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## COMMENTARY

# Lung Injury and Repair in Coronavirus Disease 2019—Related Acute Lung Injury



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The alveolar epithelium is the largest surface area of the body in continuous contact with the outside environment and is critical for effective gas exchange to support metabolism. The alveolar walls consist of three distinct layers: the alveolar epithelium, comprising type I and type II alveolar epithelial cells; a matrix scaffold; and the microvascular endothelium. Most of the alveolar surface is covered by thin type I epithelial cells that cover the matrix framework and are closely opposed to microvascular endothelial cells. Type II epithelial cells are found in each alveolar unit and produce surfactant lipids and proteins that reduce surface tension at low lung volumes and function in antimicrobial defense. The alveolar epithelium is a tight barrier that prevents fluid movement into the airspaces. Alveolar epithelial cells also reabsorb fluid from the airspaces by active transport through specialized ion channels. By contrast, the microvascular endothelial barrier changes permeability more readily in response to intravascular stimuli, resulting in interstitial edema that is cleared by lymphatic vessels. Alveolar edema forms in response to increased microvascular hydrostatic pressure, as in left heart failure, or an increase in endothelial or epithelial permeability when endothelial or epithelial cell damage occurs. Rapid repair of increased epithelial or endothelial permeability is essential to support continuing gas exchange. In some cases, such as pneumococcal pneumonia, the repair process reestablishes normal alveolar architecture without significant fibrosis, whereas in others repair leads to fibrosis, which can distort alveolar units and lead to long-term abnormalities in gas exchange and pulmonary function. Accordingly, a great deal of research has focused on identifying the mechanisms that govern normal alveolar repair versus fibrosis after various forms of acute lung injury.<sup>1</sup>

The coronavirus disease 2019 (COVID-19) pandemic has focused attention on severe lung injury, termed adult respiratory distress syndrome (ARDS). New work by

Ting et al<sup>2</sup> uses COVID-19 cases to generate a new perspective on lung injury and repair in ARDS. ARDS is a form of severe lung injury caused by a variety of different stimuli that usually results in diffuse alveolar damage, characterized by increased permeability, alveolar edema, leukocyte infiltration, type II pneumocyte proliferation, hyaline membrane formation, surfactant dysfunction, and alveolar collapse, all of which result in life-threatening hypoxemia that requires high-level respiratory support.<sup>3,4</sup> In ARDS from a variety of causes, evidence of active repair processes is present in the first days after onset of the illness, reflected by increased concentrations of procollagen III, transforming growth factor- $\alpha$ , and other growth factors in bronchoalveolar lavage fluid.<sup>5–7</sup> Although lung function improves in most survivors of ARDS, some experience a persistent fibroproliferative response that impairs pulmonary function and causes lasting disability.<sup>8,9</sup> The lungs of patients with COVID-19–related ARDS who have undergone autopsy have shown diffuse alveolar damage that is difficult to distinguish from other causes of ARDS.<sup>10,11</sup> Because COVID-19–related ARDS occurs after a well-defined viral stimulus [severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)], COVID-19 provides a relevant clinical condition in which to study factors that cause lung injury and repair, to reduce the heterogeneity inherent in studying ARDS from diverse causes.

Alveolar type II cells are the principal cells that give rise to the type I pneumocytes that make up most of the alveolar surface.<sup>1</sup> In normal homeostasis, type II cells are maintained by a subpopulation of rare progenitor cells that are

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long-lived and self-renewing. Type II progenitor cells can be isolated by unique surface markers and have distinct transcriptional and functional phenotypes.<sup>12,13</sup> They are usually found near fibroblasts that express *Wnt* genes, generating a niche for type II progenitor cells.<sup>12</sup> Following injury, remaining type II cells and the subpopulation of type II progenitor cells proliferate, then enter a non-proliferative transitional state with cuboidal or partially spread morphology, then flatten and acquire characteristics of mature type I cells.<sup>12–14</sup> Lineage tracing studies in mice with bleomycin-induced lung injury identified the transitional cells expressing the cytokeratin 8 marker (KRT8<sup>+</sup>) as key cells in the progression from type II cells to mature type I cells.<sup>15</sup> Studies of lung tissue from patients with idiopathic pulmonary fibrosis (IPF), a form of chronic fibrotic lung disease, have shown persistent transitional cells and a relative paucity of mature type I cells, leading to the hypothesis that a delay or block in progression from the transitional state to mature type I epithelial cells may be a key part of ineffective repair leading to fibrosis.<sup>15–18</sup> The role of transitional epithelial cells in ineffective repair and whether ineffective repair occurs relatively early following acute lung injury are key questions to be resolved.

To study characteristics of alveolar injury and epithelial repair in COVID-19–related lung injury, Ting et al<sup>2</sup> used autopsy material from the lungs of three patients who died within 14 days of onset of COVID-19–related ARDS. For comparison, they used archived lung tissue from three patients with non–COVID-19 ARDS, lung tissue from patients with IPF who were undergoing lung transplantation, and normal tissue from lungs rejected for transplantation. As previously reported for COVID-19 and non–COVID-19 ARDS, the lungs of all of the patients showed extensive diffuse alveolar damage, consistent with severe epithelial injury. Labeling of COVID-19 lung tissue for type II and type I epithelial cell markers [pro-surfactant protein C (pro-SPC) and human type I antigen 56 (HTI-56), respectively] showed extensive loss of mature type II cells and widespread damage to type I cells. In COVID-19 lungs, hyperplastic cuboidal epithelial cells were identified, which is a common finding in diffuse alveolar damage, but for the most part these cells did not express the pro-SPC marker, suggesting that they were not mature type II cells. Labeling of lung tissue with the KRT8 marker showed prominent labeling throughout alveolar regions, suggesting that KRT8<sup>+</sup> transitional cells were abundant in COVID-19–related as well as non–COVID-19–related injured lungs. KRT8 is an intracellular cytokeratin marker that is expressed at high levels in transitional alveolar epithelial cells of mice with bleomycin-induced lung injury.<sup>15</sup> KRT8<sup>+</sup> transitional cells have a flattened morphology and show evidence of p53 and NF- $\kappa$ B activation and have been identified in different animal models of lung injury, as well as lungs of humans with IPF. KRT8<sup>+</sup> epithelial cells have been proposed as key transitional cells in the progression from type II to type I cells, and abnormal persistence of

