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The Role of Alpha–1 Antitrypsin in Emphysema

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1. Introduction

Alpha-1 antitrypsin (AT) is a member of the serine proteinase inhibitor (SERPIN) superfamily. It is an acute phase protein produced constitutively, primarily by hepatocytes, and is secreted in to the plasma from where it diffuses into the lung. AT is the most abundant proteinase inhibitor within the lung whose main physiological role is to regulate neutrophil elastase (NE) liberated from activated neutrophils (Brantly et al., 1988a; Lomas and Mahadeva, 2002).

The importance of AT in pulmonary biology was demonstrated by the association between severe plasma deficiency and pulmonary emphysema (Laurell and Eriksson., 1963). These findings in conjunction with Gross et al., 1965 formed the basis of the proteinase-antiproteinase hypothesis for the development of emphysema and other lung diseases. It was subsequently identified that the Z variant is the commonest cause of severe AT deficiency. It results in aggregation of the protein in the hepatocyte (with a predisposition to liver disease) resulting in a secretory defect and deficiency. Initially it was presumed that NE and AT were the most important proteinase and anti-proteinase respectively within the lung, but it is now appreciated that several proteinases and inhibitors exist within the lung and other mechanisms are important e.g. apoptosis, ageing, oxidants. Nevertheless, no other PI and proteinase have been so clearly linked with pulmonary emphysema, thus emphasizing the important role of AT in lung biology. Despite this long association, epidemiological studies suggest that AT deficiency is under-recognized or misdiagnosed (Bull World Health Organ, 1997; ATS-ERS statement, 2003).

1.1 Nomenclature and detection of mutants

The AT protein is an extremely polymorphic molecule; there are over 100 variants of AT resulting from mutations in the *SERPINA1* gene. They are named by the letter of the alphabet according to the migration of the glycosylated form of the protein on isoelectric focusing (IEF). The wildtype protein is therefore termed M-AT as it is associated with normal level of serum AT and it has a medium rate of migration on IEF. Variants that migrate faster than M-AT are classified as A to L or slower than M-AT are classified as N to Z (A being the fastest and Z the slowest) (Brantly et al., 1988a; 1991; Cox et al., 1980; Fagerhol and Laurell, 1967). On the basis of their plasma level and function, the majority of individuals are M homozygotes (M1-5 subtypes).

1.2 Genotyping

Currently, diagnosis of AT deficiency is based on the measurement of AT levels in the serum and/or phenotyping by IEF of the serum within a narrow pH range on the polyacrylamide gel. The latter has been standard practice for many years, but is time consuming, difficult to interpret and limited to a few reference laboratories. Genotyping by real-time PCR and Restriction Fragment-Length Polymorphism PCR (RFLP-PCR) are highly effective, relatively inexpensive and reliable, and as a consequence are now commonly performed. Direct sequencing of coding exons of the gene can also be used as an adjunct in selected cases to clarify genotyping (Zorzetto et al., 2008; Miravitlles et al., 2010).

1.3 Alpha-1-antitrypsin gene expression

The AT gene is located on chromosome 14q31-32.1, and is co-dominantly expressed (Schroeder et al., 1985; Bull World Health Organ, 1997; Brantly et al., 1988). The gene is 12 kb in length and contains seven exons (Ia, b c and II–V) and six introns. Exon I contains the 3′ untranslated promoter sequences: Ia and Ib contains the promoter sequence for macrophage-specific, and Ic for hepatocyte-specific transcription, respectively. The coding regions (Exons II-V) are 1434 base-pairs (bp) in length and the reactive centre is within Exon V (Long et al., 1984). Aside from the promoter elements, there are other regulatory sequences including an enhancer element in the 5' and 3' flanking sequences of exonic regions of the AT gene. A polymorphism in the 3′ flanking region is associated with susceptibility to COPD (Kalsheker et al., 1987; Morgan et al., 1992). A map of single nucleotide polymorphisms (SNPs) in the 5' and 3' flanking regions showed that among the 15 SNPs, five SNPs increased the risk of COPD by 6- to 50-fold (Chappell et al., 2006). These polymorphisms within regulatory sequences are associated with normal basal plasma levels, but can result in reduced levels of AT transcription in response to stimulation *in vitro*, which is postulated to relate to the susceptibility to COPD (Henry et al., 2001; Chappell et al., 2006). However, this has not been proven *in vivo* (Mahadeva et al., 1998; de Faria et al., 2005; Courtney et al., 2006, Brennan, 2007).

