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Targeting neutrophil serine proteinases in alpha-1 antitrypsin deficiency

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Abstract

Alpha-1 antitrypsin (AAT) is the most abundant irreversible serine proteinase inhibitor in the circulation and plays a major role in protecting lung tissue against destruction from neutrophil serine proteinases. Genetic mutation of AAT leads to reduced circulating levels and AAT deficiency (AATD) which is associated with an increased risk of developing emphysema. This observation suggests that the balance between AAT and neutrophil serine proteinase is crucial in maintaining tissue homoeostasis. In AATD, the overexuberant proteinase activity resulting from inadequate AAT control creates a self-perpetuating inflammatory cycle, driving progressive tissue injury. Re-establishing this physiological balance is therefore critical for preserving lung architecture, function, and abrogating disease progression.

Several avenues within this pathophysiological pathway are being explored. This chapter addresses the pathophysiological process, current treatments targeting the pathway, and alternative approaches within the pathway that can potentially mitigate proteinase imbalance.

Keywords: Alpha-1 antitrypsin deficiency, neutrophil serine proteinase, treatment targets, emphysema, rare disease

INTRODUCTION

Alpha-1 antitrypsin [(AAT); also known as Serine Protease Inhibitor (SERPIN) family A1] is a polymorphic protein expressed in a codominant fashion by 2 alleles on chromosome 14[1]. It has a modulatory role in inflammation showing an acute phase response in its manufacture and release from the liver (the major source of production) and increased diffusion into the affected tissues/organs where it plays a primary role in protecting tissues from the destructive effect of serine proteinases.

The importance of this role is indicated by the genetic deficiency first observed in the 1960s[2]. Of the initial 5 cases identified, 3 had severe early onset basal emphysema and subsequent family studies confirmed the inheritance of the deficiency and the pulmonary association that appeared both spontaneously (with no recognised external risk factors[3]) but was accelerated by cigarette smoking[4]. Although other clinical associations such as panniculitis, systemic vasculitis, and liver cirrhosis are also recognised associations, it is the pulmonary disease that dominates.

Much has been learnt about the pathophysiology of the disease since the early observation of plasma deficiency. Over 150 variants of the protein have been identified, although most are associated with apparently "normal" concentrations and function. Deficiency can vary from partial due to heterozygosity of deficient genes with a normal gene to complete absence of AAT related mostly to point mutations and frameshift truncating mutations, although these are especially rare[5]. The majority of clinically relevant deficient cases are those homozygous for the Z variant which has a point mutation resulting in an amino acid substitution at position 342 (Glu to Lys) in the mature protein. This substitution can be identified through the genomic databases as rs28929474-p.Glu366Lys. As a result, this produces a protein that has a reduced association rate with its target enzymes[6-8]. This mutation also increases the likelihood of the protein to polymerise, particularly in the liver, impeding its secretion (by approximately 80%), resulting in plasma concentrations of ~5 μM[6-8].

The most relevant targets for AAT are serine proteinases and especially those stored in and released by circulating and migrating neutrophils. In the 1970s, the first report on the pulmonary damaging effect of neutrophil elastase was published[9]. Although this enzyme retains its importance as the direct mediator of emphysema and damage to many vital immune functions in the lung, it should be noted that two other enzymes stored in the same granules in the neutrophil (Proteinase 3 and Cathepsin G) can play similar damaging roles.

In the 1980s, the link between AAT deficiency (AATD) and emphysema was well established and the logical way to manage the condition was to enhance the circulating and hence lung concentration of AAT. Purification of plasma AAT from blood donors proved feasible and intravenous administration raised both the plasma[10] and lung[11] concentrations of AAT to safe and protective levels. Based on these studies, the United States Food and Drug Administration (FDA) approved weekly intravenous augmentation therapy for Z homozygous individuals on biochemical efficacy alone.

Because AATD is relatively rare (1 in 1500 to 1 in 5000) related to migration patterns from the Baltic areas[12], it was not felt feasible to carry out classical placebo-controlled studies to demonstrate clinical efficacy on the physiological progression of emphysema. However, observational studies[13,14] suggested a benefit. With the advent of highly sensitive quantitative computed tomographic density measurements of the lung as a direct measure of the emphysema process, the powering of studies to demonstrate the efficacy of augmentation therapy became feasible. Initial observational[13,14] and placebo-controlled studies[15-17] and a subsequent adequately powered placebo-controlled study[18] confirmed the ability of intravenous AAT to

slow the progression of emphysema.

