Delayed Alveolar Epithelialization: A Distinct Pathology in Diffuse Acute Lung Injury

To the Editor:

Diffuse alveolar damage (DAD) is the most common histologic finding in acute respiratory distress syndrome (1). DAD proceeds through overlapping phases of acute injury with hyaline membrane formation, proliferation by type 2 alveolar epithelial cells (AEC2s), and fibroplasia (2, 3). Although it has long been appreciated that variable degrees of lung repair can occur after human acute lung injury, the cellular mechanisms underlying this response remain poorly understood. Recently, mouse models have identified nests or “pods” of proliferating peribronchiolar cells that arise after lung injury and may participate in regeneration (4, 5). However, these findings have yet to be corroborated in human patients.

Here we report fulminant idiopathic acute lung injury requiring extracorporeal membrane oxygenation (ECMO) in two previously healthy young adults. Both presented with acute syndromes of fever and progressive dyspnea with bilateral airspace opacities on computed tomography (Figures 1A and 1B). Both clinically deteriorated despite treatment for presumptive community-acquired pneumonia and met Berlin criteria for severe acute respiratory distress syndrome (6). Hypoxemia worsened despite maximal medical therapy, including low tidal volume (<6 ml/kg) ventilation, paralytics, recruitment maneuvers, positive end-expiratory pressure titration, pulmonary vasodilators, and prone positioning. Extensive autoimmune and infectious studies, including PCR and sequencing assays, were unrevealing. The first patient, a 27-year-old man, underwent bilateral lung transplantation after 101 days on ECMO and has recovered and returned to work. The second, a 35-year-old woman, required ECMO support for 85 days and has slowly recovered, although with a restrictive ventilatory deficit and pulmonary fibrosis.

Wedge biopsies of the lung were obtained 4 and 2 days after intubation, respectively, which was immediately before and 1 day after ECMO cannulation, respectively, and 3 and 1 days after meeting acute respiratory distress syndrome criteria, respectively. The biopsies showed diffuse alveolar injury with very rare hyaline membranes and edematous, mildly fibrotic alveolar

walls. Regenerative AEC2s were virtually absent from gas exchange surfaces, which were instead lined by macrophages showing marked cytoplasmic spreading (Figures 1C–E). There was concomitant exuberant proliferation of p63⁺ atypical peribronchial basaloid epithelial cells, a pattern termed “squamous metaplasia” in the pathology literature (Figure 1F) (3, 7). We denote these cell clusters as peribronchiolar basaloid pods (PBPs) to distinguish them from the distinct phenomenon of large airway squamous metaplasia and to call attention to analogous findings in mice. The explanted lungs at Day 101 of patient 1 showed diffuse interstitial fibrosis with exuberant reepithelialization by hyperplastic cuboidal AEC2s (not shown). PBPs were no longer present; however, there was marked bronchiolarization (peribronchiolar bronchiolar metaplasia, or Lambertosis).

In view of the virtual absence of AEC2s at the time when PBPs were well established, we term the pattern “diffuse alveolar injury with delayed epithelialization” or DAIDE, defined by the virtual absence of hyaline membranes, and diffuse alveolar injury characterized by alveolar denudation and lining by activated macrophages. Whereas PBPs are not specific to DAIDE, they were a distinct feature at a time when AEC2s are evident in classical DAD. As controls, we reviewed 57 cases with a diagnosis of DAD, representing all surgical wedge biopsy and autopsy cases from 2011–2016 at the Massachusetts General Hospital; cases with a background of cancer or extensively fibrotic lung disease were excluded. All showed prominent AEC2s. Rare foci of denudation with macrophages lining alveolar walls were present in less than half the cases; this was in stark contrast to the two cases reported, in which diffuse alveolar denudation encompassed nearly the entire specimen.

It is known that AEC2s proliferate within days after alveolar injury and can give rise to AEC1s (4). In these two patients, the near absence of alveolar epithelium suggests alveolar reepithelialization was profoundly delayed. The cause of this impairment and eventual reepithelialization is unclear; however, it is not an early stage of DAD. We note the striking similarity between human PBPs and a reactive murine p63⁺ cell population referred to as “Krt5 pods” (reviewed in Reference 5) that proliferate in a peribronchiolar distribution after infection with influenza virus or variably after bleomycin-mediated injury. These clusters appear to originate from rare airway epithelial progenitor populations, based on stringent indelible lineage tracing (5, 8, 9). To our knowledge, no human correlate has previously been described. The roles of these “pods” after injury remains uncertain; the most recent murine findings suggest minimal alveolar contribution, and instead demonstrate that pods later form ciliated lumens with expression of some mature airway differentiation markers (10), a phenotype strikingly similar to the bronchiolarization seen in the explants of patient 1.

