Respiratory epithelial cell responses to SARS-CoV-2 in COVID-19

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ABSTRACT
COVID-19 has different clinical stages, and effective therapy depends on the location and extent of the infection. The purpose of this review is to provide a background for understanding the progression of the disease throughout the pulmonary epithelium and discuss therapeutic options. The prime sites for infection that will be contrasted in this review are the conducting airways and the gas exchange portions of the lung. These two sites are characterised by distinct cellular composition and innate immune responses, which suggests the use of distinct therapeutic agents. In the nose, ciliated cells are the primary target cells for SARS-CoV-2 viral infection, replication and release. Infected cells shed their cilia, which disables mucociliary clearance. Evidence further points to a suppressed or incompletely activated innate immune response to SARS-CoV-2 infection in the upper airways. Asymptomatic individuals can still have a productive viral infection and infect others. In the gas exchange portion of the lung, the alveolar type II epithelial cell is the main target cell type. Cell death and marked innate immune response during infection likely contribute to alveolar damage and resultant acute respiratory distress syndrome. Alveolar infection can precipitate a hyperinflammatory state, which is the target of many therapies in severe COVID-19. Disease resolution in the lung is variable and may include scarring and long-term sequelae because the alveolar type II cells are also progenitor cells for the alveolar epithelium.

INTRODUCTION
COVID-19 is a complex disease with clinical responses varying from asymptomatic to severe with catastrophic respiratory failure and death. Some therapies are effective early in the course of the disease but appear relatively ineffective in critically ill patients. Other treatments are contraindicated early in the disease but are proven to be effective late in the disease. COVID-19 and the response to therapy can be understood from the perspective of where the infection is located and which cells are infected during the clinical course of disease. The initial site of infection and viral replication is the sinonasal airway epithelium, a patchwork of luminal ciliated and mucus secretory cells that mediate mucociliary clearance. Studies show that ciliated cells are the primary target of SARS-CoV-21 and mediate initial viral replication and luminal release. At this stage, treatment needs to be designed to block infection and limit viral propagation. As the disease spreads down along the respiratory tract to the alveolar compartment, target cells and responses change and thus treatment strategies need to adapt. It is in the alveoli where the infection can turn deadly. Here, the primary cell that is infected is the alveolar type II cell, which secretes pulmonary surfactant for effective gas exchange and acts as the progenitor cell for type II and type I epithelial cells, the latter of which cover 95% of the alveolar surface. In addition to blocking viral entry and replication, treatment for SARS-CoV-2 is in the alveoli needs to focus on preventing damage to alveolar epithelial cells and the endothelial cells of the microvasculature, limiting the influx of inflammatory cells and promoting epithelial repair. This review will focus on the target cell types that are directly infected and initiate the innate immune response, summarising knowledge gained from human patients and controls, in vitro pathogen challenge models as well as in vitro studies with SARS-CoV-2-infected human respiratory epithelial cells. More detailed reviews on different aspects of SARS-CoV-2 and COVID-19 are available elsewhere and will provide more information on the virus itself and the response of recruited inflammatory cells.2,3

THE CONDUCTING AIRWAYS
Initial infection site: the sinonasal passages
Inhaled viral particles are primarily deposited on the nasal mucosa where the virus infects, replicates within and is released from epithelial target cells.4 Figure 1 These infections may be asymptomatic or may cause local symptoms. Presence of the angiotensin-converting enzyme 2 (ACE2) and membrane serine protease 2 (TMPRSS2) viral entry factor transcripts in single-cell RNA sequencing (scRNAseq) data sets was used as the first approximation of the cells that are likely targets of SARS CoV-2 within a given tissue.5,6 ACE2 is the cell surface receptor recognised by the SARS-CoV-2 spike (S) protein, and TMPRSS2 is a serine protease that cleaves the S protein for viral entry. It should be noted, however, that ACE2 expression is highly variable among cells and is expressed at low levels.

