The Serpin-tine Search for Factors Associated with COVID-19 Severity in Patients with Chronic Obstructive Pulmonary Disease

The coronavirus disease (COVID-19) pandemic, which has been driven by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, has caused significant morbidity and mortality worldwide. With a range of clinical responses from asymptomatic to severe, it has particularly targeted the elderly and those suffering from underlying disease conditions. Individuals with chronic obstructive pulmonary disease (COPD) are susceptible to a range of respiratory viral infections, and it is therefore not surprising that COPD is a risk factor for hospitalization, mechanical ventilation, severe disease, and mortality in COVID-19 (1). The effects of the COVID-19 pandemic have been globally devastating; however, as new viral strains emerge and population immunity increases, focus has turned to those groups with greater risk of developing severe disease determined early in the pandemic with risk factors including age, sociodemographic status, and preexisting comorbid conditions (2). Preexisting chronic respiratory diseases, such as asthma, interstitial lung disease, and COPD, are associated with increased risk of poor outcome although the mechanisms remain poorly described (3). The use of primary bronchial epithelial cells (PBECs) from individuals with a range of lung conditions has proved useful in understanding altered responses in these cells at baseline and following infection to delineate disease mechanisms (4). New insights published in this *Journal* by Johansen and colleagues (pp. 712–729), provide experimental evidence supporting increased susceptibility and severity of infection in patients with COPD, using a well-defined air–liquid interface model of PBECs from patients with COPD and healthy volunteers (5). The use of such patient-derived cell models provides a much clearer path to translation than cell line or murine models of disease, although it will be interesting to see how these findings translate to viral infection of type-II pneumocytes (AT2), which are tied to the development of acute respiratory distress syndrome. This will, of course, be technically challenging, but the use of alveosphere culture models may help to address this to some extent (6).

Arguably, the pivotal finding from the study by Johansen and colleagues, in addition to the diminished interferon and enhanced proinflammatory response of COPD PBECs after infection, is the discovery of a protease–antiprotease imbalance that may predispose these cells to an increased degree of infection by the SARS-CoV-2 virus and may explain, to some degree, why individuals with COPD are at greater risk of infection by this virus and severe disease (5). It is well established that some viruses, for example HIV and hepatitis C, use a variety of endogenous and therapeutically relevant viral proteases to enter or replicate within host cells (7). In addition, host proteases that assist in this process have also been identified, and expression of a number of these were elevated in COPD PBECs. Notably, furin-like proteases and TMPRSS2 (transmembrane serine protease 2) enable the initial stage of SARS-CoV-2 entry into cells (8). CTSL (cathepsin L) and CTSB (cathepsin B) may also play a role in SARS-CoV-2 virus entry, suggesting that more than one host cysteinyl cathepsin protease may be involved (9). Furthermore, a range of other proteases may also participate in SARS-CoV-2 cell entry, including trypsin-like proteases and members of the coagulation cascade including plasmin (10, 11). In addition to elevated *TMPRSS2* and *CTSB*, a significant finding by Johansen and colleagues is the demonstration of decreased expression of the serine protease inhibitors (serpins) leukocyte elastase inhibitor (*SERPINB1*), *SERPINB4*, and *SERPINB6* in COPD PBECs, which may facilitate greater uptake of SARS-CoV-2 in these cells. Although it is not clear if TMPRSS2 activity is inhibited by any of the serpins highlighted in this study, decreased concentrations of these antiproteases may be indicative of the presence of other dysregulated proteases in COPD PBECs, which may also facilitate SARS-CoV-2 uptake and replication. In support of these findings, it has recently been demonstrated that other members of the serpin family, α-1-antitrypsin (serpin A1), plasminogen activator inhibitor 1 (serpin E1), and glia-derived nexin (serpin E2), may also reduce SARS-CoV-2 infection via inhibition of TMPRSS2-mediated spike protein cleavage (12). It will be interesting to establish if SARS-CoV-2 modulates serpin expression in target cells to facilitate uptake and replication of the virus.

This study confirms the importance of using patient-derived cells to gain insight into the pathogenesis of COVID-19. Despite the importance of cell lines and healthy primary cells in establishing viral-cellular interactions, the current study underscores the importance of evaluating cells from diseased tissue as the demonstration of elevated expression of proteases and diminished levels of antiprotease protection in this disease setting (COPD) can only really be appreciated by using patient-derived cells. As suggested by Johansen and colleagues, the targeting of proteases such as TMPRSS2 and members of the cysteinyl cathepsin family may be warranted in the fight against COVID-19 infection. However, it should also be mentioned that the field of viral protease inhibitor research has yielded the development of drugs that successfully target the HIV-1 protease and the hepatitis C NS3 protease (7). SARS-CoV-2 has its own repertoire of proteases required for replication within the cell, including the main protease (M^{pro} sometimes called $3CL^{Pro}$) and the papain-like protease (PL^{pro}), and attempts to target these proteases are underway (13). As can be appreciated from clinicaltrials.gov, a significant number of clinical trials are in process, or have been completed, to target proteases in COVID-19, primarily TMPRSS2 and SARS-CoV-2 proteases, and include the use of aprotinin, camostat mesylate, α-1-antitrypsin and

Supported by funding from the Medical Research Council (MRC) (MR/P022847/1 and MR/T016760/1 CCT/SW), the National Institute of Health Research Efficacy and Mechanism Evaluation (NIHR EME) programme (NIHR134567 and NIHR130192 (CCT)), the Cystic Fibrosis Foundation (WELDON18G0). AS is Supported by Asthma + Lung UK (MCFPHD20F\2), the NIHR Health technology assessment (NIHR129593), NIHR EME programme (NIHR131600) and Medical Research Council (MR/L002736/1).