The relationship between DAIDE and PBP formation is uncertain; we hypothesize that PBPs may represent a regenerative response to some forms of severe distal airway injury (5, 10). Whereas the cause of this form of severe acute lung injury is idiopathic, it does appear to have a distinct histology from DAD

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Figure 1. Alveolar epithelialization in diffuse alveolar injury with delayed epithelialization in patient 1. (A, B) Chest computed tomography showing diffuse pulmonary infiltrates. (C) Lung biopsy H&E stain at low magnification demonstrates diffuse alveolar denudation and peribronchiolar basaloid pods (arrow). Hyaline membranes are rare and nonconspicuous, and the lung interstitium is mildly thickened. (D) The absence of alveolar epithelium in patient 1 is highlighted by the lack of cytokeratin immunostaining, whereas the peribronchiolar basaloid pods are cytokeratin positive (arrow). (E) CD163 immunohistochemistry demonstrates macrophages contiguously lining denuded alveoli. (F) Peribronchiolar basaloid pods at high power (H&E stain) shows nests of atypical cells with rapid proliferation (arrows: mitoses) surrounding and contiguous with a bronchiole (asterisk: lumen). Inset: p63 immunostain shows peribronchiolar basaloid pods are a polarized, stratified epithelium with central canalization. H&E = hematoxylin and eosin.

and may have a poor short-term prognosis. These findings require further characterization. ■

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Metagenomic Sequencing Detects Respiratory Pathogens in Hematopoietic Cellular Transplant Patients

To the Editor:

Lower respiratory tract infections (LRTIs) are a leading reason for hospitalization and mortality in hematopoietic cellular transplant (HCT) recipients (1). Despite this, the etiologic

pathogens often remain unidentified because of reduced yields of traditional diagnostics in the setting of prophylactic antimicrobials, reduced antibody titers, and infections from uncommon opportunistic microorganisms. Metagenomic next-generation sequencing (mNGS) may offer enhanced diagnostic capabilities by providing culture-independent, comprehensive measurement of the microbial composition of clinical samples (2, 3). By capturing both microbial and human RNA, mNGS also permits simultaneous transcriptional profiling of the host immunologic response and can deliver rapid (<48 h) and actionable microbiologic data for precise infectious disease diagnosis (2).

As a result of the clear need for enhanced LRTI diagnostics in HCT recipients, we sequentially enrolled 22 adult HCT recipients hospitalized for acute respiratory illnesses who underwent bronchoscopy and BAL between January 25, 2012, and May 20, 2013, under University of Michigan protocol HUM00043287. Standard-of-care BAL microbiologic testing was uniformly performed on all patients and included semiquantitative cultures for bacteria, mycobacteria, fungi, and cytomegalovirus; *Aspergillus* galactomannan assay; silver stain for *Pneumocystis jirovecii*; multiplex polymerase chain reaction influenza A/B, respiratory syncytial virus and human metapneumovirus; and human herpesvirus-6 polymerase chain reaction, as detailed in the Methods in the online supplement (4). Additional diagnostics were performed on blood and nasopharyngeal samples at the discretion of the treating physicians, as described in Table 1 and detailed in the online supplement (4).

RNA and DNA sequencing libraries were constructed from 250 µl BAL from each patient and underwent paired-end Illumina sequencing according to established methods (4). Pathogen detection leveraged a custom bioinformatics pipeline that discriminates pathogens from background microbial contaminants in clinical samples (2, 3). A ranking score consisting of the nucleotide reads aligned per million reads sequenced (rpM) multiplied by the sum of the nt and nr Z-scores for each genus relative to no-template controls was used [$\text{score} = \text{rpM}_{\text{nt}} \times (Z_{\text{nt}} + Z_{\text{nr}})$] (4).

Microbes identified were classified as confirmed pathogens if 1) both clinical testing and mNGS identified the microbe, 2) there existed literature evidence of pathogenicity in the lungs, and 3) the score was at least twofold greater than that of any other microbe of the same type (virus, bacteria, or fungus) identified in the patient. Microbes were considered new potential pathogens if mNGS alone identified the microbe and criteria 2 and 3 described here were met; all other microbes were considered unlikely or uncertain pathogens. Findings were independently confirmed by viral PCR or bacterial 16S rRNA gene sequencing, as described (4).

Standard-of-care clinical diagnostics identified microbes in seven patients (32%), six of which were considered pathogens by treating physicians, and all of which were detected by mNGS (Table 1). In addition, mNGS identified well-recognized respiratory pathogens in six patients with otherwise negative testing, including human coronavirus 229E and human rhinovirus A (HRV-A); the former, despite being a leading cause of community-acquired pneumonia (5), is still not included as a target on many clinical respiratory viral PCR panels. With respect to potential bacterial pathogens, mNGS identified *Streptococcus mitis* in patient 13, an oropharyngeal microbe known to cause bacteremia and acute respiratory distress in HCT recipients (6), and

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