Alpha-1 Antitrypsin Deficiency: Does Increased Neutrophil Adhesion Contribute to Lung Damage?

Alpha-1 antitrypsin deficiency (AATD) arises from mutations in the alpha-1 antitrypsin (AAT) antiprotease (1). The Z allele (Glu342Lys point mutation) is the most common variant that leads to disease manifestation (homozygous PiZZ state) (2). Misfolded Z-AAT polymerizes within cells, causing endoplasmic reticulum (ER) stress (3), and a low circulating concentration of functional AAT means that the proteolytic action of neutrophil-derived serine proteases is unopposed (4). The classical view is that protease-mediated tissue damage leads to emphysema and chronic airway obstruction in AATD, which can occur without smoking (5). However, AATD does not always develop full penetrance, and the lung disease is heterogeneous (5). Studies of AAT augmentation have shown variable benefit, despite achieving the putative "protective" threshold (6). Thus, there is much to learn about the development and progression of AATD; evidence is accumulating that the lack of AAT may have additional proinflammatory or immunomodulatory effects (7).

In this issue of the *Journal*, McEnery and colleagues (pp. 76–88) report on studies that examined adhesive properties of neutrophils from patients with PiZZ AATD, demonstrating that neutrophils from patients with emphysema display increased adhesion compared with healthy control individuals (PiMM genotype) (8). They elucidated that enhanced adhesion was due to increased calcium-dependent μ-calpain activity that reduced caveolin-1 transport of cholesterol to the plasma membrane. Plasma membrane proteomic assessment showed that AATD neutrophils had increased expression of integrins α-L, α-M, α-X, and β-2, as well as integrin-activating cytoskeletal proteins (e.g., talin-1). They suggest that increased integrin signaling in AATD enhances lung neutrophil infiltration, exacerbating tissue damage. Interestingly, a recent study of patients with chronic obstructive pulmonary disease showed that PiMZ heterozygotes had worse lung disease than PiMM subjects, with a higher proportion of lung neutrophils (9).

The authors provide evidence that the proadhesive neutrophil phenotype is driven by proinflammatory cytokines (CXCL8, TNF-α, and CXCL7), which are elevated in the plasma of patients with AATD. Application of these cytokines to healthy neutrophils increased integrin α-M expression and neutrophil adhesion, partly replicating the AATD neutrophil phenotype. Previously, this group has shown similarly proadhesive neutrophils, driven by the same μ-calpain/caveolin mechanism, in cystic fibrosis (CF) (10). Patients with CF also have increased circulating proinflammatory mediators but are AAT replete, adding weight to the suggestion that proinflammatory cytokines are responsible for increased neutrophil adhesion rather than the lack of AAT *per se*.

The authors performed plasma membrane proteomic assessment of neutrophils from patients undergoing AAT augmentation, comparing cells isolated on treatment Day 0 (AAT nadir) with Day 2 (AAT peak), showing modulation of several proteins related to cell migration. Functionally, Day 2 neutrophils exhibited levels of calcium, μ-calpain, caveolin, and cholesterol comparable to healthy control cells, with reduced integrin α-M expression and curtailed adhesion, indicating that AAT treatment rescues the detrimental phenotype. Because the standard weekly infusion results in AAT peaks and troughs, it is worth considering how rapidly neutrophils return to an adhesive phenotype, which was not addressed in this study. Thus, whether the beneficial effect of AAT augmentation in AATD is due to its impact on neutrophil adhesion remains to be clarified.

The authors speculate on the source of increased cytosolic calcium driving plasma membrane changes in AATD, suggesting it may be due to circulating proinflammatory mediators or ER stress from accumulated Z-AAT polymers. The former seems more biologically plausible because AAT augmentation can reduce CXCL8 and TNF-α signaling (11, 12) (and was here shown to reduce plasma levels) but does not reduce Z-AAT polymerization. Because similar results were seen after lung transplant in CF (10), where proinflammatory mediators and ER stress were both reduced, there is perhaps a common downstream pathway.

It seems the proadhesive neutrophil phenotype in AATD may be a result of neutrophil priming/activation by proinflammatory cytokines. Thus, the authors hypothesize that AAT treatment could be extrapolated to other proinflammatory respiratory pathologies, whereby AAT delivery could reduce circulating CXCL8 and TNF-α, reducing lung neutrophil infiltration. Whether additional AAT in individuals without deficiency is able to exert a beneficial biological effect is unknown, but a trial of intravenous AAT in patients with severe coronavirus disease (COVID-19) is ongoing, and the results are keenly anticipated (13).

