The Role of Neutrophils in Alpha-1 Antitrypsin Deficiency

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Abstract

Alpha-1 antitrypsin deficiency (AATD) is characterized by low levels of circulating alpha-1 antitrypsin and an increased risk for emphysema, liver disease, and panniculitis. The reduced levels of alpha-1 antitrypsin in AATD predispose the lung to unopposed proteolytic activity, predominantly from neutrophil-derived proteases, chiefly neutrophil elastase. This leads to emphysema. The mechanisms subtending the liver disease are less well understood, but are probably due to a "gain-of function" inflammatory process in the liver, stoked by intracellular retention of aberrantly folded alpha-1 antitrypsin. The panniculitis associated with AATD is most likely due to unopposed proteolytic activity in the skin. Although AATD has been traditionally viewed as a condition arising from a protease–antiprotease imbalance in the lung, it is increasingly recognized that AATD is an inflammatory disorder, both in the lung and in the extrapulmonary manifestations associated with the condition. This inflammation is predominantly neutrophil driven, and there are several alpha-1 antitrypsin–related mechanisms involved in potentiating this neutrophilic response. The rationale for AAT augmentation therapy in AATD is classically based on restoring the antiprotease balance in the lung, but its beneficial effects may also be exerted systemically, further exposing the pathogenesis of AATD-related disease and indicating a potential usage for alpha-1 antitrypsin in other inflammatory conditions.

Keywords: alpha-1 antitrypsin deficiency; augmentation therapy; inflammation; neutrophil elastase; TNF-α

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Alpha-1 antitrypsin (AAT) deficiency (AATD) is an autosomal codominant condition characterized by low circulating levels of AAT protein. People with AATD are at a high risk of developing emphysema at an early age (1) and also have a significant risk of liver disease and a lesser risk of panniculitis skin disease. The most common variants are the Z and S mutations caused by the substitution of glutamic acid for lysine or valine at positions 342 and 264 of the polypeptide, respectively (2, 3). AATD is the only proven genetic risk factor for the development of chronic obstructive pulmonary disease and even heterozygote individuals with the MZ mutation, who smoke, are at increased risk of developing lung disease (4). The most common severe variant associated with lung, liver, and skin disease is the Z mutation, occurring in greater than 95% of individuals with severe AATD (5). Mutation-induced conformational instability of the protein leads to misfolding and accumulation of Z-AAT polymers in the endoplasmic reticulum (ER) of hepatocytes and other cells (3). This results in inflammation due to "gain of function," and can occur early in life. Symptomatic lung disease, which is mainly due to "loss of function" (i.e., insufficient levels of normal AAT in the lung), usually presents in the fourth and fifth decades (6), and the classical manifestation is emphysema, which is typically panacinar and predominantly involves the lung bases (7). The loss of function may also contribute to systemic inflammation, due to the lack of AAT antiinflammatory effects.

AAT is a 52-kD glycoprotein, primarily synthesized in the liver; it is a serine protease inhibitor (Serpin), which acts primarily to inhibit neutrophil elastase (NE) in the lung, thus protecting lung tissue from proteolytic degradation. AAT also inhibits other neutrophil-derived proteases, such as cathepsin G (Cath G) and proteinase 3 (PR3) (Figure 1). Although the role of AAT in maintaining a balance between protease and antiprotease activity is essential for the protection of lung matrix from degradation and preventing emphysema, AATD is more than just a condition of unopposed proteolysis. AATD is an inflammatory disorder, and the neutrophil plays a key role in these inflammatory processes. The purpose of this review is to discuss the role that the neutrophil plays in AATD and how this cell contributes to the inflammatory phenotype associated with this condition.

Neutrophil Numbers Are Increased in the Lungs of Individuals with AATD

There is a significantly higher burden of neutrophils in the lungs of individuals with AATD compared with healthy individuals.

