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Targeting Protease Activity to Interrupt Acute Respiratory Distress Syndrome Pathogenesis

Acute respiratory distress syndrome (ARDS) is a devasting critical illness with high mortality and limited therapeutic options to interrupt its progression (1). Robust inflammation and destruction of the alveolar-capillary barrier are key features of ARDS and occur in response to a wide variety of initial stimuli, including pneumonia, sepsis, trauma, or transfusion (1, 2). In many patients, the initial infection or insult is effectively managed, but the inflammatory response propagates ongoing lung injury that contributes to high mortality and to fibrosis in ARDS survivors. In many patients with ARDS, the airspace inflammation is neutrophil predominant, and ongoing work has focused on identifying both pathways involved in neutrophil recruitment and mechanisms through which neutrophils contribute to ongoing injury.

In this issue of the *Journal*, McKelvey and colleagues (pp. 769–782) study the role of cathepsin S in triggering neutrophilic inflammation during ARDS (3). The authors use BAL samples from patients with ARDS to show clinical relevance of elevated cathepsin S. Patients with ARDS have elevated cathepsin S levels and activity and lower levels of its inhibitor cystatin SN in bronchoalveolar lavage. Patients with both pulmonary and nonpulmonary ARDS have significant changes in cathepsin S and cystatin SN, suggesting that this pathway could be of broad importance across the ARDS population. These observations are coupled with a series of elegant mechanistic studies in mouse models of acute lung injury to show that cathepsin S augments lung inflammation *in vivo*. Using models of intratracheal cathepsin S, intratracheal LPS, and cecal ligation and puncture to mimic pulmonary and nonpulmonary ARDS, the authors demonstrate that interruption of cathepsin S pathways attenuates neutrophilic inflammation and acute lung injury in mice. These studies are rigorous, using two different pharmacologic cathepsin inhibitors, cathepsin S knockout mice, and supplementation of cystatin SN. Finally, they demonstrate that the antiinflammatory effects of cathepsin S inhibition require the presence of PAR-1, a key regulator of inflammation during lung injury.

This study builds on growing literature suggesting the balance of endogenous proteases and antiproteases as a previously underrecognized mediator of acute lung injury (4). Numerous studies have investigated protease function and consequences of protease deficiencies in the pathogenesis of chronic lung diseases such as emphysema. In chronic disease, the degree of deficit of antiproteases correlates with the severity of lung destruction, and this has been therapeutically targeted to reduce the progression of disease. Previous work on proteases and antiproteases in acute lung injury and ARDS has primarily focused on the pathogenic contributions of neutrophil elastase (4). Elastase inhibitors have shown promise particularly in bacterial infection models, but clinical benefits in humans have been modest. Cathepsin S was an appealing target for further investigation because of its high expression in a variety of inflammatory cells important in ARDS and because of its potency (5, 6). Intratracheal administration of cathepsin S is sufficient to cause neutrophilic inflammation and disruption of the alveolar–capillary barrier in mice, providing evidence that targeting cathepsin S has the potential to attenuate disease.

Originally Published in Press as DOI: 10.1164/rccm.202201-0046ED on February 10, 2022

The finding that several different cystatins are depleted in the airspaces of patients with ARDS is intriguing. In addition to cystatin SN, levels of cystatins S, D, and SA were also low in the airspace of patients with ARDS. For these initial studies, the authors chose to focus on cystatin SN, because it has potent protease inhibition activity, is known to be regulated by inflammatory signals, and is expressed in the upper respiratory tract. Here, the authors show that cystatin SN inhibits cathepsin S activity. Because the therapeutic effect of synthetic cystatin SN was assessed *in vivo* only in an LPS model of injury, further investigations of whether exogenous administration of cystatin SN in other clinically relevant lung injury models are needed. Additional work is needed to explore the mechanisms leading to cathepsin upregulation and cystatin depletion in the injured lung. For example, the relative timing of cathepsin S upregulation and cystatin SN depletion from the airspace is unknown, as are the regulatory processes that control expression and release of these proteins. The authors are also planning to assess how depletion of other cystatins from the airspace affects lung inflammation.

Although these studies clearly demonstrate that pharmacologic inhibition of cathepsin S has both preventive and therapeutic benefits in biologically relevant models of acute lung injury, there remain several unknowns that need to be considered in moving toward clinical application of protease modulation as a new ARDS therapy. One major challenge with this approach is the ideal timing of intervention. Too much protease activity at the wrong time could exacerbate injury, and too little could allow the initial lung injury to blossom. The finding that targeting cathepsin S was effective in both preventative and therapeutic timeframes may suggest a long window for effective intervention. Another challenge is that the dynamics of the balance between cathepsin S and cystatin SN during progression of ARDS from the initial insult to acute inflammation through to development of either fibrosis or epithelial repair remain uncertain. This may also differ on the basis of the underlying trigger of ARDS; that the effects of cathepsin S inhibition were less prominent after cecal ligation and puncture, only affecting the balance of neutrophils and monocytes in the airway, raises some concern about whether cathepsin S inhibition will be as effective in nonpulmonary ARDS. This does not diminish enthusiasm for pursuing cathepsin S–targeted therapy; rather, it highlights the need to identify whether there are certain subsets or endotypes of ARDS that have higher likelihood to respond to this therapy. Furthermore, given that the activity of cathepsin S was present in the airspace, there may be differential effects of cathepsin inhibition with therapeutic delivery of inhibitors directly into the airspace compared with systemic therapy, and the ideal route to manipulate protease balance is unknown.

Overall, this exciting study identifies that tipping the balance between cathepsin S and cystatin SN may reduce detrimental inflammation and be a promising new therapeutic approach to interrupt a key element of ARDS pathogenesis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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The P2X7 Receptor in Cystic Fibrosis Monocytes
Linking CFTR Deficiency to Inflammation

Cystic fibrosis (CF) lung disease is characterized by an intense mucopurulent process driven in large part by IL-1β (1). The neutrophil-dominant inflammatory response results in the accumulation of active neutrophil elastase (NE) in CF airway secretions. Both IL-1β and neutrophil elastase are highly predictive of bronchial destruction or bronchiectasis, which is the hallmark of CF lung disease (2, 3). Several key clinical studies have identified that this mucopurulent process appears early in infants, is much more severe than would be expected for the initial airway bacterial burden, and is sustained despite repeated or continuous antibiotic suppression (4, 5). These characteristics point to a probable link between the CF basic defect, deficient cystic fibrosis transmembrane conductance regulator (CFTR), and the inflammatory response. Despite the identification of several potential molecular pathways to explain why CFTR deficiency is associated with excessive airway inflammation, the lack of effective

Originally Published in Press as DOI: 10.1164/rccm.202201-0008ED on February 9, 2022