1.4 Variants associated with alpha-1 antitrypsin deficiency

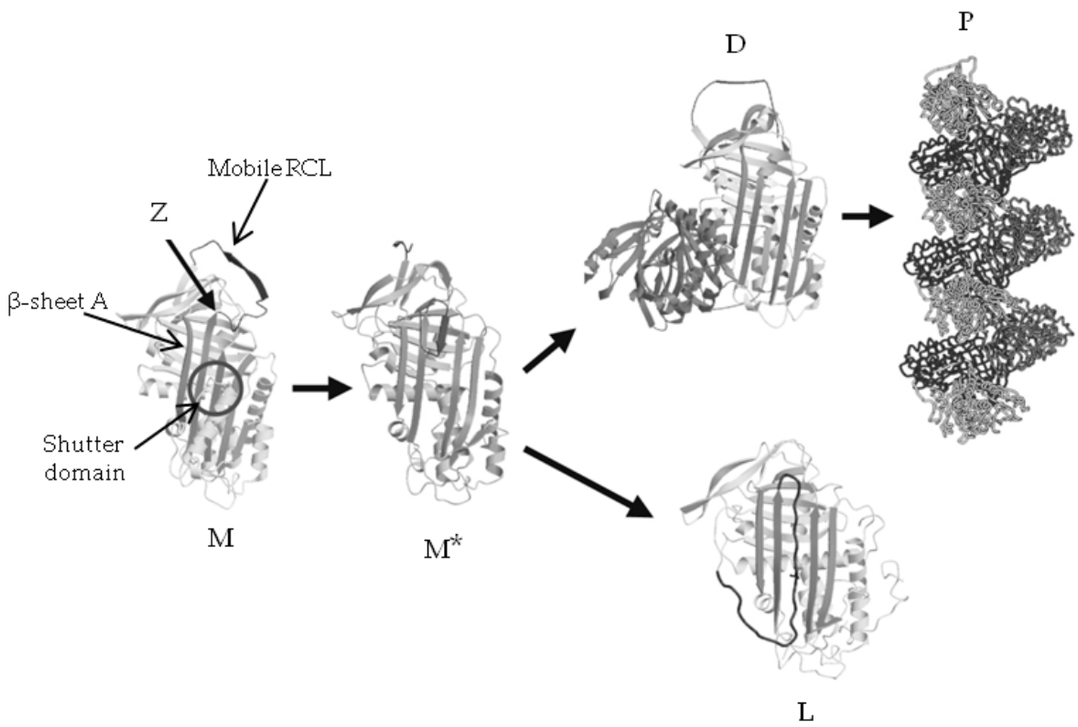
The commonest cause of severe deficiency in Caucasians is Z-AT (Glu342Lys). Four percent of North Europeans carry this variant, and amongst them 1/2000 are PiZ homozygotes (Fagerhol, 1974). The frequencies of PiZ are 1/2700 in USA and 1/5000 in UK (Cook, 1975; de Serres, 2002; Brantly et al., 1988a). The distribution of the genetic types (PI alleles) of AT has been investigated in many populations. Some variants are only common in specific populations; the Z mutant is rare in Asian and African populations, whereas the S (364 Glu-Val) variant is more frequent in the Mediterranean area. The plasma level of the principal AT phenotypes are MM (20-39 μmol/L), MS (19-35 μmol/L), SS (14-20 μmol/L), MZ (13-23 μmol/L), SZ (9-15 μmol/L), ZZ (2-8 μmol/L). About 20 variants are associated with lower but detectable AT in plasma (Table 1). The dysfunctional Pittsburgh (M358R) variant converts AT from an elastase inhibitor to a thrombin inhibitor due to mutation in the active site. The Null (QO) variants occur as a result of insertion or deletion of nucleotides. They are associated with only trace amounts (less than 1%) of AT in plasma and associated with increased risk for emphysema (Bull Health World Organ, 1997; ATS-ERS, 2003).

