REVIEW

The discovery of α1-antitrypsin and its role in health and disease

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Summary
α1-Antitrypsin (AAT) is the archetype member of the serine protease inhibitor (SERPIN ) supergene family. The AAT deficiency is most often associated with the Z mutation, which results in abnormal Z AAT folding in the endoplasmic reticulum of hepatocytes during biogenesis. This causes intra-cellular retention of the AAT protein rather than efficient secretion with consequent deficiency of circulating AAT. The reduced serum levels of AAT contribute to the development of chronic obstructive pulmonary disease (COPD) and the accumulation of abnormally folded AAT protein increases risk for liver diseases. In this review we show that with the discovery of AAT deficiency in the early 60s as a genetically determined predisposition to the development of early-onset emphysema, intensive investigations of enzymatic mechanisms that produce lung destruction in COPD were pursued. To date, the role of AAT in other than lung and liver diseases has not been extensively examined. Current findings provide new evidence that, in addition to protease inhibition, AAT expresses anti-inflammatory, immunomodulatory and antimicrobial properties, and highlight the importance of this protein in health and diseases. In this review co-occurrence of several diseases with AAT deficiency is discussed.

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1-Antitrypsin (AAT)

The protease inhibitor activity of human plasma was first discovered by Fermi and Pernossi in 1894. However, the isolation and characterization of individual proteins have only emerged much later with the availability of new techniques. Ultimately, the so-called serum trypsin inhibitor responsible for antiprotease activity was first isolated in 1955 by Schultze and named $\alpha_1$-antitrypsin because of its occurrence in the $\alpha_1$-globulin fraction and its ability to inhibit trypsin.

$\alpha_1$-Antitrypsin (AAT), also referred to as alpha$_1$-proteinase inhibitor or SERPIN1, and is the prototypical member of the SERPIN (an acronym for serine proteinase inhibitor) family of protease inhibitors. The term SERPIN was introduced as by Carrell and Travis in 1985 to describe a superfamily of serine protease inhibitors of mammalian plasma. Serpin-like genes have subsequently been identified in animals, poxviruses, plants, bacteria and archaea, and over 1500 members of this family have been described to date.

AAT synthesis and regulation

AAT is mainly produced (70–80%) by liver cells, but it is also synthesized by monocytes, macrophages, pulmonary alveolar cells, and by intestinal and corneal epithelium. De novo synthesis of AAT has also been demonstrated in human cancer cells.

The normal plasma concentration of AAT ranging from 0.9 to 1.75 g/L and the protein is cleared with a half-life of 3–5 days. AAT is also present in saliva, tears, milk, semen, urine and bile. The distribution of the protein is not uniform: for example, in the epithelial lining fluid of the lower respiratory tract its concentration is suggested to be about 10% of plasma levels. As an acute-phase reactant, plasma AAT levels increase rapidly (3–4 fold) in response to inflammation or infection. The concentration of AAT in plasma also increases during oral contraceptive therapy and pregnancy. During an inflammatory response, tissue concentrations of AAT may increase as much as 11-fold as a result of local synthesis by resident or invading inflammatory cells. For example, human alveolar macrophages can contribute to tissue AAT levels in response to inflammatory cytokines (IL-6, IL-1 and TNF$\alpha$) and endotoxins. The expression of AAT by alpha and delta cells of human islets and intestinal epithelial cells is also enhanced by cytokines. AAT synthesis by corneal epithelium, on the other hand, appears to be under the influence of retinol, IL-2, fibroblast growth factor-2, and insulin-like growth factor-1. Interestingly, AAT expression also shows some degree of substrate and/or auto-regulation with enhanced synthesis following exposure to elastase alone or as a complex of AAT.