Along with patient samples, primary airway epithelial cell cultures derived from nasal brushings have provided much of our understanding of SARS-CoV-2 infection mechanisms and cellular responses. Primary cultured epithelia are readily infected with SARS-CoV-2 and release a substantial amount of virus from the apical cell surface.5,6,9 In the nasal epithelium, both ciliated and mucous-secreting goblet cells express ACE2 and TMPRSS2.10 ACE2 protein has only been detected on cilia and the apical ciliated cell surface.11 Tropism and viral entry likely depend on additional molecules and structures. Other proteases such as cathepsin B and L and furin may also be involved in infection. Pinto

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Received 3 May 2021
Accepted 9 July 2021
et al reported that SARS-CoV-2 attaches to the actin-rich microvilli on the ciliated cell apical surface and not the cilia themselves. 12 It will be important to localise ACE2 more precisely and to better define the functions of ciliated cell structures. Importantly, the airway epithelium has altered cellular composition and contains structurally and functionally aberrant ciliated cells in chronic inflammatory airway diseases such as asthma, cystic fibrosis, chronic rhinosinusitis and chronic obstructive pulmonary disease. 13–17 Furthermore, ACE2 expression is stimulated by IL-1β and IFN-β and inhibited by IL-13 in bronchial epithelial cells. 7 It will be critical to understand whether this impacts infection and COVID-19 disease outcomes in patients with chronic airway diseases.

Target cells for SARS-CoV-2 infection in the upper airways

Infected ciliated cells shed their ciliary axonemes, 12 18 which disables mucociliary clearance and likely enables disease progression. However, the nasal epithelium itself should be able to recover, since the basal stem cells are relatively spared from infection. Epithelial cells within the nasal submucosal glands also remain uninfected. 9 The one unusual feature of COVID-19 in some patients is anosmia, the loss of sense of smell. This is likely due to infection of the epithelial cells in the olfactory bulb. 5 19 There is concern that infection of the nerves in the nasal bulb may lead to retrograde passage of the virus into the central nervous system, but this requires additional study. 5 We need more information on the rate of recovery of the sense of smell and whether these symptoms are associated with a distinctive clinical outcome.

Dampened innate immune response to SARS-CoV-2 infection

The innate immune response to SARS-CoV-2 in the nasal passages is complex and has recently been reviewed. 20 Evidence points to a dampened interferon response, which may be related to disease severity. ScRNAseq of cells obtained from nasal brushings of COVID-19 patients 10 failed to detect type I (interferon alpha or beta) or type III (interferon lambda) transcripts, although cells did express interferon responsive antiviral genes (MX1 and IFITM3). Interferon gene expression was markedly lower in patients with severe COVID-19 infection than in those with mild disease. Fiege et al used scRNAseq of cultures of human tracheal cells and not only demonstrated primary infection in ciliated cells and a reduced interferon response but also observed a markedly reduced rate of infection after treatment with interferon beta. 21 Pizzorno et al showed that both nasal and bronchial primary airway epithelial cultures produced minimal interferon and interferon-regulated gene expression at 24 hours after SARS-CoV-2 infection, 22 although expression rose at later time points. Gamage et al measured multiple secreted cytokines after SARS-CoV-2 infection of nasal epithelial cells, and, with the exception of CXCL10, also reported dampened cytokine secretion. 8 This innate immune response was significantly reduced from what was observed with influenza A infection. 8

The reason for the dampened interferon response to SARS-CoV-2 is not known and is currently being investigated. The signalling cascades in infected epithelial cells that lead to cytokine production begin with recognition of the virus by foreign pattern-recognition molecules such as Toll-like Receptor-3 (TLR-3) and TLR-7 in the endosome and RIG-I and MDA5 in the cytoplasm. 23 This leads to activation of the latent transcription factors interferon regulatory factors 3 and 7 and nuclear factor-kappa β, which ultimately initiate transcription of type I and type III interferons and other cytokines. 23 Interferon beta signals through a JAK/STAT pathway to stimulate hundreds of interferon stimulated genes in infected and uninfected bystander cells. While the precise cause for the reduced interferon signal is not known, viruses such as SARS-CoV-1, Middle East Respiratory Syndrome (MERS) and influenza A have viral proteins