	Variants	Mutations	Mechanism of deficiency	Clinical disease	References
Severe					
	Z	(E342K)	polymerization in liver cells; reduced inhibitory activity; deficiency	Emphysema Liver disease	Oakeshott et al., 1985; Carell, 1990; Ogushi et al., 1987
	Siiyama	(F53S)	polymerization in liver cells and plasma; deficiency	Emphysema Liver disease	Seyama et al., 1991
	Mmalton	(52Phe deleted)	polymerization in liver cells; deficiency	Emphysema Liver disease	Cox and Billingsley, 1989;Roberts et al., 1984
	Mheerlen	(P369L)	intracellular degradation; deficiency	Emphysema	Kramps et al., 1981
	Mprocida	(L41P)	decreased inhibitory activity; deficiency	Emphysema	Takahashi et al., 1988; Holmes et al., 1990 (a)
	P	(D256V)	intracellular degradation; deficiency	Emphysema	Holmes et al., 1990 (b)
	Pittsburgh	(M358R)	altered substrate specificity	Serous bleeding	Owen et al., 1983
Mild					
	S	(E264V)	incorrect splicing of mRNA; abnormal intracellular degradation; polymerization in liver cells		Elliott et al., 1996a; Schindler, 1984; Engh et al., 1989
	I	(R39C)	polymerization; slightly decreased inhibitory activity; mild deficiency	Emphysema	Mahadeva et al., 1999; Graham et al., 1989; Baur and Bencze, 1987
	$M_{mineral\ Springs}$	(G67E)	aberrant posttranslational biosynthesis; loss of conserved Gly; disturbed packing; degraded in liver cells; decreased inhibitory activity; deficiency	Emphysema	Curiel et al., 1990
	F	(R223C)	polymerization; decreased inhibitory activity		Okayama et al., 1991; Hayes et al., 1992

Table 1. Alpha-1-antitrypsin variants associated with plasma deficiency

1.5 Molecular structure of α_1-antitrypsin

AT consists of three *β*-sheets, eight α-helices, and a reactive centre loop (RCL), which contains the residues that directly interact with the proteinase substrate (Fig. 1). β-Sheet A is composed of five strands spreading along the long axis of the protein: the first strand has 5-6 residues and the other strands have 12-15 residues. In the native conformation, the 17 amino acid RCL locates at an external position in relation to the body of the molecule between the C-terminus of β-sheet A3 and the N-terminus of β-sheet C1. The N-terminal side of the reactive loop including M358 (P1) is directly related to the recognition and binding of the substrate (Fig. 1) (Song et al., 1995; Silverman et al., 2001; Elliott et al., 1996a; Ryu et al., 1996; Kim et al., 2001)



Pathways of polymerization of α1-antitrypsin. The structure of α1-antitrypsin is centred on β-sheet A and the mobile RCL. Polymer formation results from the Z-AT (E342K at P17; Z) or other mutations in the shutter domain, which open β-sheet A to favour partial loop insertion and the formation of an unstable intermediate (M*). The patent β-sheet A can accept either the loop of another molecule, to form a dimer (D), which then extends into polymers (P), or else its own loop, to form a latent conformation (L). The individual molecules of AT within the polymer are shown in different shades of grey. Reproduced from Lomas and Mahadeva, 2002.

Fig. 1. Mechanism of Z α1-antitrypsin polymerization

1.6 Physiology and function of α_1-antitrypsin

AT is a 394 amino acid (52 KDa) glycoprotein produced primarily by hepatocytes. Other cells produce the protein to a lesser extent in peripheral blood monocytes, alveolar macrophages and bronchial epithelial cells and gastrointestinal mucosa. (Brantly et al.,

1988a; Cichy et al., 1997). Daily production of AT is 34 mg/kg. The large amount of AT in the circulation and lung is primarily present to control the activity of elastase in the lung. NE is the main substrate for AT, accordingly it inhibits 90% of the NE in circulation and interstitium of lung. AT also inhibits the serine proteinases cathepsin G and proteinase 3.

