dACE2*, but not full-length *ACE2*, in various human cell lines, while overexpressed *dACE2* does not bind to SARS-CoV-2. STAT1 was one of the best predictors for *ACE2* expression in the airway epithelium (33). Interestingly, phosphorylation of STAT1 was blocked in infected lower airway cells, while uninfected bystander cells showed STAT1 phosphorylation and nuclear translocation, resulting in ISGs induction (52).

EPITHELIAL CELL-RELATED THERAPEUTIC OPTIONS

Blocking the cellular entry of SARS-CoV-2

ACE2 acts as a receptor of viral entry of SARS-CoV-2 and SARS-CoV, while protecting from lung injury (98). Human recombinant soluble ACE2 (hrsACE2) was treated with SARS-CoV-2 infected Vero E6 cells, and hrsACE2 inhibited the viral infection in a dose-dependent manner (99). The protective role of hrsACE2 has been proved in acute respiratory distress syndrome (100,101). TMPRSS2 inhibitor (camostat mesylate) and endosomal cysteine proteases CatB/L inhibitor (ammonium chloride or E-64d) block SARS-CoV-2 priming and entry in primary HBECs and Calu-3 cells (16). An anti-CD147 monoclonal Ab, meplazumab, can block SARS-CoV-2 amplification in Vero E6 and BEAS-2B cell lines (102). COVID-19 patients treated with meplazumab had a shorter recovery time and lower severity of the disease (103).

Therapeutic role of IFNs

To overcome viral IFN evasion strategies, antiviral activity of type I and III IFNs have been studied in SARS-CoV and MERS-CoV (104,105). In human Calu-3 and Vero E6 cell line, IFN-α and IFN-λ inhibited SARS-CoV-2 replication, while only IFN-α blocked SARS-CoV (106). Another study on Calu-3 and Vero E6 cells pretreated with IFN-α demonstrated that only SARS-CoV-2 was attenuated and failed to phosphorylate of STAT1 and express ISGs (107). Overall, SARS-CoV-2 is more sensitive to IFN treatment than SARS-CoV (108). Type III IFN, also known as IFN-λ, plays an essential role as a first defense line at the mucosal barrier surface, such as respiratory, gastrointestinal, and genitourinary tracts (109). Unlikely type I

IFNs, IFN-λ receptor, IFNLR, is restricted to epithelial cells and neutrophils (110). Therefore, treatment with IFN-λ at an early stage of COVID-19 would elicit ISG and antiviral response in epithelial cells (111). However, Broggi et al. (112) recently reported that IFN-λ produced by dendritic cells in the lower airway in SARS-CoV-2 infection, and chronic exposure of IFN-λ on lung epithelial cells compromise barrier function, predisposing secondary bacterial infection. Taken together, further meticulous studies are warranted to evaluate the therapeutic roles of IFNs in COVID-19.

CONCLUSION

Airway epithelial cells are crucial for cell entry and viral replication of SARS-CoV-2. Expression of ACE2, the host cellular receptor for SARS-CoV-2, is regulated by a variety of host factors, such as age, sex, smoking, and comorbidities. Once exposed to SARS-CoV-2, the amount of virus entering the host tissues depends on the expression level of principal receptors in the airway epithelial cells. The subsequent immune response and inflammatory substances secreted by infected epithelial cells play an essential role in initiating infection and triggering inflammation. Therefore, ACE2 expression and epithelial cell-derived inflammatory markers are associated with the clinical course and prognosis of COVID-19. Reduced or blocked type I IFN response leads to an imbalance of the immune system in COVID-19 patients, and it can be a biomarker of impairment of innate immunity to SARS-CoV-2 infection. Further studies on the interaction between SARS-CoV-2 and airway epithelial cells and their potential as therapeutic targets are needed.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Research Foundation of Korea (2020R1A4A2002903 to H.W.S. and 2020R1I1A3075353 to G.R.) and a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant No. HI17C1669). This work was supported by the Soonchunhyang University Research Fund.

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