Figure 1  SARS-CoV-2 infection of the upper airway epithelium (A) SARS-CoV-2 infects the ACE2 expressing ciliated cells. (B) SARS-CoV-2 replicates, is released apically, and infects neighbouring cells. (C) The ciliated cells initiate the innate immune response by secreting type I and type III interferons and other cytokines. It should be noted that the events in (B) and (C) are shown to illustrate the infection process. However, they occur nearly simultaneously in an infected tissue. The time from infection to initial release of virus is estimated to be about 6 hours. SARS-CoV-2 has the ability to dampen or delay the interferon and cytokine response compared with similar infections with influenza. (D) The infected ciliated cells shed their cilia leading to impaired mucociliary clearance. In some areas, the epithelial barrier is severely damaged. The basal cells are spared from infection and can proliferate and restore the damaged epithelium.
that can inhibit the interferon response. The NS1 protein of influenza A markedly reduces interferon-stimulated gene expression. Comparable candidate proteins in SARS-CoV-2 are open reading frame 3b (ORF3b) and ORF6 but defining the functions of these and other non-structural proteins requires more investigation. The host microbiome and other pathogen exposures may also play a role in the dampened immune response. The nose is the entry point for a variety of inhaled viruses, pollens, pollutants and other antigens. It supports an extensive microbiome-containing cultivable and non-cultivable bacteria. Because of ongoing exposure to microbes and their pathogen-associated molecular patterns, the innate immune response in the nose is likely dampened. Finally, the relatively lower temperature at the sinonasal airspace and epithelial surface may also contribute to the dampened immune response in cells in spite of high levels of viral shedding. V’kovski et al reported that SARS-CoV-2 grew to higher titres at 33°C compared with 37°C in airway epithelial cells, and this was associated with a reduced interferon transcriptional response. This also provides additional rationale as to why even asymptomatic or mildly symptomatic individuals can shed a significant amount of SARS-CoV-2 virus from their nasal passages.

Therapeutic strategies for early infection in the upper airways

Treatments for early, mild infections are designed to block virus interaction with host ACE2 or to inhibit subsequent viral replication. Monoclonal antibodies to the receptor binding domain of the SARS-CoV-2 spike protein inhibit viral docking to the ACE2 receptor. This treatment is very effective if administered in the first few days of clinical infection. However, administering these antibodies as outpatients is logistically difficult, and some of these antibodies may not recognise the new and emerging variants. Drugs that block viral replication such as remdesivir of these antibodies may not recognise the new and emerging variants. This may be particularly facilitated in the elderly, conducting airways, likely by microaspiration of pharyngeal secretions. This may be particularly facilitated in the elderly, conducting airways, likely by microaspiration of pharyngeal secretions. This may be particularly facilitated in the elderly, conducting airways, likely by microaspiration of pharyngeal secretions. This may be particularly facilitated in the elderly, conducting airways, likely by microaspiration of pharyngeal secretions.

Infection of the lower conducting airways—target cells, cellular responses and therapeutic strategies

The next stage of the disease occurs after the virus enters the conducting airways, likely by microaspiration of pharyngeal secretions. This may be particularly facilitated in the elderly, who have diminished cough reflexes and reduced baseline mucociliary clearance. Infection in the bronchi produces epithelial injury, cellular infiltrates and in some cases marked denudation of the epithelial surface. The cells that express ACE2 and TMPRSS2 in the conducting airways are ciliated cells, mucus secreting cells and club cells. Club cells, identified by uteroglobin (SCGB1A1) as well as surfactant protein (SP)-A and SP-D expression, are present in only the very small bronchioles in humans, although they are more widespread in the airways of hamsters and mice. As in the nose, ciliated cells are the primary infected cell type. In pathologic specimens, ciliated and secretory epithelial cells appear infected, but progenitor basal cells are spared. This is supported by the primary bronchial epithelial cell culture model, where ciliated cells are the main cells infected at early times and then the infection spreads to secretory cells.

Similar to the nasal epithelium, Belanço-Melo et al found only a modest cytokine response to SARS-CoV-2 in cultured bronchial cells in contrast to influenza A or treatment with Interferon beta (IFN-β). Type I and type III interferons were undetectable, and there was only a modest increase in CXCL10 expression, a marker of interferon stimulation. Ravindra et al showed that using scRNAseq type I and type III interferon gene expression was restricted to the infected ciliated cells with no detectable expression in neighbouring bystander cells. However, both infected and bystander cells expressed interferon responsive genes, implying a response to secreted interferon by the bystander cells. Hui et al demonstrated reduced CXCL10 and multiple other cytokines at the transcript and secreted protein level, respectively, in bronchial cultures compared to infections with influenza. Hence, as in the nose, the interferon and cytokine responses are only modest following SARS-CoV-2 infection. Chua et al used scRNAseq of nasopharyngeal and bronchial samples from patients with COVID-19 to better define the interactions of epithelial cells and immune cells and reported that patients with critical COVID-19 had an enhanced inflammatory response and highlighted the importance of C-C Motif Chemokine Receptor 1 (CCR1) and CCR5 pathways for the recruitment of macrophages.