KRT8<sup>+</sup> cells is thought to be a key feature in the progression from acute lung injury to chronic fibrosis.<sup>15,16</sup>

The findings of Ting et al<sup>2</sup> show that type II epithelial cells are lost and KRT8<sup>+</sup> transitional epithelial cells appear early after the onset of acute lung injury in humans, but raise the question of whether the appearance of transitional cells is a marker of impending fibrosis. Despite the widespread loss of type II cells and the abundance of transitional cells, the lungs from COVID-19 and non–COVID-19 ARDS decedents did not show evidence of collagen deposition, myofibroblast accumulation, or distortion of the underlying alveolar matrix structure, all of which are common findings in IPF and fibroproliferative ARDS.<sup>8</sup> Although most KRT8<sup>+</sup> transitional cells did not express the HTI-56 type I cell marker, HTI-56 was detected on rare KRT8<sup>+</sup> cells in the COVID-19 lungs, suggesting incomplete progression of transitional cells to the mature type I epithelial phenotype. Further labeling of lung sections with markers of cell cycle arrest and senescence showed that although epithelial markers of cell cycle arrest (CDKN1A, CDKN2B, TP53, and CCND1) were detectable in lung tissue of COVID-19 and non–COVID-19 ARDS patients, only the lung tissue from patients with IPF expressed a relatively specific marker of senescence, p26 (CDKN2A). Taken together, these findings led the authors to hypothesize that in patients with early COVID-19 and non–COVID-19 ARDS, proliferating type II epithelial cells or related progenitor cells become transitional cells in a state of cell cycle arrest and then differentiate into mature type I cells to restore normal alveolar architecture, whereas in fibroproliferative ARDS or in IPF, the transitional cells become senescent and lose the capacity for type I differentiation, leading to the development of fibrosis. These findings are important, because they show that transitional cells arise relatively early in the course of severe lung injury due to COVID-19 as well as non–COVID-19 etiologies, before evidence of fibrosis can be detected. Whether fibroproliferative changes might have occurred in the lungs of these patients over a longer period cannot be determined from these autopsy samples.

The findings of Ting et al<sup>2</sup> are important because they provide human evidence in support of translational studies of alveolar regeneration in mice.<sup>15</sup> They focus attention on transitional cells in lung epithelial repair and the factors that govern the progression from type II cells to transitional cells and then normal type I epithelial cells, versus the development of senescence in transitional epithelial cells with associated fibrosis. The inflammatory milieu of the injured lung is a complex environment, containing a variety of growth factors and cytokines that influence the balance between normal epithelial regeneration and fibrosis, including tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , transforming growth factor- $\alpha$ , keratinocyte growth factor, granulocyte-macrophage colony-stimulating factor, and others, and persistent inflammation has been proposed as an important factor promoting fibrosis.<sup>5,19–22</sup> Sorting out the key factors that promote progression from KRT8<sup>+</sup> transitional cells to mature type I pneumocytes

versus the alternative mechanisms that activate fibrosis is an important goal of future research.

The study by Ting et al<sup>2</sup> has some limitations that are inherent in studies of human autopsy material. First, the COVID-19 and non-COVID-19 ARDS lung samples were obtained at single times within 2 weeks of onset of acute lung injury, so the data do not provide insight into the progression of the findings over longer times. Second, although the investigators performed comprehensive analyses on the lung tissue samples, they studied tissue from a limited number of patients, raising the question of how uniform the findings might be in a larger population. Third, although the index cases of COVID-19 ARDS all had a common primary viral injury, all of them also were mechanically ventilated, adding a second and different injurious stimulus that must be considered. Although the authors did not find evidence of persistent viral infection in the lungs, how initial viral damage to type II pneumocytes might interact with ongoing injury from mechanical forces in the lungs remains an important question. The study was not designed to consider how other levels of complexity, including damage to the alveolar wall matrix and epithelial mesenchymal cross talk, might contribute to aberrant repair.<sup>23–25</sup> Last, although the idea that development of senescence in KRT8<sup>+</sup> transitional cells due to repetitive injury may be linked with the onset of fibrosis is intriguing, the IPF tissue samples were collected much later in the course of that illness, so the direct relevance of the findings for the evolution from acute to chronic fibroproliferative lung injury remains to be proven.

Despite these limitations, the study by Ting et al<sup>2</sup> provides important directions for future research on lung repair. The factors that favor the maturation of KRT8<sup>+</sup> transitional cells into normal type I epithelial cells versus factors that lead to a senescent state need to be clarified and studied in a larger number of patients as well as relevant animal models. The results could provide clues about new therapeutic approaches to promote normal repair and/or prevent fibrosis. The results may also be useful in evaluating whether currently available therapies for chronic fibrosis might be helpful early after the onset of acute lung injury.

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