1.7 Other biological effects of alpha-antitrypsin

In addition to acting as an antiproteinase, AT plays important role in modulating inflammation. It may inhibit immune responses, and fibroblast-proliferation and fibroblast procollagen production thereby contributing to repair and matrix production (Dabbagh et al., 2001), and have antibacterial activities (Hadzic et al., 2006), and also blocks the cytotoxic and stimulatory activity of defensins (Hiemstra et al., 1998). AT also has direct and indirect anti-apoptotic properties by inhibiting caspase-3 or NE mediated apoptosis, respectively (Petrache et al., 2006). AT is also involved in calcium-induced activation mechanisms; AT inactivates calpain I (μ-calpain), induces a rapid cell polarization and random migration of neutrophils The role of AT in neutrophil regulation was further supported by its ability to transiently increase calcium from intracellular stores, which is linked to neutrophil polarization. AT modulated increase in intracellular lipids, activation of the Rho GTPases, Rac1 and Cdc42, and extracellular signal-regulated kinase (ERK1/2) all these kinases are indeed found to be activated or phosphoryated in polarized neutrophils with significant mobility (Al-Omari et al., 2011). Furthermore, a recent study demonstrated that AT can control immune complex–mediated neutrophil chemotaxis by inhibiting ADAM-17 (TACE) activity and preventing the release of glycosylphosphatidylinositol-linked (GPI-linked) membrane protein, FcγRIIIb, from the cell (Bergin et al., 2010). The same study also demonstrated *in vivo,* that AT is a potent inhibitor of neutrophil chemotaxis in Z-AT individuals compared with M-AT individuals correlating with increased chemotactic responses of both CXCR1 and immune complex receptor (FcγRIIIb) (Bergin et al., 2010).

1.8 Mechanism of proteinase inhibition

The process of inhibition is initiated by the specific binding of the proteinase to the RCL of AT to form a non-covalent Michaelis complex and is one of "suicide substrate inhibition" (Gettins, 2002). The inhibitory mechanism of AT relies upon cleavage of the methionine-serine P1-P1′ by NE (1:1 AT-elastase complex). The protease is then swung 70 Å (1 Å = 0.1 nm) from the upper pole to the lower pole of the protein in association with the insertion of the reactive loop as an extra strand into β-sheet A. The complex inactivates the protease by distortion of the catalytic triad at the active site (Huntington et al., 2000; Wilczynska et al., 1997; Stratikos and Gettins, 1997). The stable complex is subsequently recognized and cleared by the liver. The complexes are short lived (a few hours) in the circulation compared with the native AT (5-6 days), and the low-density lipoprotein receptor related protein (LRP) on liver cells appears to be the principal receptor for clearance of the AT-proteinase complexes (Kounnas et al., 1996).

1.9 Mutations and their effect on conformation of AT

Molecular mobility and the P1 methionine is essential for elastase inhibitory behaviour, but is also its Achilles heel making the molecule vulnerable to the effects of critically situated

point mutations and oxidation (Stein and Carrell, 1995). AT molecules can undergo conformational transitions, which not only inactivate is antiproteinase function, but also confers it with other biological properties.

2 Conformations and their effect on structure and function of α_1-antirypsin

2.1 Conformations of α_1-antirypsin

In vivo AT can exist in different conformational forms; native, oxidized, polymerized, oxidized-polymers, RCL cleaved and latent, and the AT-elastase complex. The conformational changes can be the result of inflammation, such as the cleavage by non-target proteinases and oxidation by reactive oxygen species (ROS). Mutaations e.g Z, S predispose it to polymerization. Whilst these conformations result in a loss of proteinase inhibitory activity, they can have biological effects such as inflammatory cell activation and chemotaxis, cytokine release or apoptosis (Janciauskiene, 2001).