Protease inhibitory activity of AAT

Until recently it was thought that inhibition of neutrophil elastase and proteinase 3 (PR3) is the primary function of AAT. Current studies demonstrate that AAT is an irreversible inhibitor for kallikreins and $\alpha_1$-proteinase, and that AAT also inhibits intra-cellular and cell-surface proteases. Matriptase, a cell-surface serine protease involved in the activation of epithelial sodium channels, is one such protease. Findings that AAT inactivates the catalytic domain of matriptase in vitro and inhibits epithelial sodium transport both in vitro and in vivo support the notion that the inhibition of matriptase by AAT may offer a pharmacologic target for improving mucociliary clearance in both COPD and cystic fibrosis. Several studies have shown that AAT inhibits the activity of caspase-3, an intra-cellular cysteine protease which plays an essential role in cell apoptosis. A recent study by Bergin and collaborators, provides new evidence that AAT modulates neutrophil chemotaxis in response to soluble immune complexes by inhibiting ADAM-17 activity, also called TACE (tumor necrosis factor-$\alpha$-converting enzyme).

Other biological activities of AAT

AAT has been reported to play an immunoregulatory role, to inhibit neutrophil superoxide production, to enhance insulin-induced mitogenesis in various cell lines, and to induce a IL-1 receptor antagonist expression. In vivo, AAT has been shown to protect against TNF$\alpha$ or endotoxin-induced lethality. Findings that AAT enhances the
synthesis of both transferrin receptor and ferritin revealed a role of AAT in iron metabolism.\(^1\) AAT has also been found to bind to the secreted enteropathogenic *Escherichia coli* proteins (EspB, EspD), thereby reducing their hemolysis of red blood cells.\(^3\) An interaction between AAT and *Cryptosporidium parvum*,\(^4\) a protozoan parasite, has been shown to inhibit *Cryptosporidium parvum* infection, suggesting a potential role for AAT in cryptosporidiosis. Aerosolized AAT also suppressed bacterial proliferation in a rat model of chronic *Pseudomonas aeruginosa* lung infection and in patients with cystic fibrosis.\(^5\) It has recently been demonstrated that a specific 20-residue fragment of AAT (C-terminal peptide, residues 377–396, referred to as VIRIP) binds to the gp41 fusion peptide of HIV-1 and prevents the virus from entering target cells, thereby inhibiting HIV-1 infection.\(^4\) These findings suggest that AAT may play a protective role in HIV-1-infected individuals.\(^4\)

In vitro, AAT was demonstrated to inhibit endotoxin-stimulated TNF-\(\alpha\) and to enhance IL-10 expression in human monocytes, mediated by signaling through the cAMP-dependent protein kinase A pathway which is targeted by a number of anti-inflammatory drugs used and/or currently under development for COPD.\(^44\) AAT also expresses a broad anti-inflammatory profile in gene expression studies on primary human lung microvascular endothelial cells, including the suppression of self-induced TNF-\(\alpha\) expression.\(^45\) Animal studies provide further evidence that AAT therapy prolongs islet graft survival in transplanted allogeneic diabetic mice.\(^46,47\) Current findings show that AAT stimulates insulin secretion and protects \(\beta\)-cells against cytokine-induced apoptosis, and these effects of AAT also seem to be mediated through the cAMP pathway. In view of these novel findings, it is suggested that AAT may represent an anti-inflammatory compound to protect \(\beta\)-cells under immunological attack in type 1 diabetes and also therapeutic strategy to potentiate insulin secretion in type 2 diabetes.\(^48\)

Interestingly, complexes between AAT and other proteins have been associated with specific diseases. In sera from patients with myeloma, Bence-Jones proteinemia complexes between AAT and the kappa light chain of immunoglobulins were detected.\(^49\) In plasma from diabetic subjects, complexes of AAT with factor Xla and with heat shock protein-70 (HSP70), as well as glycosylated forms of AAT were found.\(^50,51\) Moreover, complexes between immunoglobulin A and AAT have been detected in the sera and synovial fluid of patients with rheumatoid arthritis, systemic lupus erythematosus and ankylosing spondylitis.\(^52\) Human tissue kallikrein 3, known as a prostate-specific antigen which correlates with prostate hypertrophy and malignancy, is also found to bind to AAT in sera of subjects with high prostate-specific antigen concentrations.\(^53\) Localization of AAT and AAT-low-density-lipoprotein complexes in atherosclerotic lesions, and enhanced degradation of AAT-low-density-lipoprotein by macrophages suggested the involvement of the complex in atherogenesis.\(^54\) So far, the factors driving assembly of AAT with other proteins and the biological roles of such complexes warrant further investigations.

