The multifaceted protease-anti-protease imbalance in COVID-19

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In this issue of *eBioMedicine*, Dr. McElvaney and colleagues report altered protease/anti-protease balance in moderate to severe COVID-19 ARDS by the disease processes as well as therapies targeting IL-6 signaling.[1] The authors use relevant clinical samples (plasma, tracheal aspirate, and lungs) of COVID-19 subjects to measure alpha-1 antitrypsin (AAT) level and its activity against the protease neutrophil elastase (NE) before and after tocilizumab, an IL-6 receptor antagonist used in COVID-19 treatment.[1]

Robust inflammation and disruption of alveolar-capillary barrier are key features of ARDS lung injury and are independent of the initial insult, which can either be lung-specific (bacterial pneumonia or COVID-19) or systemic (trauma or non-pulmonary sepsis). In ARDS patients the inflammation is driven by neutrophil-predominant airspace recruitment and the pro-inflammatory milieu of proteases, cytokines, and metabolites secreted by the neutrophils or the structural cells of the airways and distal lung after interaction with the pathogen (e.g. SARS-CoV-2).

Among the proteases involved in ARDS: NE, cathepsins, matrix metalloproteinases, and type II transmembrane serine proteases (TTSP),[2] NE is the main one driving the high protease burden, and if unbalanced by anti-proteases, leaves the airways and distal lung unprotected against NE-induced damage.[3] In COVID-19 excessive proteases are also associated with priming of the viral spike protein by TMPRSS2, a member of TTSP family, thus increasing viral epithelial cell uptake via ACE-2 mediated mechanism.[2,4]

In comparison, anti-proteases such as AAT, the most abundant serine protease inhibitor in the lung, or secretory leukocyte protease inhibitor (SLPI) inhibit NE and modulate neutrophil's pro-inflammatory cytokines and metabolites secretion.[2] Here, Dr. McElvaney et al. report that despite higher AAT level in the circulation, AAT undergoes hyper-glycosylation, degradation, and less AAT-NE complex formation in the tracheal aspirate of COVID-19 ARDS subjects, suggesting an unbalanced protease/anti-protease ratio in the lungs of COVID-19 vs. non-COVID-19 ARDS individuals.

Dr. McElvaney's study builds on the growing literature[5,6] that inflammation in ARDS, particularly in COVID-19 ARDS, might be different in the lung compartments vs. the systemic circulation. Thus, anti-inflammatory therapies that target systemic inflammatory mediators (e.g. IL-6) might not achieve the expected outcomes in the lung. Dr. McElvaney et al. previously described higher circulating levels of IL-6 in COVID-19 ARDS vs. community acquired pneumonia ARDS.[7] Similar to Sarma et al. who did not find elevated IL-6 signaling when studying the transcriptomics of tracheal aspirate in COVID-19 vs. non-COVID-19 subjects,[6,8] here, we see no difference in the IL-6 level of moderate COVID-19 subjects pre- or post-treatment with the IL-6 receptor inhibitor, tocilizumab. Moreover, they measure lower AAT, SLPI, and a higher IL-6/AAT ratio in tocilizumab-treated patients and Hep2G cells treated with COVID-19 plasma post-tocilizumab treatment. These findings suggest that therapies targeting indiscriminately IL-6 signaling may in fact worsen the protease-anti-protease imbalance in the lung compartments. Thus, if we wish to target this pathway in COVID-19, use of therapeutics aimed at IL-6 trans-signaling (which leave the classical IL-6-hepatic-AAT-synthesis pathway unaffected) with concomitant aerosolized AAT may better address the reality of protease/anti-protease compartmentalization in COVID-19 ARDS.

While this study clearly demonstrates the utility of a compartmentalized assessment of the ARDS inflammation in COVID-19, there remain several unknowns to be considered in developing more personalized approaches to risk-stratify and treat moderate-severe COVID-19. Judging inflammation compartmentalization in COVID-19 ARDS poses challenges: (1) Accounting the time from the initial insult to the plasma and tracheal aspirate collection time; (2) Capturing the dynamics of protease/anti-protease ratio as ARDS develops and progresses; and (3) Interpreting the results in the context of