However, intravenous augmentation therapy is both expensive and inconvenient for the patient and remains unavailable in many countries. Research has continued into the pathophysiology of chronic lung disease including the processes relevant to AATD that suggest alternative strategies may be equally or potentially more effective in correcting the imbalance that occurs when enzymes such as elastase are released into an environment where deficient AAT fails to adequately control the enzyme activity exceeding its physiological role and leading to excessive tissue damage. The mechanism and potential strategies to enhance protection in AATD are discussed below.

The pathophysiology of emphysema

The proteinase/antiproteinase balance theory

AAT is the most abundant serine proteinase inhibitor in the circulation and plays a major role in the lung by largely entering the tissues via simple transudation and therefore increases in the presence of local inflammation[19]. In healthy individuals, plasma concentration of AAT ranges between 20 to 40 μM resulting in interstitial concentrations of roughly 80% of that in plasma. The other significant lung inhibitor is the secretory leukocyte proteinase inhibitor (SLPI) produced by mucous glands and bronchoepithelial cells and can be secreted basally into the interstitium[20]. Although a reversible inhibitor (unlike AAT, which is irreversible), it is better at protecting elastin from neutrophil elastase than AAT[21] despite being unable to inhibit Proteinase 3[22].

When the physiological balance between these enzymes (as in AATD) is disturbed, excessive tissue breakdown occurs as the proteinases will have a longer duration and radius of activity[23,24]. During neutrophilic inflammation, the neutrophil traffic and elastase load to the lung is increased. In those with normal AAT, the acute phase response increases AAT production and inflammation increases its penetration into the lung leading to modulation of inflammation. In AATD, the AAT concentration is too low at baseline and inflammation is greater than in patients with normal AAT which is associated with a poor acute phase response[25]. This potentially leads to greater and more persistent tissue damage, destruction of elastin, and the development and progression of emphysema.

When AAT-deficient individuals were reported to have increased susceptibility to emphysema development[2], researchers quickly recognised the balance between AAT and a destructive enzyme/s[3] (subsequently identified as a feature of neutrophil serine proteinases[9,26]) was key to maintaining elastin homeostasis and that emphysema reflected a disturbance in this balance where proteinase activity prevails.

The proteinase/antiproteinase imbalance between AAT and its cognate proteinase is reflected by the excessive amount and activity of neutrophil elastase in AAT-deficient individuals[27]. Neutrophil elastase load in the lung tissue is directly associated with the pathological severity of emphysema[28]. Furthermore, the systemic footprint of neutrophil elastase measured as a neutrophil elastase-specific fibrinogen cleavage product (AaVal360) is significantly higher in AAT-deficient individuals and correlates with the severity and progression in the early stages of AATD lung disease[29,30].

In the most prevalent form of AATD, it is the replacement of Glu to Lys at position 342 (hinge loop region) of the Z variant AAT protein that increases the likelihood of spontaneous polymerisation[6]. As a consequence, retention in the liver reduces secretion and hence the plasma and lung concentration, increasing susceptibility to tissue damage by neutrophil serine proteinases. In addition, polymerised AAT aggregates can be found in the lung tissue[31], associated with the accumulation and activation of neutrophils

in the localised areas halting their migration into the airways, and hence causing more localised tissue damage. Furthermore, after degranulation, proteinases can become bound to neutrophil cell membranes (especially in AATD[32]) and remain active while being resistant to AAT inhibition, which further complicates the control of local proteinase activity, especially for AATD individuals.

The role of neutrophil elastase in the pathophysiology of emphysema is widely recognised. However, emerging evidence suggests that Proteinase 3 may play a greater role in driving emphysema in AATD. Proteinase 3 is stored at 3-4 times greater concentrations in the azurophilic granule than neutrophil elastase[33]. In addition to replicating pathological changes similar to emphysema in animals[26,34,35], mathematical modelling indicated that Proteinase 3 possessed a greater potential for injury than neutrophil elastase diffusing over a greater radius for longer before reaching an enzyme inhibitor equilibrium, especially in AATD[23]. Neutrophils from AATD subjects also expressed more Proteinase 3 on the cell membrane[32], which is replicated by treating healthy neutrophils with deficient Z as opposed to normal M AAT plasma, suggesting that AAT itself modulates cell membrane localisation.