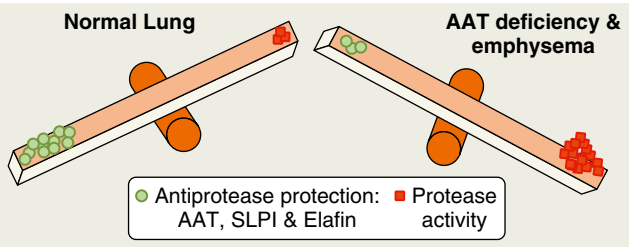

Figure 1. Protease–antiprotease balance in respiratory epithelial lining fluid. Antiprotease protection is provided by alpha-1 antitrypsin (AAT), secretory leukoprotease inhibitor (SLPI), and/or elafin. Protease activity includes neutrophil elastase (NE), cathepsin G (Cath G), and/or proteinase 3 (PR3).

In this regard, Hubbard and colleagues (8) demonstrated that there was not only an increased number of neutrophils in AATD bronchoalveolar lavage fluid, but also that the neutrophil chemotactic index was elevated. Moreover, it has been demonstrated that individuals with AATD homozygous and heterozygous for the Z allele (9) have increased neutrophil influx into the airways (10). A number of mechanistic models have been put forward to aid our understanding of the cause of the increased neutrophil count in the lungs of individuals with AATD. Accordingly, it has been demonstrated that Z-AAT polymers may deposit both in alveolar and bronchial epithelial cells, and that these polymers can act as potent chemoattractants, comparable to IL-8 *in vitro* (11). It should also be noted that NE can induce the expression of IL-8 in bronchial epithelial cells via Toll-like receptor 4, subsequently leading to neutrophil chemotaxis and increasing the inflammatory burden in the lung (12). Evidence of the vital role that AAT plays in the control of neutrophil influx is demonstrated by its ability to modulate chemotaxis in response to IL-8 and soluble immune complexes (sICs) (13). Results demonstrate that AAT binds IL-8 via glycan moieties, and subsequently this AAT–IL-8 complex prevents chemokine receptor 1 engagement, with resultant downstream signaling effects on calcium flux, cytoskeleton rearrangements, and F-actin formation, with subsequent decreased neutrophil chemotaxis (Figure 2). AAT also affects neutrophil migration mediated by sIC. After sIC engagement, increased a disintegrin and metalloproteinase-17 (ADAM17) activity leads to release of the glycosylphosphatidylinositol-anchored Fc receptor FcγRIIIb from the cell surface, thereby directing downstream chemotaxis signaling events. AAT controls sIC-mediated neutrophil chemotaxis by inhibiting ADAM17 activity and preventing release of FcγRIIIb from the cell membrane (13) (Figure 2). In AATD, this