**Discovery of \(\alpha\)1-antitrypsin deficiency (AATD)**

In 1952, C-B Laurell at Malmö University Hospital made outstanding contributions to protein research by introducing plasma protein electrophoresis as a tool for clinical investigations. By using electrophoresis, clinicians in respiratory medicine in Malmö unexpectedly noted the absence of the alpha1 band in two patients, both of whom suffered from severe respiratory insufficiency caused by emphysema. This observation, published in 1963 by C-B Laurell and S Eriksson, established that AAT deficiency (AATD) is inherited and linked to emphysema. In the following years, several research groups in Europe and the USA demonstrated that AAT is an effective inhibitor of neutrophil elastase, thereby implicating elastase in the pathogenesis of emphysema. Based on these developments, it was generally accepted that the protease-antiprotease balance is an essential requirement for respiratory health.\(^55\) Later, in a classical paper from 1978 by Larsson and collaborators, it was convincingly demonstrated that the age of onset of emphysema in AATD persons who were also current or ex-smokers was very rapidly reduced.\(^56\)

Since AAT is produced mainly in the liver, investigators in Malmö expected to find abnormal liver function in AATD patients. Eriksson wrote that in 1962 they had seen a patient with cryptogenic, decompensated cirrhosis who also lacked the AAT band in the electrophoresis strip. Unfortunately, the finding was misinterpreted in the belief that the patient had developed an acquired AATD secondary to a severely impaired liver function.\(^56\) The association between liver diseases and inherited AATD was documented in the USA by Sharp and colleagues in 1969.\(^57\) These observations were explained in the 1990s when Lomas and his group in Cambridge showed that the mutant AAT in deficient patients forms intra-hepatic polymers due to the insertion of the reactive center loop of one molecule into the opened B-sheet of the next.\(^58\)

The detection of AAT variants became the greatest challenge from 1963 to 1978. The Pi-system was developed, based on the migration of the AAT variants in an electric field. The position of the migrated proteins is identified by a letter, where M is normal, while the positions of the slower-moving proteins are marked by letters before M in the alphabet and these of the faster-moving proteins are denoted by letters after M. Soon it became apparent that the underlying abnormalities of the AAT variants were due to single amino acid substitutions. By 1975, Carrell and co-workers were able to show that the S variant is due to the substitution of a glutamic acid by a valine at position 264 in AAT.\(^59\) Soon after this, the abnormality of Z AAT (glutamic acid substitution by lysine) was explained by Jeppson in Malmö.\(^60\) Moreover, it was shown that the mutation in Z AAT does not affect protein synthesis, but causes a perturbation of structure that results in defective secretion of the protein.\(^61,62\) Thus, cases of inherited AATD traceable to genetic mutations firmly demonstrated the clinical importance of AAT in determining susceptibility to chronic inflammatory conditions, including COPD and liver diseases (Fig. 1).