An additional factor influencing the role of Proteinase 3 is that SLPI (the predominant antiproteinase in airway secretion) is a poor inhibitor of Proteinase 3 and suggests that Proteinase 3 likely has a more important role in tissue destruction, especially in AATD[22-24]. This is supported by the persistent Proteinase 3 activity in lung secretions of AATD patients compared to those with non-deficient chronic obstructive pulmonary disease (COPD) even when elastase activity is undetectable[32,36] and the greater plasma concentrations of the Proteinase 3 activity footprint than that of the neutrophil elastase footprint as measured by the specific fibrinogen cleavage products[37].

Neutrophils and emphysema

Neutrophil serine proteinases, particularly neutrophil elastase and Proteinase 3 are essential to neutrophil migration through the lung interstitium[24,38]. The movement of neutrophils is facilitated by the mobilisation of proteinases to the leading edge of the cell[39]. During this process, the concentration of the released proteinases in the pericellular space overwhelms their inhibitors, causing an area of obligate destruction closely surrounding the neutrophils until the proteinases diffuse away and an equilibrium is reached[24,32]. In AATD, the destructive effect is amplified as cell surface proteinases are less controlled, potentially causing a greater area of damage[40].

Accumulation of neutrophils in the lung is well documented in AATD. This neutrophilic accumulation is in response to locally released chemoattractants. Leukotriene B4 (LTB4) is generated and released by alveolar macrophages in response to excess neutrophil elastase activity[41]. Similarly, CXCL8 can be released from epithelial cells in response to an elastase challenge[42]. Both these potent chemoattractants have been detected at high levels in lung secretions of AATD[43]. Additionally, tissue degradation products generated by elastase from elastin can also add to the chemoattractant signal gradient[44], providing an amplification loop to the lung neutrophilic response, thus enhancing tissue damage.

Understanding the whole pathway involved in tissue damage in AATD (See Figure 1) provides a series of potential therapeutic interventions to restore a physiological balance and protect the lung.

With this whole pathway in mind, there are several defined points at which the process can potentially be modulated.