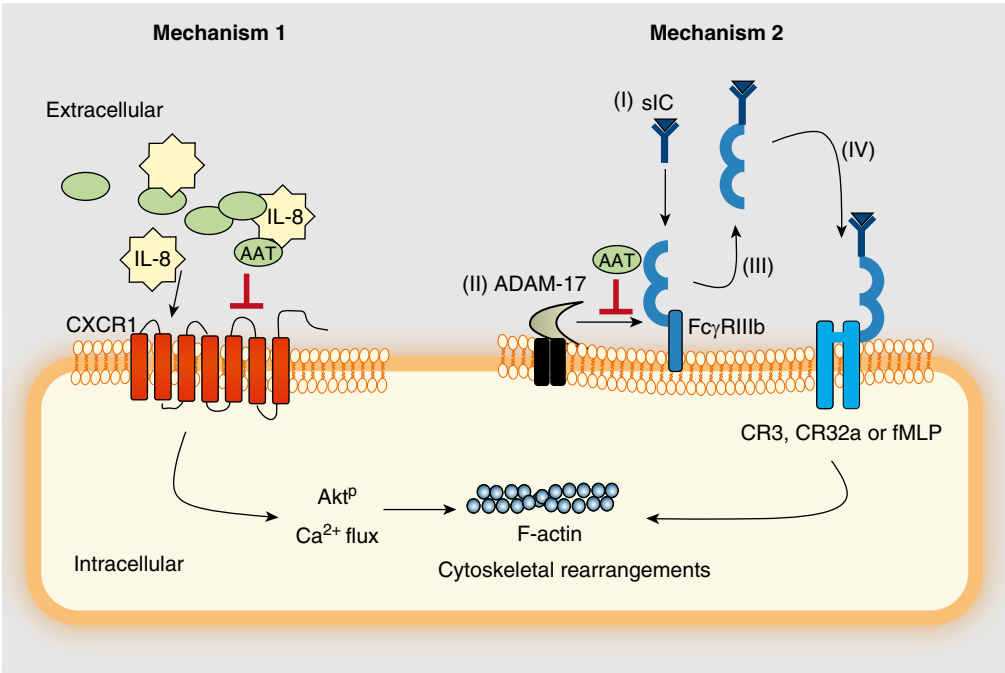

Figure 2. Alpha-1 antitrypsin (AAT) regulates neutrophil chemotaxis in response to IL-8 and soluble immune complex by two distinct mechanisms. *Mechanism 1*: in response to IL-8, cell activation results in Akt phosphorylation (Akt^{P}), calcium (Ca^{2+}) flux, and F-actin formation, required for cell chemotaxis. *In vivo*, the circulating cell is bathed in a high concentration of AAT (27.5 μM). Glycan moieties of AAT can bind IL-8 and modulate chemokine receptor (CXCR) 1 engagement. *Mechanism 2*: (*I*) in response to soluble immune complex (sIC), (*II*) a disintegrin and metalloproteinase-17 (ADAM-17) activity is increased, (*III*) causing release of FcγRIIIb from the cell surface. Possible signaling mechanisms include cross-linking with complement receptor (CR) 3, CD32a, or the *N*-formylmethionyl-leucyl-phenylalanine (fMLP) receptor, (*IV*) causing downstream signaling events. AAT modulates the sIC-induced chemotactic response of neutrophils by inhibiting ADAM-17 activity, resulting in diminished release of FcγRIIIb from the cell membrane.

immune-modulatory effect of AAT on both IL-8– and sIC-induced chemotaxis is greatly reduced. Moreover, the alveolar macrophage also plays a role in the increased neutrophil burden in the AATD lung. NE stimulates the release of leukotriene B_4 (LTB_4), another potent neutrophil chemoattractant from alveolar macrophages (Figure 3). A recent study by O'Dwyer and colleagues (14) demonstrated that the plasma levels of LTB_4 are significantly increased in individuals with ZZ-AATD compared with healthy control subjects. This study also showed that AAT can directly bind LTB_4, abrogating its activities, a mechanism significantly compromised in the setting of AATD (14).

The Effect of Increased Neutrophils in the Lungs of Individuals with AATD

The significant neutrophil burden in the AATD lung contributes to increased proteolytic activity and inflammation (10, 13). The recorded increased number of phagocytes correlates with decreased lung function and with high levels of proinflammatory cytokines, including IL-8, IL-6, and IL-1β (9). Although other proteases released from neutrophils, such as Cath G and PR3, may also be important, their role, as yet, needs further confirmation, and it is generally agreed that NE is the major protease in the proteolytic cascade in the AATD lung. Of major significance, smoking has been confirmed to further exacerbate the imbalance between proteases and antiproteases (15, 16) by rendering AAT inactive (17), and Z-AAT, even in its unpolymerized form, has diminished anti-NE capacity (18). Furthermore, in mice, it has been demonstrated that NE and other neutrophil-derived serine proteases damage lung epithelium and also increase the activity of additional destructive proteases, including matrix metalloproteases 9 (19). In addition, by cleaving complement receptor 3 (20, 21) and chemokine receptor 1 (22) on neutrophils, NE directly impairs the ability of neutrophils to kill bacteria. NE also affects innate immunity by cleaving epithelial cell surface receptors, including T cell Ig and mucin domain-containing molecule-3, involved in the innate immune response to infection (23, 24), and humoral immunity by cleaving Igs (25). NE also cleaves signaling cytokines, including the IFN-γ–inducing factor, IL-18 (26), thus potentiating the airway inflammatory milieu. Furthermore, NE is a secretagogue and promotes increased mucus secretion (27), increased mucus exocytosis (28), and mucus cell hyperplasia (29). NE has also been shown to induce the expression of MUC5AC (30, 31), while also interrupting mucociliary clearance by decreasing the ciliary beat frequency of bronchial epithelial cells, hence adding to mucus burden in the lung (32). Furthermore, NE interacts with other lung cells, such as macrophages, to activate and induce other damaging proteases, such as cysteinyl cathepsins and metalloproteases (33). These proteases have been shown to degrade locally produced antimicrobials in the lung, such as defensins,