2.2 Oxidized AT

Oxidation of AT is a sulphoxide modification of the methionine residues of AT (Johnson and Travis, 1979; Beatty et al., 1980; Taggart et al., 2000). Methionine can be attacked by various oxidants, such as peroxide, hydroxyl radicals, hypochloride, chloramines and peroxynitrite (Vogt, 1995; Rahman and MacNee, 1996), which are mainly produced by activated inflammatory cells. Oxidized AT (Ox-AT) *in vivo* has been confirmed by the finding that the inactive AT purified from the inflammatory synovial fluid contains methionine sulphoxide residues, and that 41% of total AT in the fluid is inactive, oxidized and/or cleaved (Zhang et al., 1993). Smoking is a major external source of oxidants (Rahman and MacNee, 1996; Church and Pryor, 1985; Schaberg et al., 1992). In smoking-related emphysema, 5-10% of total AT is in the oxidized state (Wong and Travis, 1980). Oxidation of the P1 methionine (M358) significantly reduces the activity of AT against NE to 1/2000 of the normal (Johnson and Travis, 1979). Recent data demonstrates that oxidation of Z-AT promotes polymerization of Z-AT thus increasing the risk of emphysema of Z-AT deficient patients (Alam et al., 2011). Ox-AT has also been shown to stimulate release of MCP-1 and IL-8 from lung epithelial cells (Li et al., 2009) and stimulate monocyte activation, inducing an elevation in MCP-1, IL-6, TNF-α expression and NADPH oxidase activity (Moraga and Janciauskiene., 2004)

2.3 Polymerized form

The Z-variant accumulates in the hepatocyte involving a process of loop-sheet polymerization whereby the RCL of one molecule inserts into β-sheet A of a second and so on to form chains of Z-AT polymers (Lomas et al., 1992; Mahadeva et al., 1999). M-AT has not been found to polymerize *in vivo* (Mahadeva et al., 2005). Polymerization can occur in other variants of AT, such as Siiyama, Mmalton, I, and S (Mahadeva et al., 1999; Janciauskiene et al., 2004; Elliott et al., 199b). I α_1-antitrypsin and S-AT polymerize slower than Z-AT but faster than M-AT, and hence are associated with less severe plasma deficiency (Dafforn et al.,1999).

The occurrence of Z-AT polymerization *in vivo* has been confirmed by the finding of AT polymers in lungs (Elliot et al., 1998b; Mulgrew et al., 2004). Polymers of Z-AT are found in

emphysematous alveolar walls (Mahadeva et al., 2005). Polymers of Z-AT are chemotactic for neutrophils (Parmar et al., 2002; Mulgrew et al., 2004). Polymers of Z-AT are also ineffective anti-inflammatory molecules or inhibitors of NE, (Alam et al., 2011; Bergin et al., 2010; Al-Omari et al., 2011). Recent findings show that ER accumulation of Z-AT polymers is associated with up-regulation of PKR-like ER kinase (PERK), regulator of G-protein signaling (RGS) 16, and calnexin, and NF-κB activation and secretion of inflammatory mediators; IL-6 and IL-8 in keeping with activation of the ER overload response (EOR) linked to excess inflammatory activity of the Z-AT cell (Alam et al., Unpublished observation).

3. Mechanisms of disease and pathology

3.1 Alpha-1-antitrypsin associated diseases

The normal plasma concentration of AT is about 30 μM, providing 24 μM in lung interstitium, which is thought to be critical in inhibiting elastase. It has been calculated that a concentration of 11 μM of plasma AT is the threshold for providing sufficient AT in the lung (Wewers et al., 1987; Stockely, 2003). Hence, although some phenotypes of AT are associated with abnormally low concentrations of AT in the plasma; PiMS: 80% of normal, PiSS: 60%; PiMZ: 57.5%, only PiSZ, 40%, and PiZ:10-15% and Null variants have been linked to the development of lung disease (Brantly et al., 1988a;b). AT deficiency appears to be under-diagnosed in some populations (Bull World Health Organ, 1997; de Serres, 2003) with only a small proportion of those predicted according to allele frequencies to have AT deficiency to have been identified: 4.5% in UK, 6% in Sweden, and 5% in USA (Tobin et al., 1983; Larsson, 1978; Silverman et al., 1989).

3.2 Z α_1-antitrypsin associated lung disease

Cigarette smoking is the most important independent risk factor for the development of emphysema in the Western world. A landmark study (Fletcher et al., 1977) showed that 15 to 25% of smokers with normal AT develop clinically significant COPD, and that the rate of FEV1 (forced expiratory volume in 1 second) decline was around 50 ml/year in smokers compared with 25 ml/year in non-smokers. Among the AT-deficient population, the decline of FEV1 is 70 ml/year in current smokers compared with 41 ml/year in ex-smokers (Piitulainen and Eriksson, 1999). Smokers with severe deficiency of AT develop symptoms of emphysema 10-15 years earlier than those non-smoking individuals and have a higher mortality (Buist et al., 1983; Janus et al., 1985).