As in the nose, current treatment at this stage of the infection is directed at preventing viral infection with monoclonal antibodies and limiting viral propagation with antiviral agents. Inhaled beta interferon has been tested to stimulate the innate immune response in a phase 2 clinical trial, but additional larger clinical trials are required to determine its efficacy. Hamsters are susceptible to SARS-CoV-2 infection, which produces extensive pulmonary infiltrates but is not lethal. Treatment of hamsters with interferon alpha markedly reduces the viral burden and pulmonary inflammation. These findings provide additional support for inhaled type I or type III interferons as possible therapeutic agents. Systemic steroids are not indicated early in the infection, but there remains an interest in the role of inhaled steroids at this stage awaiting a large, controlled clinical trial.

THE GAS EXCHANGE UNITS

Infection of the alveolar epithelium: potential for structural and functional damage and severe illness

The extension of SARS-CoV-2 infection into the gas exchange portions of the lung is the primary cause of severe morbidity and mortality in patients with COVID-19 (figure 2). Infection in this region produces progressive hypoxia associated with pulmonary infiltrates. Early pathologic changes include alveolar flooding and inflammatory cell infiltrates. However, the disease rapidly progresses to acute respiratory distress syndrome (ARDS) with diffuse alveolar damage, hyaline membranes, epithelial and microvascular injury and, in some cases, thrombi of small and large vessels. The pathologic alterations are very similar to what was reported with SARS-CoV-1. The main cell type that expresses ACE2 and TMPRSS2 in this region is the alveolar type II cell, although scRNAseq shows that only a small proportion of type II cells (1%–3%) have detectable levels of ACE2 transcripts. Infection is demonstrated by the presence of SARS-CoV-2 antigens and mRNA within alveolar type II cells.

Type I cells express less ACE2, but in primates, they can be infected. Alveolar type I-like cells (dedifferentiating type II
Tight junctions between alveolar epithelial cells function as a major barrier for fluid flux from the microvasculature and interstitium into the alveolar space. Type II and type I cells actively transport fluid from the alveolar subphase to the interstitium driven by the basolateral Na/K ATPase. Based on the studies with SARS-CoV-1 and influenza, alveolar type II cells can also initiate the innate host response subsequent to viral infections and secrete type I and type III interferons and chemokines. These cells are among the first responders to respiratory viruses in the gas exchange portion of the lung. In addition, human alveolar type II cells express Human Leukocyte Antigen DR-isotype (HLA-DR), CD80 and CD86, which are required for antigen presentation to T cells, and, thus, are thought to serve as antigen-presenting cells during viral infections to initiate T cell responses but are less efficient than dendritic cells.

**Target cells for SARS-CoV-2 infection in the alveoli**

The infection of and damage to type II cells by SARS-CoV-2 in pathologic reports likely have multiple physiologic consequences, which can be extrapolated from similar infection by influenza and SARS-CoV-1. Type II cells produce pulmonary surfactant, which is required to produce the low surface tension at the air–liquid interface on the alveolar surface. The low surface tension markedly decreases the work of breathing and facilitates gas exchange. Surfactant also contains SP-D, which are multivalent lectins that are important in host defence proteins. SP-D is critical in protecting the host against influenza A infections. There is some evidence that SP-D may also protect against coronavirus infections, but more definitive studies need to be conducted. A subset of type II cells undergoes self-renewal during homeostasis and after injury and also serve as progenitor cells for type I cells. Hence, damage to type II cells can significantly impede epithelial repair mechanisms, as shown in mice infected with influenza A/PR8/1934 (H1N1). Tight junctions between alveolar epithelial cells form a major barrier for fluid flux from the microvasculature.