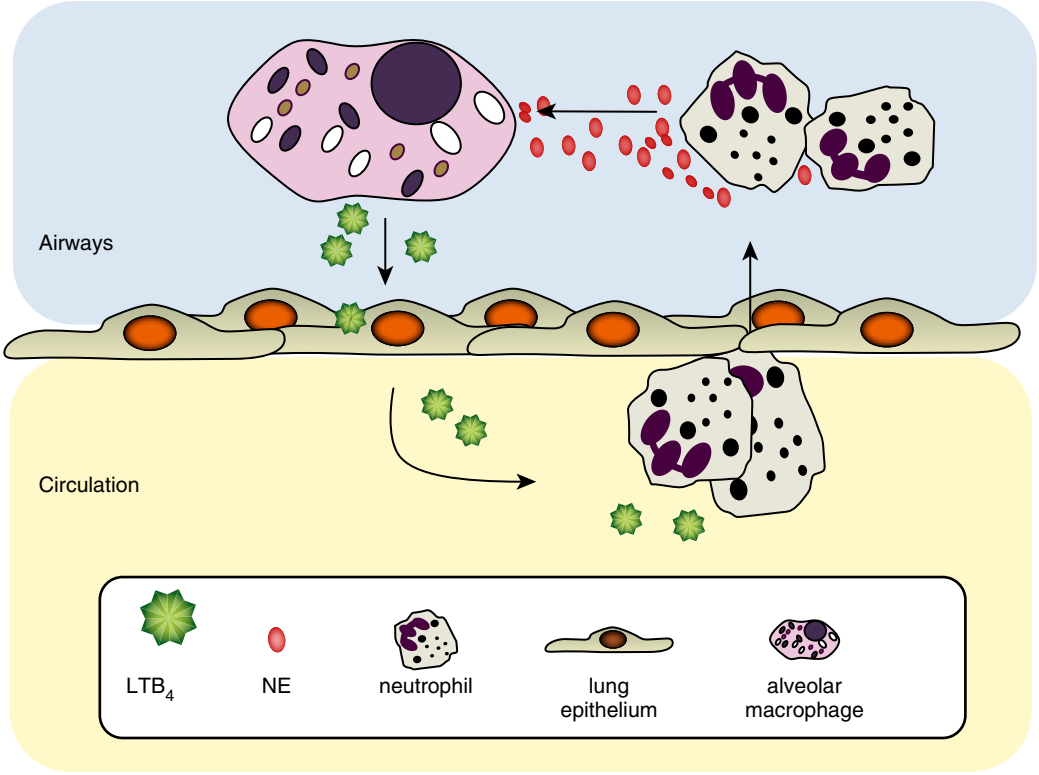

Figure 3. Leukotriene (LT) B_4 cycle of inflammation. In alpha-1 antitrypsin (AAT) deficiency (AATD), the lack of antiprotease protection results in increased levels of uninhibited neutrophil elastase (NE) activity. This free NE can bind to macrophages and stimulate the cell to release LTB_4, drawing further neutrophils in to the airways and compounding tissue damage.

secretory leukoprotease inhibitor, and lactoferrin, all of which have significant antimicrobial and antiinflammatory effects (34–37). NE can also induce cytokine expression in bronchial epithelial cells, subsequently leading to neutrophil chemotaxis and increasing the inflammatory burden in the lung (12, 38).