**Prevalence of AATD**

The highest prevalence of the Z deficiency variant of AAT is recorded in Northwest European countries and North America\(^63\) (Table 1). The Swedish neonatal screening performed in 1972–74 greatly contributed to our knowledge of AATD epidemiology,\(^64\) and permitted determination of a Z
gene frequency of 0.026. Another survey in Denmark yielded a Z frequency of 0.049. The prevalence of the Z AAT genotype decreases throughout Europe along a north-west→south-east gradient. The distribution of the S variant of AAT differs markedly from that of Z, the highest frequency being reported in Southern Europe, and peaking in the Iberian Peninsula (frequency > 0.1400). On the other hand, these mutant gene frequencies are very low in the Far East (0.00002 in Japan, 0 in China, and 0.0061 in South Korea). The frequencies of Z and S AAT variants in North America, Australia and New Zealand are very similar (0.012 and 0.035, respectively), which is further support for the hypothesis that AATD arose in European population. Some scientists estimate that the Z AAT mutation arose between 7000 and 4000 years ago in Southern Scandinavia, and subsequently was spread to other European countries. In contrast, the high prevalence of the S mutation in the Iberian peninsula indicates that this variant most likely arose in the Portuguese population.

Diagnosis of AATD

To date, approximately 100 different alleles of the AAT gene (SERPINA1; MIM# 107400) have been identified and named according to the Pi classification scheme. Moreover, available AAT variants have been categorized as normal (M), deficient (most common Z and S), dysfunctional or null variants. With regard to AATD, the “null” alleles are associated with no detectable AAT in the serum, while the “deficient” alleles lead to a decrease in plasma AAT. The AAT genes are inherited as co-dominant alleles (products of both
genes can be found in the circulation). Therefore, individuals heterozygous for the Z allele (MZ) have 50–60% while individuals homozygous for the Z allele (ZZ) have only 10–15% of the normal MM AAT value (<0.8 mg/ml or ≤11 μmol/L).  

Guidelines for the diagnostic strategies and management of AATD were published by the ERS/ATS in 2003. In general, measurement of the serum AAT concentration is the first step in the algorithm for diagnosis of AATD. However, AAT is an acute-phase protein and its synthesis may be up-regulated during inflammation. Therefore, it is recommended that C-reactive protein (CRP) levels are determined simultaneously and that AAT concentration results are rejected if CRP levels are abnormal. On the other hand, if the serum concentration of AAT is below normal, characterization of AAT genotype and phenotype is recommended. All newborns with prolonged jaundice or nonspecific signs of liver disease should be screened for AATD. Adults with chronic respiratory diseases, such as COPD, not fully reversible bronchial asthma, bronchiectasis, immotile cilia syndrome, frequent airway infections or indeterminate liver disease, should be tested once in their lives. In addition to the index case, relatives need to be screened in order to identify symptom-free carriers, and, if necessary, to recommend prophylactic measures.

Clinical manifestations of AATD

Lung disease in AATD

It is generally accepted that ZZ AATD comprises about 1–2% of all COPD cases, and that heterozygous MZ and MS have no risk for development of COPD. However, a recent German study using a targeted screening program including 2696 subjects identified 268 (ZZ), 488 (MZ), 97 (MS) and 53 (SZ) with AATD, and 16 with rare genotypes. The data from the study carried out in Denmark showed that the frequency of AATD among COPD patients is 0.8% for ZZ and 2.4% for MZ genotype. In the Lithuanian cohort, consisting of 1167 patients with moderate to severe COPD, it was found that 40 (3.4%) are MZ, 39 (3.3%) MS, 1 (0.1%) SS, 3 (0.3%) SZ and 8 (0.7%) ZZ. Current literature indicates that asthma signs and symptoms are common in AATD with or without COPD and that bronchodilator response is a risk factor for FEV1 decline. The British Registry of patients with AATD reported the presence of asthma in about 11% of cases, and an increased prevalence of the MS (18%) and MZ (7%) genotypes. AATD was found in Puerto Rican children attending an asthma clinic. These findings suggest that not only severe ZZ, but also mild, heterozygous (MS, MZ, SZ) AATD may be a risk factor for developing airways pathology.