Originally Published in Press as DOI: 10.1164/rccm.202205-0956ED on May 25, 2022

lopinavir or ritonavir (HIV protease inhibitors). The use of protease inhibitors to ameliorate lung disease has had a difficult history with limited success to date. However, recent studies have shown more success including the use of α-1-antitrypsin to slow the progression of emphysema in patients with α-1-antitrypsin deficiency (RAPID trial) (14) and the use of the cathepsin C inhibitor, brensocatib, which has shown some success in patients with bronchiectasis (15). In conclusion, future therapeutic strategies to treat COVID-19 infection could incorporate the use of viral and host-directed protease inhibitors, and the development, and repurposing, of protease inhibitors to this end should be a focus of COVID-19 treatment strategies. ■

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

Aaron Scott, Ph.D.
Institute of Inflammation and Ageing
University of Birmingham
Birmingham, United Kingdom

Sinéad Weldon, Ph.D.
Clifford C. Taggart, Ph.D.
Wellcome-Wolfson Institute for Experimental Medicine
Queen's University Belfast
Belfast, Northern Ireland, United Kingdom

ORCID ID: 0000-0001-5628-6624 (S.W.).

References

1. Gerayeli FV, Milne S, Cheung C, Li X, Yang CWT, Tam A, *et al.* COPD and the risk of poor outcomes in COVID-19: a systematic review and meta-analysis. *EClinicalMedicine* 2021;33:100789.
2. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, *et al.* Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020;584:430–436.
3. Aveyard P, Gao M, Lindson N, Hartmann-Boyce J, Watkinson P, Young D, *et al.* Association between pre-existing respiratory disease and its treatment, and severe COVID-19: a population cohort study. *Lancet Respir Med* 2021;9:909–923.
4. Guo-Parke H, Linden D, Mousnier A, Scott IC, Killick H, Borthwick LA, *et al.* Altered differentiation and inflammation profiles contribute to enhanced innate responses in severe COPD epithelium to rhinovirus infection. *Front Med (Lausanne)* 2022;9:741989.
5. Johansen MD, Mahbub RM, Idrees S, Nguyen DH, Miemczyk S, Pathinayake P, *et al.* Increased SARS-CoV-2 infection, protease, and inflammatory responses in chronic obstructive pulmonary disease primary bronchial epithelial cells defined with single-cell RNA sequencing. *Am J Respir Crit Care Med* 2022;206:712–729.
6. Mulay A, Konda B, Garcia G Jr, Yao C, Beil S, Villalba JM, *et al.* SARS-CoV-2 infection of primary human lung epithelium for COVID-19 modeling and drug discovery. *Cell Rep* 2021;35:109055.
7. Agbowuro AA, Huston WM, Gamble AB, Tyndall JDA. Proteases and protease inhibitors in infectious diseases. *Med Res Rev* 2018;38:1295–1331.
8. Baggen J, Vanstreels E, Jansen S, Daelemans D. Cellular host factors for SARS-CoV-2 infection. *Nat Microbiol* 2021;6:1219–1232.
9. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, *et al.* SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181:271–280.e8.
10. Jaimes JA, Millet JK, Whittaker GR. Proteolytic cleavage of the SARS-CoV-2 spike protein and the role of the novel S1/S2 site. *iScience* 2020;23:101212.
11. Ji HL, Zhao R, Matalon S, Matthay MA. Elevated Plasmin(ogen) as a common risk factor for COVID-19 susceptibility. *Physiol Rev* 2020;100:1065–1075.
12. Rosendal E, Mihai IS, Becker M, Das D, Frängsmyr L, Persson BD, *et al.* Serine protease inhibitors restrict host susceptibility to SARS-CoV-2 infections. *mBio* 2022;13:e0089222.
13. Yang H, Yang J. A review of the latest research on M^{pro} targeting SARS-COV inhibitors. *RSC Med Chem* 2021;12:1026–1036.
14. Chapman KR, Burdon JGW, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, *et al.*; RAPID Trial Study Group. Intravenous augmentation treatment and lung density in severe α1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015;386:360–368.
15. Chalmers JD, Haworth CS, Metersky ML, Loebinger MR, Blasi F, Sibila O, *et al.*; WILLOW Investigators. Phase 2 trial of the DPP-1 inhibitor brensocatib in bronchiectasis. *N Engl J Med* 2020;383:2127–2137.

Outcomes from COVID-19 Clinical Trials in Hospitalized Patients
Seeking the Truth That Matters

The coronavirus disease (COVID-19) pandemic has resulted in remarkable progress in understanding the disease through research and innovation at a pace far faster than possible pre-2020. For clinical trials, a key challenge has been the trade-off between "quick" answers versus those that have a longer time horizon and require more data collection. Understanding the implications of these approaches is critical when the aim is measuring sustained patient recovery.

In this issue of the *Journal*, Douin and colleagues (pp. 730–739) highlight the potential pitfalls of using hospital discharge as an endpoint in trials by comparing several approaches to outcome measurement (1). The authors compared the performance of three different measures of recovery with different time horizons. Their aim was to establish whether studies that considered discharge from hospital alone as a successful outcome might under-represent important outcomes occurring in the following weeks such as hospital readmission or post-discharge death.

Originally Published in Press as DOI: 10.1164/rccm.202205-0907ED on May 24, 2022