**Robust innate immune response to alveolar infection**

There have been a number of in vitro studies of SARS-CoV-2 infection with human type II cells. For example, Huang et al used a model system of induced pluripotent stem cell-derived type II cells to study the impact of SARS-CoV-2 infection. In this study, there was a large increase in expression of inflammatory cytokines 24 hours after inoculation including IL-6, TNF, CXCL2 and CXCL8 and by 96 hours postinfection, type II cell differentiation genes were downregulated, including surfactant-associated genes (SFTPC, SFTPA1 and LAMP3). The interferon response was surprisingly low in this model system with no detectable interferon beta or lambda response at 1 or 4 days after inoculation and CXCL-10 expression was variable. Youk et al used alveolar organoids of expanded human type II cells and demonstrated a high level of infection associated with type I and type II interferon signals at 1 day postinoculation.
and induction of a broad range of interferon-stimulated genes 3 days postinfection.64 Similarly, Katura et al used human type II cell organoids derived from primary lung tissue to detail a robust interferon-mediated inflammatory response, reduction of SFTPB and SFTPB SP expression and type II cell apoptosis in response to SARS-CoV-2 infection in vitro.65 Hence, alveolar type II cells appear to have a more rapid and robust innate response to SARS-CoV-2 infection compared with nasal or bronchial epithelial cells.

An animal model that shows the intense and catastrophic effect on type II cells would be beneficial for investigating the pathogenesis, the interaction with the immune system and response to therapy. Leist et al developed a mouse-adapted strain of SARS-CoV-2 (SARS-CoV-2 MA 10), which will be useful in defining the host response in the distal mouse lung in vivo.26 These investigators demonstrated profound damage to type II cells and bronchiolar club cells in response to infection,26 including reduced expression of Stgpb and Stfpc (SP-B and SP-C homologs), suggesting a critical loss of pulmonary surfactant. Because there are so many reagents and genetic constructs available in mice, this will serve as a powerful platform going forward.

Comparison of airway versus alveolar infection mechanisms and outcomes

Several more factors may indicate why SARS-CoV-2 infection is more devastating in the alveolar region compared with the nasal passages. In addition to robust activation of inflammatory cytokines during the ARDS phase of COVID-19, the complement cascade may also be activated, similar to what has been shown following SARS-CoV-1 infections in mice.66 Alveolar epithelial cells produce and secrete several components of the complement pathways, including C2, C3, C4 and C5.67 There is also a difference in the clearance mechanisms between the conducting airways and the alveolar region. Debris in the airways can be cleared by mucociliary clearance and coughing. Clearing debris in the alveolar compartment is more difficult, and most of the debris is phagocytosed by macrophages locally and transported to regional draining lymph nodes. Finally, major differences exist between the alveolar epithelium and the nasal and bronchial epithelium with respect to epithelial repair. Type II cells are the primary progenitor cells for the alveolar epithelium, which is a simple epithelium, and if they are damaged, the repair mechanisms are compromised. In the conducting airways, basal cells are the progenitor cells, and are not infected by SARS-CoV-2. Thus, the spared basal cells can proliferate and differentiate into secretory and ciliated cells to repair damage and reinitiate mucociliary clearance in this pseudostratified epithelium.

Mechanisms of severe disease with advanced age

For COVID-19 as well as SARS, the elderly have been particularly vulnerable to infection and have had a markedly worse clinical outcome. The reason for the susceptibility of this population in COVID-19 is not known. Some strains of SARS-CoV-1 are lethal in aged but not young mice.68 When aged mice are infected with lethal SARS-CoV-1 strains, they demonstrate an earlier and more robust interferon and acute phase response within 24 hours following infection characterised by a substantial increase in CXCL-10, IFN-β and IL-6 expression.68 The implication is that early elevation of these cytokines in the lung might indicate a worse clinical outcome in the elderly.69 Congruent with these data, ACE2 expression in alveolar type II cells has been demonstrated to increase with age.7 Furthermore, single cell analyses of airway epithelial cells from adult and paediatric patients identified reduced interferon responses in epithelial cells and a higher proportion of innate lymphoid and naïve T cells in peripheral blood in COVID-19 paediatric patients compared with adults.70