Severe pulmonary impairment, manifesting as COPD and panacinar lung emphysema (Fig. 2), may be expected if the AAT serum concentration is below the protective threshold of 35% of the normal mean value (<0.8 mg/ml or ≤11 μmol/L). Pulmonary symptoms, such as cough, sputum expectoration, and dyspnea may occur by the age of 30–40 years. Up to 20% of symptomatic patients develop bronchial hyperreactivity. The course of the disease is progressive and may lead to severe respiratory insufficiency. As in COPD, which is unrelated to AATD, typical findings are repeated acute exacerbations with increase of symptoms and worsening of the general condition. In the majority of cases, the cause of exacerbations is believed to be airway infection. The frequency of exacerbations seems to be associated with the progression of pulmonary emphysema and correlates with the decline of pulmonary function. The disease may progress faster if additional risk factors are present, such as tobacco smoking or (occupational) air pollutants. The mean life expectancy of ZZ AATD patients is 48–52 years for smokers and 60–68 years for nonsmokers. Today it is widely accepted that the most important problem behind AATD-related emphysema is a reduction in anti-elastase defense. Either an increase in elastase burden (which might happen with cigarette smoking or pulmonary infection) or the quantitative deficiency of an inhibitor of neutrophil elastase (inherited AATD) can lead to an elastase/anti-elastase imbalance that accelerates lung tissue breakdown. Most recent experimental data show that cigarette smoke accelerates polymerization of Z AAT by oxidative modification, which in so doing further reduces pulmonary defenses and increases neutrophil influx into the lungs (Fig. 3). These novel findings provide a molecular explanation for the striking observation of premature emphysema in ZZ AATD subjects who smoke and raise the prospect of anti-oxidant therapy in AATD-related COPD.

Liver disease in AATD

Today it is well established that the Z, and also Siliyama (Ser53Phe) and Mmalton (52Phe del) variants of AAT, form polymers and are retained in the endoplasmic reticulum (ER) of hepatocytes. The polymers of Z-mutant AAT can be identified by microscopy in diastase resistant inclusion bodies reacting positively with PAS-staining (Periodic acid-Schiff). This accumulation of abnormally folded AAT protein is thought to be associated with increased risk for liver diseases. Research from several laboratories has identified a possible role for ER stress in Z AAT-induced hepatocyte
The pathogenesis of AATD-related liver disease is based on the aberrant folding and polymerization of AAT protein in the hepatocytes. Polymeric Z AAT is also found in the plasma, bronchoalveolar lavage fluid and in the alveolar walls of Z AATD individuals. Cigarette smoke accelerates polymerization of Z AAT by oxidative modification, and further reduces anti-elastase defense and increases neutrophil influx into the lungs.

In infants liver disease associated with ZZ AATD typically presents as neonatal cholestatic jaundice, which often subsides spontaneously after a few months. However, the outcome in childhood and adolescence varies a lot, ranging from normalization of liver test results to early cirrhosis requiring liver transplantation. Even in children with an apparently favorable outcome, a significant degree of liver fibrosis may be present. It is well known that ZZ AATD individuals, who do not develop liver disease in childhood, have increased risk of liver cirrhosis and hepatocellular carcinoma later in adulthood. Notably, AATD may be a relevant cause for liver cirrhosis, after viral hepatitis, alcohol abuse, and chronic cholangitis. From retrospective studies it is known that up to 25% of those with ZZ AATD may suffer from liver cirrhosis or liver cancer in late adulthood. However, in adults the association between the ZZ genotype and liver disease is still unclear, and the incidence of such an association varies considerably. Cirrhosis in ZZ AATD individuals may become clinically apparent at any age, with the peak incidence typically occurring in elderly never-smokers who have survived without developing severe emphysema. Aside from low plasma AAT levels, laboratory and other clinical features of affected individuals are indistinguishable from those with cirrhosis of any etiology. Therefore, in the absence of specific biomarkers and lack of firm evidence regarding optimal follow-up and preventive strategies, the regular assessment of simple liver function tests is typically recommended in elderly individuals with AATD who lack liver symptoms.

