Thoughts on the alveolar phase of COVID-19

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Mason RJ. Thoughts on the alveolar phase of COVID-19. Am J Physiol Lung Cell Mol Physiol 319: L115–L120, 2020. First published June 3, 2020; doi: 10.1152/ajplung.00126.2020.—COVID-19 can be divided into three clinical stages, and one can speculate that these stages correlate with where the infection resides. For the asymptomatic phase, the infection mostly resides in the nose, where it elicits a minimal innate immune response. For the mildly symptomatic phase, the infection is mostly in the pseudostratified epithelium of the larger airways and is accompanied by a more vigorous innate immune response. In the conducting airways, the epithelium can recover from the infection, because the keratin 5 basal cells are spared and they are the progenitor cells for the bronchial epithelium. There may be more severe disease in the bronchioles, where the club cells are likely infected. The devastating third phase is in the gas exchange units of the lung, where ACE2-expressing alveolar type II cells and perhaps type I cells are infected. The loss of type II cells results in respiratory insufficiency due to the loss of pulmonary surfactant, alveolar flooding, and possible loss of normal repair, since type II cells are the progenitors of type I cells. The loss of type I and type II cells will also block normal active resorption of alveolar fluid. Subsequent endothelial damage leads to transudation of plasma proteins, formation of hyaline membranes, and an inflammatory exudate, characteristic of ARDS. Repair might be normal, but if the type II cells are severely damaged alternative pathways for epithelial repair may be activated, which would result in some residual lung disease.

ACE2; SARS-CoV-2; type II cells

INTRODUCTION

COVID-19 is a devastating disease, but details on the pathogenesis of this disease are not known. The respiratory epithelium is the initial site of the infection (22, 29). One can formulate a perspective of what is likely happening based on previous studies with SARS. Both SARS-CoV-1 and SARS-CoV-2, the designation for the virus that causes the disease COVID-19, use ACE2 as the receptor. By single-cell RNA profiling, ACE2 expression level in lung cells is low (33). The site of infection is thought to most likely occur in cells that express both ACE2 and the serine protease TMPRSS2, and these sites are corneal conjunctiva in the eye, ciliated and secretory cells in the nose, ciliated and secretory cells in the conducting airways, and alveolar type II cells in the gas exchange area of the lung (33). ACE2 is the docking site whereby the virus attaches and can enter the cell and start replicating. The role of the protease activity of ACE2 in the pathogenesis of the disease has been postulated but is complex and unresolved (12, 18, 23). ACE2 is protective and can block the development of acute lung injury (14, 18). Shed ACE2 or recombinant ACE2 might be useful both to decrease lung inflammation and to be a decoy to inhibit viral uptake (12). On the other hand, HCoV-NL63 is a relatively innocuous respiratory coronavirus that also uses ACE2 for viral entry (23).

PHASES OF PATHOGENESIS

The pathogenesis of COVID-19 can be divided into three phases based on the site of the respiratory epithelium infected. The first phase of the disease is in the nose. Here the virus likely infects ciliated and secretory cells, replicates, but apparently does not induce a vigorous innate immune response (33). This is the stage for asymptomatic individuals who can transmit the disease. Respiratory viruses that circulate in the community grow intracellularly and need a host to survive. It is reasonable to assume that the nasal cavity that houses these respiratory viruses has a blunted innate immune response (41). We need to learn more about the magnitude of the innate immune response to the virus located in the nasal passages compared with viral infection in the intrapulmonary conducting airways (41). It might be informative to add PCR tests for interferons, interferon response genes, and viral sensing genes to the positive nasal swabs to identify subjects who have a higher risk of disease progression (26, 48).

The second phase of COVID-19 takes place along the conducting airways, the bronchi and bronchioles. The virus infects ciliated cells as the disease progresses deeper into the lung. SARS-CoV-1 infects ciliated but not mucus cells in air-liquid interface cultures (15, 30). Similarly in nonhuman...
primates, SARS-CoV-2 infects ciliated cells (29). When the infection reaches this region, one can speculate that a more vigorous innate immune response is generated and the patient feels ill. Likely the infection and recruitment of inflammatory cells impairs clearance and allows the infection to spread deeper into the lung. Keratin 5-expressing basal cells are apparently spared from direct infection (29). These basal cells are the progenitor cells for the airway epithelium, and hence the epithelium of the conducting airways can readily repair and recover from the infection. Primate, hamster, and single-cell expression studies for ACE2 have all indicated that club cells in the bronchioles might be an important site of infection (3, 29, 33). Direct studies of club cells have not yet been reported, but they are located in the very distal bronchioles in humans (24).