Therapeutic strategies for alveolar infection

During the alveolar phase of COVID-19, the goals of treatment are to inhibit viral replication, to preserve type II cell survival and function, and to dampen the inflammatory response to maintain the architecture of the gas exchange units. Although endothelial cells are not likely to be direct targets of SARS-CoV-2, they are clearly damaged, thus treatments to prevent endothelial injury and microvascular thrombi are also very important. The two treatments that have been studied in detail are dexamethasone and anticytokine therapies. Corticosteroids are administered primarily to diminish tissue inflammation, but they also stimulate surfactant production in vivo and type II cells in vitro and induce structural maturation of the lung in the newborn.71–73 Dexamethasone is beneficial in hospitalised patients requiring mechanical ventilation or supplemental oxygen but not in those not requiring respiratory support.74 If a goal is to preserve injured type II cells, factors that stimulate cultured adult type II cells or organoids of type II cells in vitro should be considered. These factors include, but are not limited to, dexamethasone, cyclic Cyclic adenosine monophosphate (cAMP) (isobutylmethylxanthine, cholera toxin) and mitogenic growth factors (KGF, Hepatocyte growth factor (HGF), Transforming growth factor alpha (TGFα)).75 76 77 However, stimulating type II cell proliferation may be detrimental by increasing the number of ACE2 expressing cells and thereby increasing viral replication, similar to what has been reported in mice treated with KGF prior to infection with influenza.7 Type II cells are also the primary target cell of influenza infection in the alveolar region.78 There have been many attempts to inhibit individual components of the resultant hyperinflammation (previously termed “cytokine storm”), but these therapies have only been partially successful. Baricitinib, an inhibitor of Janus kinase 1 and 2, blocks several cytokines elevated in COVID-19 and is promising in patients receiving high-flow oxygen or non-invasive ventilation.78 Tocilizumab and sarilumab are monoclonal antibodies that block the IL-6 receptor. In the recent open-label Randomised, Embedded, Multi-factorial Adaptive Platform Trial for Community-Acquired Pneumonia with rapid enrollment of COVID-19 patients with ICU and greater than 80% receiving corticosteroids, tocilizumab and sarilumab increased respiratory and blood pressure support-free days to day 21 as well as survival and met the predefined criteria for efficacy.79 In an earlier standard controlled clinical trial prior to the widespread use of glucocorticoids, tocilizumab was not effective for preventing intubation or death in a cohort of moderately ill hospitalised patients with COVID-19.80 Rubin et al commented in an accompanying editorial on the differences in study design between the two trials and stressed the importance of context of the trials at different times in the COVID-19 pandemic both in terms of the concomitant use of glucocorticoids and in the evolving practice patterns in intensive care medicine for patients with COVID-19.81

SUMMARY

Treatment of COVID-19 depends on the stage of the disease and the cell types that are infected. The mortality and long-term sequelae of SARS-CoV-2 infection depend on the extent of damage to the alveolar epithelium. One of the major differences
for the long-term effects of SARS-CoV-2 infections is damage to the progenitor cells for the epithelium in the conducting airways and the alveoli. In the conducting airways, basal stem cells are spared; in the alveoli, type II cells are damaged. There remain a number of important questions to be answered. What is the mechanism for this purported dampened immune response in the nose and bronchi compared with the lower respiratory tract? At what stages of the disease would enhancing or inhibiting the innate immune system be beneficial? What treatments can preserve type II cell survival and function in the lung and enable them to fully restore the alveolar epithelium after injury? What is the cellular and molecular basis for the impaired response to the virus in the elderly? There are many unknowns in the pathogenesis of COVID-19 that are currently under study and will be reported in the near future.

Acknowledgements
This is a rapidly expanding field of research and we may have missed some critical reports. We apologise for this oversight.

Contributors
JPB: wrote and approved the manuscript. EKV: wrote and approved the manuscript. HH: edited and approved the manuscript. RJM: wrote and approved the manuscript.

Funding
The authors are supported in part from funds from the National Institutes of Health (HL136343 JPB), Cystic Fibrosis Foundation (EKV), and NeuroRx corporation (JPB and RJM). EKV is a Boettcher Early Career Investigator.

Competing interests
None declared.

Patient consent for publication
Not required.

Provenance and peer review
Not commissioned; externally peer reviewed.

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