Contrary to what one would expect, the increased neutrophil number in the AATD lung does not correlate with an increased ability to protect the lung against bacteria. The AATD neutrophil has an impaired ability to kill bacteria, such as *Pseudomonas aeruginosa* (39), and exhibits reduced production of reactive oxygen species (ROS) (40). Neutrophils normally produce ROS via the nicotinamide adenine dinucleotide phosphate oxidase pathway, which is essential for the killing of bacteria and fungi in phagocytic vacuoles. However, if there is excessive, uncontrolled release of ROS into the extracellular space, this can lead to damage of the lung parenchyma. It has been demonstrated that AAT can modulate the levels of ROS released by neutrophils by acting as a direct ROS scavenger (17), by acting on components of the nicotinamide adenine dinucleotide phosphate oxidase complex (41), or through the inhibition of membrane-bound proteases involved in ROS production (42). This function of AAT further highlights the integral role that AAT plays in neutrophil function, and the potential effect a lack of AAT has upon neutrophil dysfunction leading to an inflammatory state in individuals with AATD. Finally, the AATD neutrophil is also dysfunctional, due to a gain of function, whereby the misfolded Z-AAT protein accumulates in the ER of neutrophils, leading to ER stress and accelerated neutrophil apoptosis, which may, in turn, contribute to increased susceptibility to infection (39).

Functional Abnormalities of the Neutrophil and Systemic Interactions

Until recently, much of the work on the neutrophil in AATD has focused on its role in lung inflammation. More recent data have emerged to show systemic abnormalities in the AATD neutrophil, which contribute to the pathogenesis of this condition. One of the most striking examples of neutrophilic inflammation outside the lungs in AATD is panniculitis. This condition is rare, estimated to occur in 1 in 1,000 individuals with PiZZ AATD, and is characterized by painful inflammation of the subcutaneous fat (panniculus adiposus). It can present rapidly and cause significant morbidity (43). Skin biopsy of these lesions demonstrates the classic features of neutrophilic inflammation: foamy macrophages and fat necrosis. Panniculitis in the setting of AATD responds rapidly and effectively to systemic administration of high-dose plasma–purified AAT, suggesting a role for proteases in its pathogenesis (44). Similarly, there is an association between AATD and vasculitis, in particular anti-PR3 or cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCAs)–positive antibody–associated vasculitis. The prevalence of a Z allele in these groups has been reported as being three to nine times higher than in healthy individuals (45, 46). More recent genome-wide association studies of granulomatosis with polyangiitis (formerly known as Wegener's granulomatosis) have identified a genetic link between PR3-ANCA–associated vasculitis and serpin peptidase inhibitor, clade A (alpha-1 antiprotease, antitrypsin), member 1 (SERPINA1) for the Z allele. The mechanism for the development of anti-PR3 antibodies in patients with abnormal AAT phenotypes is not clear, but may relate to the increased propensity of unbound and uninhibited PR3 to stimulate autoantibody production (45). In an experimental model, exogenous AAT has been shown to prevent ANCA from interacting with the PR3 on neutrophils and abrogating the usual resultant oxidant burst, a potential model for treatment of the condition (47). In line with this concept, increased levels of neutrophil-derived lactoferin have been reported in ZZ-AATD plasma, with 27% of patients recruited to the study testing positive for anti-lactoferrin IgG (48). Of major importance, individuals receiving AAT augmentation therapy for 4 years illustrated a significant decrease in titer of autoantibodies directed against lactoferrin (48).