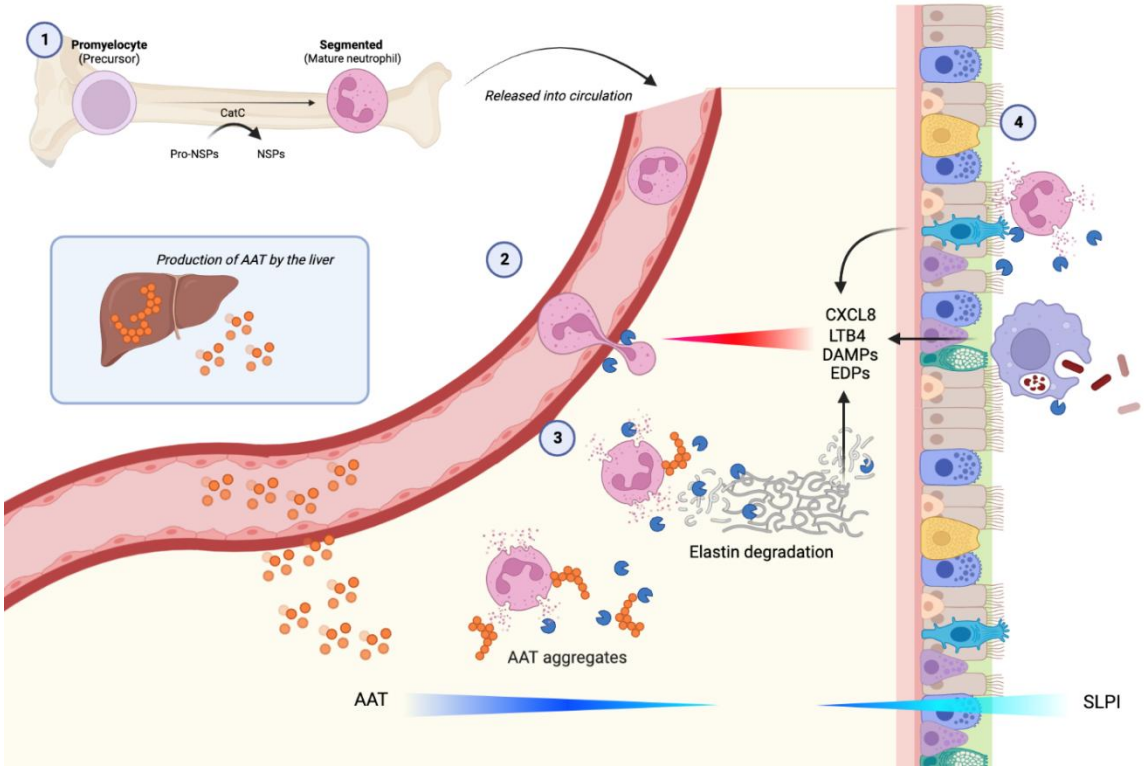

Figure 1. (1) Neutrophil proteinases are transcribed and packaged early in cellular differentiation in the bone marrow. Cell division later in differentiation divides the preformed packages (azurophil granules) amongst daughter cells and the mature cells are released into the circulation. (2) When the circulating cell senses a chemotactic gradient, it adheres to the endothelium via specific adhesion molecules and transmigrates through endothelial junctions. Granules migrate to the leading edge and the cell releases elastase at the point of transmigration, facilitating movement into the tissues, degrading a connective tissue pathway while leaving enzyme in its wake. (3) The presence of AAT polymers in AATD patients can potentially drive further neutrophil recruitment and local activation, which in the presence of deficiency amplifies local tissue damage, generating chemotactic fragments that add to the chemoattractant signal[31]. (4) Once in the airway, further release of elastase generates additional chemoattractants such as matrikines[44] and DAMPs[45], amplifying neutrophil recruitment. CatC: Cathepsin C; NSPs: neutrophil serine proteinases; DAMPs: damage-associated molecular patterns; ECM: extracellular matrix; CXCL8: C-X-C Motif Chemokine Ligand 8; LTB4: leukotriene B4; AAT: Alpha-1 antitrypsin; AATD: Alpha-1 antitrypsin deficiency; SLPI: secretory leukocyte proteinase inhibitor.

Augmentation of the antiproteinase screen

Since the genetic deficiency is likely central to the disturbance of the physiological balance with neutrophil serine proteinase in the lung, the most logical step is to restore the missing protein to normal/safe levels. Gadek and colleagues[10] demonstrated this was feasible with AAT purified from plasma, given weekly at 60 mg/kg to achieve a putative "protective" nadir level (≥ 11 μM)[24,46,47].

Registry data found that patients receiving AAT augmentation had slower lung function decline, especially in a limited midrange detected by the forced expiratory capacity volume in 1 sec (FEV_1) as a surrogate marker of emphysema progression[13,48]. This observation was supported by the slowing of lung density decline in later controlled clinical trials through measuring computed tomographic densitometry, a more direct marker for emphysema[15-17]. However, despite AAT infusion reducing the rate of lung density decline, a more recent randomised control trial did not observe an effect on halting deteriorating lung function stabilising or improving health status or reducing exacerbations[18], although the study was not statistically powered for such outcomes[49].

Assessment of the clinical efficacy of AAT augmentation therapy is complicated by different study designs and standard outcome measurements. The longstanding debate concerning the clinical impact of AAT augmentation therapy was more recently addressed by the European Respiratory Society. A comprehensive meta-analysis was undertaken and concluded a clinical benefit in reducing emphysema progression assessed by computed tomographic densitometry and the slowing of lung function decline[50], although a longer study of augmented and non-augmented patients showed no apparent benefit of augmentation as determined by the decline in FEV_1[51]. Nevertheless, although the benefit of augmentation on the loss of lung tissue is now accepted, the progression continues even with this therapy, albeit at a slower rate, suggesting that other factors are at play[52].

Modified AAT forms are also being explored for possible improved stability and better cost-effectiveness. INBRX-101 (also known as rhAAT-Fc) is a modified AAT that is more resistant to oxidative inactivation[53]. Intraperitoneal administration of INBRX-101 in mice was more protective than using pooled plasma AAT in emphysema induced by elastases (pancreatic porcine elastase and neutrophil elastase) or cigarette smoke[54]. Recently, the phase 1 trial of intravenous INBRX-101 every three weeks showed that it restored functional AAT levels in the plasma of AATD patients, comparable to those of healthy individuals (40.4 μM), which was sustained over the course of the study period[55]. However, the full results or any potential biochemical efficacy have yet to be released[55]. INBRX-101 may be more sustainable than traditional AAT augmentation therapy and possibly have a greater effect than conventional plasma-purified AAT in abrogating disease progression.