Another intriguing finding of the study by McEnery and colleagues is that although patients with AATD without emphysema had increased plasma CXCL8, sTNFR1, and CXCL7, their neutrophils displayed a distinct plasma proteome profile compared with patients with emphysema: Integrins α-X and α-M, as well as talin-1, were all similarly increased, but not integrins α-L and β-2. Although functional properties of neutrophils from this patient subgroup were not assessed, it is likely that they would not exhibit increased adhesion, because individuals lacking functional β-2 integrin have leukocyte adhesion deficiency, with impaired neutrophil adherence and extravasation (14). This finding may

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explain why some patients with AATD develop accelerated lung function decline, whereas others with similarly deficient AAT concentrations remain stable. Could this be (in part) due to selective effects on neutrophil plasma membrane integrin expression? However, it is not clear how the differential proteomic results could be explained in the context of a similarly elevated proinflammatory cytokine profile. Further research is needed to dissect this conundrum because it also has implications for potential benefits of AAT augmentation. Indeed, β-2 integrin was not modified by AAT treatment in this cohort, although the study may have been underpowered to detect a difference.

It is also possible that the above findings could be explained by clinical and demographic differences between the patients with and without emphysema or that the patients without emphysema were earlier in their disease trajectory. A limitation of this study was that patients with AATD with emphysema were older than those without emphysema and the healthy control individuals. Sapey and colleagues have shown that neutrophils from older individuals (without lung disease) have aberrant migration with increased tissue damage capacity, as well as increased protease activity and systemic inflammation (15). This is an important potential contribution to lung disease in older patients with AATD that may partly explain why they have developed emphysema.

In summary, the study by McEnery and colleagues demonstrates that systemic inflammation in AATD promotes a proadhesive neutrophil phenotype. Enhanced lung neutrophil infiltration may therefore contribute to lung tissue damage. AAT augmentation can reverse this detrimental neutrophil phenotype and may have some utility in other inflammatory lung diseases. ■

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References

1. Laurell CB, Eriksson S. The electrophoretic α_1-globulin pattern of serum in α_1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963;15:132–140.
2. Jeppsson JO. Amino acid substitution Glu leads to Lys α_1-antitrypsin PiZ. *FEBS Lett* 1976;65:195–197.
3. Lomas DA, Evans DL, Finch JT, Carrell RW. The mechanism of Z α_1-antitrypsin accumulation in the liver. *Nature* 1992;357: 605–607.
4. Fazleen A, Wilkinson T. The emerging role of proteases in α_1-antitrypsin deficiency and beyond. *ERJ Open Res* 2021;7: 00494-2021.
5. Stockley RA, Edgar RG, Pillai A, Turner AM. Individualized lung function trends in alpha-1-antitrypsin deficiency: a need for patience in order to provide patient centered management? *Int J Chron Obstruct Pulmon Dis* 2016;11:1745–1756.
6. Stockley RA, Bayley DL, Unsal I, Dowson LJ. The effect of augmentation therapy on bronchial inflammation in α_1-antitrypsin deficiency. *Am J Respir Crit Care Med* 2002;165:1494–1498.
7. Cosio MG, Bazzan E, Rigobello C, Tinè M, Turato G, Baraldo S, *et al.* Alpha-1 antitrypsin deficiency: beyond the protease/antiprotease paradigm. *Ann Am Thorac Soc* 2016;13:S305–S310.
8. McEnery T, White MM, Gogoi D, Coleman O, Bergin D, Jundi B, *et al.* Alpha-1 antitrypsin therapy modifies neutrophil adhesion in patients with obstructive lung disease. *Am J Respir Cell Mol Biol* 2022;67: 76–88.
9. Ghosh AJ, Hobbs BD, Moll M, Saferali A, Boueiz A, Yun JH, *et al.*; COPDGene Investigators. Alpha-1 antitrypsin MZ heterozygosity is an endotype of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2022;205:313–323.
10. White MM, Geraghty P, Hayes E, Cox S, Leitch W, Alfawaz B, *et al.* Neutrophil membrane cholesterol content is a key factor in cystic fibrosis lung disease. *EBioMedicine* 2017;23:173–184.
11. Bergin DA, Reeves EP, Meleady P, Henry M, McElvaney OJ, Carroll TP, *et al.* α-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. *J Clin Invest* 2010; 120:4236–4250.
12. Churg A, Wang X, Wang RD, Meixner SC, Pryzdial ELG, Wright JL. α_1-Antitrypsin suppresses TNF-α and MMP-12 production by cigarette smoke-stimulated macrophages. *Am J Respir Cell Mol Biol* 2007;37: 144–151.
13. McEvoy NL, Clarke JL, Mc Elvaney OJ, Mc Elvaney OF, Boland F, Hyland D, *et al.* A randomised, double-blind, placebo-controlled, pilot trial of intravenous plasma purified alpha-1 antitrypsin for SARS-CoV-2-induced acute respiratory distress syndrome: a structured summary of a study protocol for a randomised, controlled trial. *Trials* 2021;22:288.
14. Hogg N, Stewart MP, Scarth SL, Newton R, Shaw JM, Law SKA, *et al.* A novel leukocyte adhesion deficiency caused by expressed but nonfunctional β2 integrins Mac-1 and LFA-1. *J Clin Invest* 1999;103:97–106.
15. Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, *et al.* Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. *Blood* 2014;123:239–248.