The abnormal systemic response of the AATD neutrophil could be due to both gain of function (as noted with ER stress leading to apoptosis) or loss of function leading to unopposed systemic inflammation, and not just in the lung as previously believed. One of the major systemic modulators of systemic and local inflammation is TNF-α. AAT has a major role in managing TNF-α expression and activity. TNF-α self-regulates its own gene expression (49), as well as modulating the expression of other important inflammatory cytokines (50, 51). AAT prevents TNF-α from interacting with its receptor, and thus can down-regulate TNF-α gene expression in response to exogenous TNF-α, and block nuclear factor of kappa light polypeptide gene enhancer in B cell inhibitor α (IκBα) degradation in neutrophils, hence inhibiting NF-κB signaling (48). In AATD, this mechanism is significantly reduced, with neutrophils isolated from people with AATD showing increased levels of IκBα compared with healthy control subjects. In addition, in AATD, the lack of AAT controlling TNF-α biosynthesis results in increased TNF-α signaling and excessive neutrophil degranulation. This is evidenced by the high levels of soluble TNFR1 in AATD plasma and the increased expression of TNF-α on the membrane of AATD neutrophils (48). In addition, secondary to increased neutrophil degranulation in AATD, there are elevated plasma levels of neutrophilic granule contents, which leads to the development of autoantibodies, specifically against lactoferrin, an important antibacterial peptide involved in the innate immune response (48). Anti-lactoferrin antibodies are associated with increased ROS and disease activity in inflammatory diseases, such as rheumatoid arthritis (52), systemic lupus erythematous (53), and inflammatory bowel disease (54). These elevated autoantibodies in AATD are indicative of a severe inflammatory phenotype, and highlight the contribution of the neutrophil to the inflammatory burden in AATD (Figure 4).

The Effect of Alpha-1 Antitrypsin Augmentation Therapy on Neutrophil Dysfunction in AATD

In AATD, the logical treatment approach is to augment the low circulating and pulmonary AAT levels. Classically, AAT augmentation therapy involves administration of plasma-purified human AAT intravenously once weekly at a dose of 60 mg/kg body weight. This has been shown to be safe and well tolerated (55, 56), and results in increased levels of AAT in the serum and bronchoalveolar lavage fluid of individuals with AATD (57, 58). Several