ALTERNATIVE STRATEGIES FOR AATD INFUSION

Increase Alpha-1 antitrypsin secretion

Alpha-1 antitrypsin folding drug

In Z AATD, the reduced circulating levels of AAT are not due to a lack of production of AAT, but rather a result of intracellular Z AAT aggregation impeding secretion into the circulation. Based on this concept, drugs such as ZF874[56] and VX864[57] were developed to help rescue the misfolding of the Z AAT, thereby potentially preventing aggregation and increasing the release of monomeric AAT into the bloodstream and, thus, partially restoring inhibitory capacity. Phase 2 trials for both drugs have recently been completed. Results for both drugs are currently undocumented, although details relating to side effects have been released. It was announced that high-dose treatment of VX864 in patients with Z AATD over 28 days lowered plasma Z AAT polymers by approximately 90% and the level of functional plasma AAT was increased. However, the magnitude of this increase was deemed insufficient to protect the lung adequately[57]. A 48-week trial is currently underway to assess the long-term effects of VX864 in AATD patients.

Inhaled therapy

Delivering AAT directly to the airways might have a greater impact on airway inflammation than giving it intravenously, thereby focussing the distribution in the lung. The direct effect (if any) on emphysema remains unknown. A randomised controlled trial that assessed the effects of aerosolised AAT over 50 weeks in AATD patients with severe emphysema did not reduce the time to exacerbation (the primary outcome) compared to the placebo cohort[58], although post hoc analysis suggested a benefit on FEV_1. While inhalation studies have shown that AAT levels rise in lavage fluid[59,60], the levels are unlikely to reach the alveolar region where emphysema occurs and subsequently penetrate through epithelial tight junctions into the interstitium.

Another strategy to control excessive proteinase activity would be to give SLPI, a proteinase inhibitor already enriched in airway secretions produced locally by bronchoepithelial cells[20]. SLPI also inactivates neutrophil elastase via direct 1:1 inhibition[61] and can additionally inhibit elastin-bound neutrophil elastase which is AAT-resistant[21,62]. SLPI levels in sputum or epithelial lining fluids are, however, influenced by local neutrophil elastase falling during inflammation[61,63]. This is also a feature of AATD as significantly lower levels of SLPI are present in the sputum of such individuals, likely a result of the increased local serine proteinase activity[36] and hence forming a potentiating inflammatory proteinase-rich loop.

SLPI has been given by inhalation for other conditions with excessive airway neutrophilic inflammation (such as in cystic fibrosis) showing an antiproteinase effect[64], but again in AATD where proteinase burden is high, a high concentration of SLPI will also have to reach the distal airways and penetrate into the interstitium. Furthermore, SLPI has no inhibitory activity on Proteinase 3[22] and Proteinase 3 also degrades SLPI[65], suggesting that SLPI may be less appropriate, especially as Proteinase 3 potentially has a major or even greater impact on driving disease progression than neutrophil elastase.

Neutrophil elastase inhibitors

Reagents (especially oral ones) capable of direct inhibition of neutrophil elastase would be a strong potential strategy based on the current understanding of elastase being a key direct mediator of emphysema and disease progression in AATD. Neutrophil elastase also orchestrates a series of proinflammatory responses, including cleavage activation of metalloproteinases[66], inducing the release of danger signals[67], and stimulating aberrant growth factor release[68] as well as impairing lung host defence mechanisms[69]. Thus, in addition to directly suppressing elastolysis, inhibiting neutrophil elastase could also prevent amplification of the inflammatory response and improve host defences.

AZD9668 is described as a potent selective inhibitor of neutrophil elastase[70]. Though the exact mechanism of action is currently unpublished, *in vitro* studies of AZD9668 successfully reduced plasma neutrophil elastase activity following whole blood stimulation by inhibiting both membrane-bound and liberated neutrophil elastase[70]. The disease-modifying potential of AZD9668 was validated in rodent models as oral administration of the drug attenuated systemic inflammation and neutrophil elastase-induced injury[70]. Furthermore, it was recently announced that oral administration of AZD9668 (Alvelestat or MPH966) successfully suppressed plasma evidence of neutrophil elastase activity in a phase 2 study in patients with Z AATD, demonstrating a progressive decline in the systemic fibrinogen biomarker of neutrophil elastase activity (AaVal360) in the high-dose treatment arm[71]. However, any effect on clinical outcomes has yet to be reported.

Modulation of neutrophils

Neutrophil chemotaxis

AAT-deficient neutrophils appeared to be inherently primed. When compared to healthy neutrophils, more AAT-deficient neutrophils spontaneously adhered to the endothelium[72] and displayed enhanced chemotactic response toward the chemoattractants LTB4 and CXCL8 which are abundant in the AATD lung[43]. The high chemoattractant burden, increasing neutrophil influx to the lung, and the resultant tissue damage suggest that modulating neutrophil migration to attenuate neutrophil-driven inflammatory effects could be advantageous. In the inflamed lung *milieu*, where a multiplicity of chemoattractants are present, chemokine receptor blockade offers an alternative approach to modulating elastase-mediated tissue damage.