The third phase of the disease is the lethal phase, as the infection spreads into the gas exchange portion of the lung and infects alveolar type II cells (Fig. 1). The patient is now hypoxic and has scattered subpleural ground glass densities on radiographic imaging. The gas exchange structures are an exquisite example of biologic engineering. There is close apposition of the endothelium to the type I cells to allow rapid exchange of oxygen and carbon dioxide as blood passes through the pulmonary capillaries. However, this anatomic arrangement is very delicate. Type I cells are extremely large flat cells that are easily damaged in many forms of lung injury. These cells are critical for gas exchange and transepithelial ion movement to keep the alveolus relatively free of fluid. Type I cells use CLIC5 as the chloride channel, whereas type II cells use CFTR. Both cell types express SCNN1A, SCNN1B, and SCNN1C as the sodium channel and ATP1A1 and ATP1B1 as the Na-K-ATPase. Both cell types likely contribute to the active resorption of alveolar fluid (5, 20). Rapid flooding of alveoli early in the disease suggests that type I cells are also

Fig. 1. Infection of human type II cells with SARS-CoV. Human type II cells were cultured at an air-liquid interface so as to maintain their state of differentiation and infected with SARS-CoV-1 (26). The viral particles (white arrows) are seen in vesicles near normal-appearing lamellar bodies and mitochondria. There was no observed cytopathic effect under the conditions of these cultures.
damaged or infected (22, 26). Both type I cells and type II cells appear to be infected by SARS-CoV-2 in nonhuman primates (22, 29). SARS-CoV-1 infects highly differentiated type II cells more readily than poorly differentiated type II cells, which is also reflected in their relative ACE2 protein expression (21, 26). The protein expression of ACE2 in freshly isolated type II cells is highly variable and may indicate susceptibility to more severe disease (26). Type II cells make and secrete pulmonary surfactant, which is required for effective gas exchange. One of the early changes in surfactant in the development of ARDS is a switch in the minor anionic phospholipids from phosphatidylglycerol to phosphatidylinositol (8, 11). The composition and biophysical properties of surfactant will likely be impaired as in other forms of ARDS, lead to higher surface tension, and promote alveolar flooding in the diseased portions of the lung (2, 8, 11).

**PHASES OF ALVEOLAR INVOLVEMENT**

There are apparently two phases for the alveolar involvement in COVID-2 (6). The early phase is characterized by profound hypoxia, normal compliance, and focal alveolar flooding, and the late phase is similar to traditional inflammatory ARDS. In the early phase, the compliance is normal because most of the lung is normal and uninvolved, and the patient is breathing close to normal functional residual capacity at the lower end of the pressure-volume curve. The physiological alterations that cause the initial hypoxia require additional studies. Focal alveolar flooding occurs, likely due to anatomic factors, to both the epithelium and the endothelium. In SARS, endothelial cells are not thought to be infected directly in spite of the fact that they express some ACE2 (35). We need more detailed studies on possible infection of endothelial cells. The flooding will likely be impaired as in other forms of ARDS, lead to higher surface tension, and promote alveolar flooding in the diseased portions of the lung (2, 8, 11).

**L117ALVEOLAR REPAIR IN COVID-19**

**Fig. 2.** Bystander amplification and multiple cell-cell interactions in the cytokine response. The cytokine response is very complex. Virus-infected epithelial cells release a variety of cytokines during infection, and nearby bystander cells amplify the response, especially in response to interferon beta and lambda. Soon thereafter the recruited inflammatory cells, immune cells, and other organs such as the liver produce additional mediators and cytokines and further amplify the response.

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cells proliferate and migrate into the lung parenchyma, forming clusters seen in paraffin-embedded sections (Fig. 3D) (19, 36, 43, 47). Lineage tracing has shown that these cells are heterogeneous (17, 36, 45, 47). There are nonlineage labeled airway cells that express keratin 5 and migrate into the alveolar compartment (27, 36, 43). Club cell-derived stem cells can also proliferate, migrate, express keratin 8, and migrate into the alveolar compartment (32, 46, 47). However, it is not clear that these alternatively derived epithelial cells can fully differentiate into functional type I and II cells (27, 45). During COVID-19 infection there will likely be different repair pathways present in different parts of the lung. In the normal pathway for the transition of type II cells to type I cells, resting type II cells are activated, express keratin 8, and differentiate into type I cells (16, 32). The normal pathway will likely be indicated by transitional cells that express keratin 8 and markers of both type I cells and type II cells such as RAGE and Muc1, and the areas of alternate pathways of repair will be indicated by cells expressing club cell antigens and keratin 8 or clusters of keratin 5 cells in the alveolar walls (4, 16, 32). If these alternate pathways for epithelial repair are activated in the most severe disease, there may be some scarring and residual disease. The degree of scarring and residual disease after COVID-19 is not currently known. However, both non-human primates and hamsters apparently recover fully and would not likely use these alternative pathways (3, 22).

SUMMARY

In summary, COVID-19 can be easily understood by considering where the infection is located. The responses in the nose, the conducting airways, and the alveoli will be different. We need to learn more about the innate immune response in these three sites. We also need to know more about the infection and response in club cells, which have a restricted location in the human lung (24, 37). The disease in the gas exchange portions of the lung can be very severe and may take a long time to fully recover. Now we just have to await the development of effective therapeutics and a vaccine.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

R.J.M. prepared figures; drafted manuscript; edited and revised manuscript; and approved final version of manuscript.

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