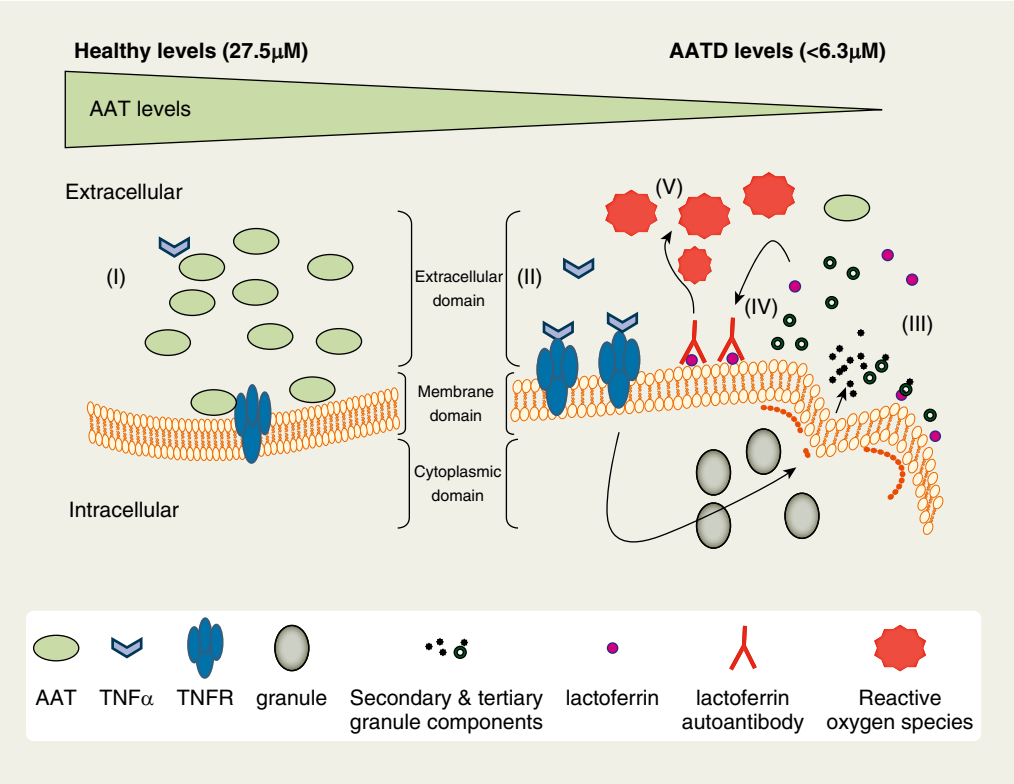

Figure 4. Alpha-1 antitrypsin (AAT) modulates TNF-α signaling in neutrophils. (*I*) Physiological serum levels of AAT (27.5 μM) controls TNF-α biosynthesis and signaling in circulating neutrophils. In ZZ-AATD, the low level of AAT results in increased TNF-α signaling (*II*) and increased degranulation of secondary and tertiary granules (*III*). The described excessive degranulation can lead to development of autoantibodies against released granule proteins, including lactoferrin (*IV*). These autoantibodies can lead to activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase of neutrophils in ZZ-AATD, resulting in production of reactive oxygen species (ROS) (*V*). AAT augmentation therapy restores the concentration of AAT in the circulation to healthy control levels, thereby modulating TNF-α signaling, preventing neutrophil degranulation and averting an autoimmune response.

studies have demonstrated an effect upon lung function; but usually only in subgroups with pulmonary function within certain parameters. More recently, studies seeking to show clinical efficacy of AAT augmentation therapy have focused on demonstrating decreased loss of lung density, as measured by computed tomography (59–61). The recent, large, randomized, placebo-controlled trial of augmentation therapy in alpha-1 proteinase inhibitor deficiency (RAPID) trial, and RAPID extension study, has the most impressive results to date, demonstrating that 60 mg/kg of plasma-purified AAT is effective in slowing the loss of lung density, as measured by high-resolution computed tomography thorax densitometry (62).

Only a small number of studies have investigated the effects of AAT augmentation therapy upon systemic markers of inflammation (63, 64). Within the circulation, AAT can bind IL-8, thereby inhibiting neutrophil chemotaxis. The ability of AAT to directly bind IL-8 is charge related and glycosylation dependent, which has implications for augmentation therapy. As previously discussed, AAT can also modulate neutrophil chemotaxis in response to sIC by inhibiting ADAM17 activity. Accordingly, neutrophils isolated from clinically stable patients with AATD are characterized by increased membrane ADAM17 levels (39), low membrane expression of FcγRIIIb, and increased chemotaxis in response to sIC. In individuals with AATD receiving augmentation therapy, there are increased plasma levels of AAT, and this functions to bind FcγRIIIb-expressing neutrophils, thereby decreasing the neutrophil chemotactic response in AATD to that of healthy control levels (13) (Figure 2). These findings highlight the systemic effect of AAT on circulating neutrophils in AATD. Moreover, we have previously shown that AAT coordinates TNF-α intracellular signaling and neutrophil degranulation of tertiary and secondary granules via modulation of ligand–receptor interactions. Treatment of individuals with ZZ-AATD with AAT augmentation therapy results in decreased neutrophil membrane TNF-α expression and decreased plasma levels of granule antigenic proteins and IgG class autoantibodies. These results demonstrate a mechanism by which AAT augmentation therapy affects TNF-α signaling in the circulating neutrophil, indicating promising potential of this therapy for other TNF-α–related diseases. The inhibition of TNF-α by AAT can also have intracellular effects on the neutrophil, whereby AAT augmentation therapy, by decreasing the TNF-α effect on neutrophils, reduces caspase-8 and associated caspase-3 cleavage, thereby normalizing neutrophil apoptosis (39).