CXCR2 is a membrane chemokine receptor expressed on neutrophils involved in regulating neutrophil chemotaxis. Preclinical studies investigating CXCR2 antagonism successfully prevented CXCL8-mediated chemotaxis[73]. The effect of CXCR2 blockade was also shown in a phase 2 trial with 615 COPD patients

successfully reducing neutrophilic inflammation. However, more frequent episodes of exacerbation and pneumonia infections were reported in the high-dose treatment arms, implying that retaining adequate physiological responses is crucial for retaining host defence[74].

Neutrophil adhesion

An alternative approach to modulate neutrophil migration would be targeting endothelial adhesion. LTB4 mediates neutrophil adhesion by upregulating a b2-integrin (Mac-1) on endothelial cells as well as promoting neutrophil chemotaxis and degranulation[75,76]. Although LTB4 receptor antagonists have been studied in neutrophilic inflammatory diseases including cystic fibrosis and COPD, so far, no evident clinical benefit has been reported[77], even though Gompertz and Stockley[78] showed an anti-inflammatory effect. In addition, directly blocking the adhesion molecules could have the same effect, although both strategies run the risk of impairing this important secondary host defence as indicated above for CXCR2.

Proteinase production

A further approach would be to target upstream regulators required for maturating neutrophil serine proteinases before packaging into granules, thereby reducing the proteinase payload delivered per neutrophil. Cathepsin C (CatC) is an essential endopeptidase regulating the activation and subsequent packaging of neutrophil serine proteinases. An uncleaved signal peptide in the zymogens is signalled for degradation and when it is removed by CatC, the neutrophil serine proteinases become activated and packaged into the azurophilic granules. This packaging process occurs early in neutrophil maturation[79].

Recently, it was discovered that azurophilic degranulation is also significantly enhanced in AATD neutrophils through Rac2 signalling induced by elastase activity on the proteinase-activated receptor 2 on the neutrophils[80], adding further amplification to neutrophil elastase release. CatC is therefore an appealing target to simultaneously abrogate neutrophil serine proteinase activity and control the magnitude of degranulation. More importantly, CatC inhibition does not seem to interfere with neutrophil migration and antibacterial properties, as individuals with severe CatC deficiency (Papillion-Lefèvre syndrome) do not experience increased susceptibility toward major bacterial infections[81]. CatC blockade, therefore, should cumulatively reduce the quantity of neutrophil elastase and Proteinase 3 packaged into each azurophilic granule, lessen the amount of proteinase released and prevent excessive degranulation restoring a proteinase/antiproteinase balance to a level consistent with AATD while retaining neutrophilic defence properties. However, this should be balanced to avoid severe deficiency associated with the Papillion-Lefè vre syndrome.

CatC inhibition as a strategy is supported by AZD7986 (Brensocatib), a reversible CatC inhibitor used in a 24-week phase 2 trial in non-cystic fibrosis bronchiectasis where it reduced sputum neutrophil elastase activity by 40% while lowering exacerbation rates. In AATD, this approach may have not just been a beneficial effect on emphysema decline targeting both neutrophil elastase and Proteinase 3 but also an added benefit to the 30% of AATD patients with both emphysema and bronchiectasis[82].

SUMMARY

Neutrophil serine proteinases are responsible for driving inflammation and tissue destruction in the lung, especially in chronic lung diseases, such as AATD. This process is partly modulated by AAT replacement in AATD.

Replenishing AAT to restore protection against proteinase-driven damage is a logical and straightforward strategy but is costly and requires weekly infusions. Although AAT has broad immunomodulatory functions, it plays a passive role in preventing downstream injury as a side effect of excess neutrophil trafficking in response to chemoattractants. In comparison to the greater than $100,000 per patient per year for delivering augmentation therapy, novel oral drug candidates are likely to be cheaper but, importantly, less invasive and therefore more convenient for the patients.

CONCLUSION

Understanding the steps that lead to neutrophil migration, degranulation and tissue damage offers several potential strategies to modulate the proteinase/antiproteinase balance, although this has to ensure the important secondary host defensive functions are retained.

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Both authors declared that there are no conflicts of interest.

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Consent for publication

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