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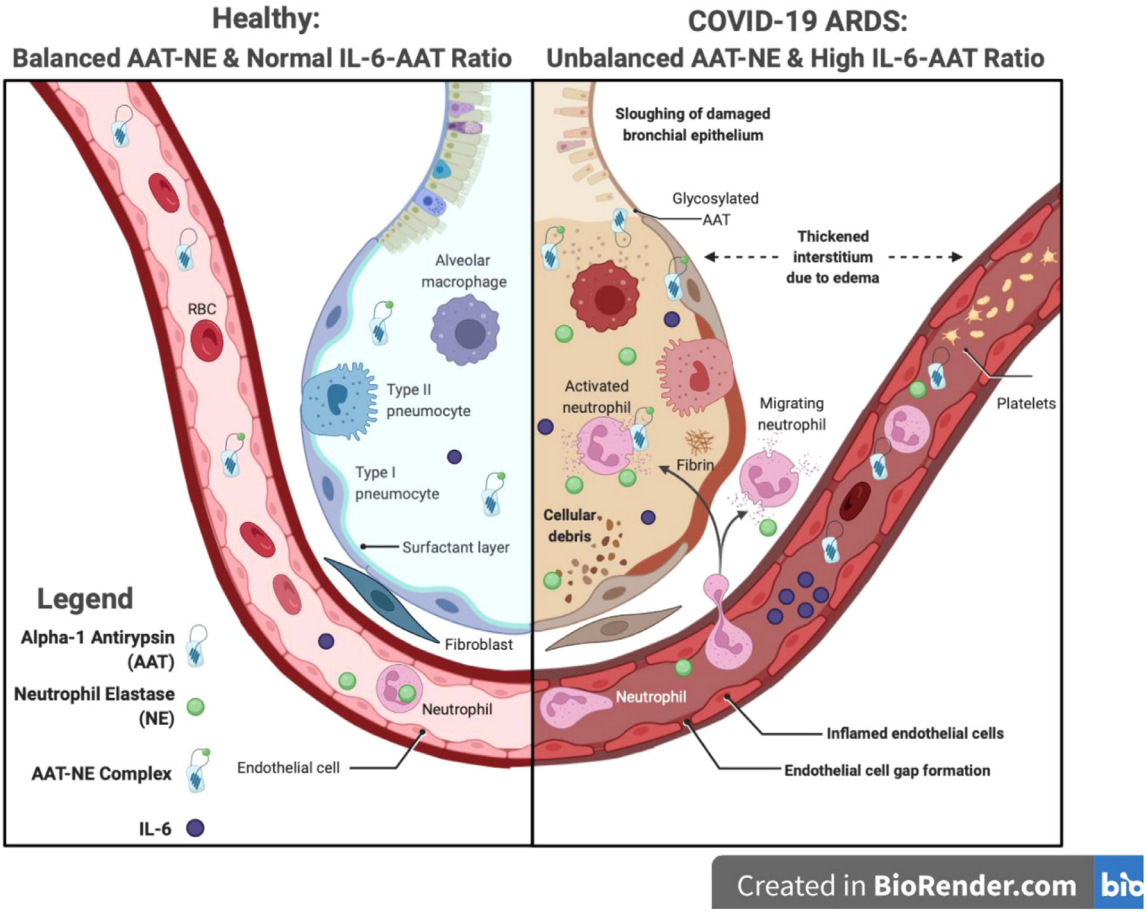

Figure 1. Schematic of the vascular / systemic and alveolar compartments during homeostasis vs. acute inflammation of COVID-19 ARDS.

limited information about prior treatments, such as outpatient treatment with glucocorticosteroids, which can extensively modulate the inflammation during COVID-19 and ARDS.[6] Another challenge is the limited insight into the alveolar compartment, the site of main injury in COVID-19. In Dr. McElvaney's study, this data predominantly comes from the post-mortem lung examination of COVID-19 non-survivors. In COVID-19 ARDS lungs we learn that NE and AAT co-localize inside the neutrophils and alveoli,[1] without further assessment of whether AAT inhibits NE and cytokines extracellularly or triggers anti-inflammatory pathways intracellularly.[9] Nevertheless, these limitations do not diminish the enthusiasm about targeting protease-anti-protease balance as a way to risk-stratify COVID-19 subjects and identify those that would preferentially benefit from anti-protease therapies.

Furthermore, given the interconnection between AAT and IL-6 signaling, a careful assessment of the current anti-COVID-19 therapies should be considered, since targeted anti-viral and anti-inflammatory therapies could synergies with tocilimizumab. In fact, instead of focusing on the downstream inflammatory cytokines; exogenous AAT to match the increased needs in COVID-19 ARDS, may serve as anti-inflammatory and anti-viral molecule. Wettstein et al. identified by mass spectrometry, that among molecules of pooled bronchoalveolar lavage fluid and homogenized human lung that inhibit SARS-CoV-2, AAT is the main compound that decreased SARS-CoV-2 infection of airway epithelium at physiologic concentration.[10]

In conclusion, Dr. McElvaney's recent study highlights the importance of considering the protease/anti-protease imbalance and its compartmentalization (see Figure 1) in the assessment and treatment of COVID-19 ARDS and paves the foundation for further investigations studying AAT as a therapeutic option.

Declaration of interests

KAS received honoraria for CME presentations from IWAA, for an educational event from The France Foundation. She received travel support from IWAA and serves on Alpha-1 Foundation Grant Advisory Committee and Medical Advisory and Scientific Committee, on ATS-RCMB Program and Website Committees as well

as on the National Jewish Health IBC committee (all these being unpaid roles). KG does not report any potential conflict of interest.

Contributors

KG: literature search, figures, data interpretation, writing.

KAS: literature search, figures, study design, data interpretation, writing.

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