### Disease manifestations other than the lung and liver

Diseases other than lung and liver diseases have been associated with AATD, but their association with AATD is less clear. Indeed, the multiple protective role(s) of AAT described above suggest that the deficiency of that protein may worsen the pathogenesis of other diseases rather than causing them. To date fewer than 50 cases of panniculitis associated with various phenotypes of AATD have been reported. Panniculitis characteristically presents as red, painful nodular lesions and clinical features that distinguish the AATD-associated panniculitis include higher frequency of ulceration, a vigorous neutrophilic response and histological evidence of both necrosis and elastin breakdown. In support of AATD as a contributor to the inflammatory pathogenesis of panniculitis, a few case reports provide evidence that the infusion of purified pooled human AAT induces a rapid clinical resolution of panniculitis.

The putative association between AATD and vasculitis (Wegener’s granulomatosis) is based on the fact that AAT variants occur more frequently among individuals with multisystem vasculitis anti-neutrophil cytoplasmic antibodies (C-ANCA) or anti-protease-3 (PR-3) and glomerulonephritis. Moreover, since AAT plays an important role in inhibiting PR3, it has been suggested that AATD could trigger an autoimmune response due to increased extracellular exposure to PR3. Alternatively, although unproven, it is conceivable that circulating Z AAT polymers could prompt a vascular response.

Several reports have described AATD patients having both liver disease and rheumatoid arthritis and larger studies...
from Canada and Sweden have shown an increased frequency of AATD phenotypes in patients with rheumatoid arthritis compared to population frequencies.\textsuperscript{101,102} In addition, in the setting of AATD-associated chronic liver disease, renal disease is often seen in children and young adults.\textsuperscript{103,104} Numerous reports have also associated AATD and pancreatitis, inflammatory bowel disease, celiac disease, peptic ulcer disease, fibromuscular dysplasia, aneurysms and coronary atherosclerosis. AATD association with psoriasis, chronic urticaria, multiple sclerosis, fibromyalgia, ocular allergy, acute uveitis and other conditions have been reported (Table 2). However, further investigations are needed before these putative associations between AATD and various diseases are confirmed. In general, one can predict that in AATD, the protective effects of AAT protein are much lower and therefore a more aggressive inflammatory course of disease can be seen in AATD carriers.

Finally, recent studies have raised the possibility of an association between AAT and diabetes. In support of this, a few clinical studies demonstrated that plasma AAT levels and activity are lower in diabetic patients than in non-diabetic controls.\textsuperscript{105} It has also been demonstrated that over-expression of AAT significantly reduces insulin and prevents the development of overt hyperglycemia in non-obese diabetic (NOD) mice.\textsuperscript{46} In addition, it has been shown that administration of clinical-grade human AAT prolongs pancreatic islet allograft survival and exhibits cytoprotective effects.\textsuperscript{59} Studies examining the role of AAT in diabetes are an area of intense and ongoing investigation.

Conclusions

Despite tremendous progress made in the field of AATD since its first description by Laurell and Eriksson,\textsuperscript{140} key challenges still remain. Clearly the role of AAT in inflammatory processes in the lung has until recently been over-simplified. Although we believe that the effects of AAT are predominantly anti-inflammatory, the actual impact of AAT on inflammatory disease may depend not only on the relative concentration of native, inhibitory and non-inhibitory, modified forms of AAT, but also on the inflammatory context in which they are expressed. The formation of post-translational modified forms of AAT may lead to a functional deficiency of AAT, even if the individual has a normal level of AAT, and the modified forms of AAT may themselves express biological activities. Studies characterizing the inhibitor-independent activity of AAT and its modified forms are incomplete, particularly with regard to an understanding of the signaling mechanisms involved. Delineation of these will not only help us to further understand the role of AAT in the pathogenesis of inflammatory disease, but may also facilitate the identification of new and effective anti-inflammatory therapies.

Conflict of interest

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1138 S.M. Janciauskiene et al.


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