A recently published study demonstrated that AAT can directly bind LTB_4, abrogating the proinflammatory effects of this lipid mediator (14). The ability of AAT to bind LTB_4 involved the

hydrophobic region located between strand 2 of β-sheet A and helices D and E of the molecule. Upon exposure to LTB_4, neutrophils from individuals with AATD released significantly higher levels of primary, secondary, and tertiary granule components due to a lack of AAT. Moreover, ZZ neutrophils exposed to AAT in *in vitro* experiments, or isolated from patients after AAT augmentation therapy, released significantly lower levels of granule enzymes in response to LTB_4 (14). Furthermore, by inhibiting NE, AAT reduced the NE-mediated release of LTB_4 by both macrophages (8) and neutrophils (14), thus impacting upon the levels of LTB_4 in the airways of individuals with AATD (64).

Although most of these antiinflammatory effects of AAT have been demonstrated after systemic administration, it is also possible that aerosol delivery of AAT directly to the lungs may have similar beneficial effects. A number of studies have shown that aerosolized AAT, either recombinant or plasma purified, has the ability to inhibit NE on the epithelial surface of the lung and restore antiprotease inhibitory capacity (65). Furthermore, aerosolization of AAT to people with AATD, by inhibiting NE, can have downstream effects on the induction and activation of other proteases, such as cysteinyl cathepsins and metalloproteases (33). However, limitations to AAT replacement therapy may exist with regards to targeting the proinflammatory and chemotactic effects of Z-AAT polymers present in epithelial cells of the alveoli and airways (66), thereby endorsing the therapeutic use of antiinflammatory cotreatment (phosphodiesterase inhibitors plus histone deacetylase-2 activators) with chaperon molecules capable of depolymerizing the abnormally folded Z-AAT protein (67). However, further research in this field is required, and a mechanism for the effective deposition of functional concentrations of chaperon peptide or nonpeptide compounds (68) has yet to be determined. With regards to AAT therapy, in a number of studies in patients with cystic fibrosis, aerosolization of AAT inhibits NE, decreases IL-8 and neutrophil numbers, and also restores the ability of neutrophils to kill bacteria by preventing NE-induced cleavage of receptors on neutrophils (22). This has implications for AATD. The effects of AAT on TNF-α activity in the circulation may also be pertinent for the lungs as TNF-α levels have been shown to be elevated in inflamed lungs (69, 70). This concept is supported by a study that demonstrated that aerosolized AAT decreases NE activity, neutrophil numbers, bacterial load, and levels of inflammatory cytokines, including TNF-α, in cystic fibrosis airways (71).

Conclusions

The lung disease in AATD is characterized by neutrophillic inflammation and increased proteolytic activity (72), predominantly through the unopposed activity of serine proteases released from neutrophil granules, specifically NE, but also including Cath G and PR3 (73) and other nonneutrophil-derived proteases. AAT is an effective antiprotease, but also possesses a number of important antiinflammatory and immune-modulating properties, which are of pivotal importance in understanding the pathogenesis of the inflammatory lung disease in AATD and further systemic manifestations. The role of AAT in modulating neutrophil chemotaxis and degranulation points to a systemic effect, as does its ability to decrease autoantibody production. This has significant implications for our understanding of AATD moving us away from a compartmentalized version of the protease–antiprotease imbalance to a broader understanding of an ongoing interaction between loss of function and gain of function. This concept perhaps is best exemplified by the AATD neutrophil with excess Z-AAT in its ER, exhibiting ER stress while, at the same time, being exposed to an unopposed TNF-α–mediated inflammation, ultimately leading to premature apoptosis. Finally, it should be noted that many of these antiinflammatory effects of AAT operate through different pathways, some through hydrophobic interactions between AAT and the signaling molecule, and others through electrostatic bonds, through direct AAT binding, and also through binding of AAT to related receptors. This exciting antiinflammatory repertoire lends itself to pharmacological manipulation, which should result in more specific antiinflammatory therapeutic options in the future. ■

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

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