Severe AT deficiency usually due to Z-AT accounts for about 2% of cases of emphysema (Morse, 1978), and has also been linked to asthma and bronchiectasis (Parr et al., 2007; Eden et al., 1997; King et al., 1996; Bleumink and Klokke, 1985). Individuals, who have never smoked, rarely develop symptoms before the age of 50. Twenty-40% of patients have chronic bronchitis and bronchiectasis, and about half have exacerbations (Needham and Stockley, 2004). Most PiZ non-index cases have normal or slightly abnormal lung function in the absence of symptoms (Tobin et al., 1983). The development of lung disease is intimately related to cigarette smoking. However, the severity of lung disease can show some variability: lung function is well maintained in some AT-deficient smokers, while can be impaired in non-smokers (Brantly et al., 1988b; Janus et al., 1985). It is also

postulated that host factors, such as individual bronchodilator reversibility, baseline lung function, sex, age, and other unidentified genetic factors as well as other environmental factor such as dust-exposure and recurrent respiratory infections may influence the clinical phenotype (Needham and Stockley, 2004). A recent familial study estimated heritability for FEV_1/forced vital capacity (FVC) in 378 ZZ homozygotes from 167 families identified cigarette smoking as the genetic modifier in the pathogenesis and severity of COPD (DeMeo et al., 2009).

3.3 MZ α_1-antitrypsin associated lung disease

The serum levels of AT in MZ heterozygotes is lower than in MM homozygotes (Section 1.4), but whether MZ individuals have an increased risk of COPD remains controversial. Increased COPD risk in this group may have public health implications because there are about 117 million of MZ and MS phenotypes worldwide (de Serres 2002; Brantly et al., 1991). Many studies have addressed the risk of lung function reduction and increased risk of COPD in MZ heterozygotes, but the results have not been consistent. A meta-analysis demonstrated increased risk of COPD in MZ compared to MM, but there was no difference in mean FEV_1 between MZ and MM individuals when combining the results from population-based studies (Hersh et al., 2004), which is in agreement with a cohort of MZ heterozygotes analyzed from the Danish Alpha-1-Antitrypsin Deficiency Registry (Seersholm et al., 2000) and a longitudinal study of the general population in Arizona (Silva et al., 2003). Many of the previous studies have been limited by small sample sizes, varying phenotype definitions, or failure to adjust for smoking. However, recent studies investigated two large, well characterized populations of current and ex-smokers, a case-control study and a multicenter family-based study using quantitative CT scan measurements of emphysema and airway disease, established an association of reduced FEV_1/FVC in MZ compared to MM (Sandhaus et al., 2008; Sørheim et al., 2010). This suggests that at least some MZ heterozygotes are more susceptible to the development of COPD. Interestingly, MZ with a low smoking history (<20 pack-year) had more severe emphysema on chest CT scan. It remains to be established whether all MZ individuals have an increased risk or whether a subset is more susceptible because of other genetic or environmental factors.

3.4 Mechanism of Z α_1-antitrypsin-related emphysema

The majority of emphysema occurs in cigarette smokers who have normal AT concentrations and smoking can injure the lungs by many mechanisms, such as, A. increasing the oxidant burden (Alam et al., 2011; Church and Pryor, 1985; Carp and Janoff, 1978); B. direct stimulation of neutrophils and macrophages to produce proteinases (Bracke et al., 2005; Hautamaki et al., 1997); C. inactivation of AT and other proteinase inhibitors by oxidation (Alam et al., 2011; Wong and Travis, 1980); D. interfering the repairing process by repeated damage (Janoff et al., 1983). These mechanisms can all occur in Z-AT individuals. However, there are some noteable differences in emphysema due to Z-AT compared to those with normal AT. Firstly, the emphysema has a predilection (although not exclusively) at least initially to affect the lower lobes in Z-AT emphysema compared with the upper lobes in M-AT emphysema. Secondly, the emphysema in Z-AT

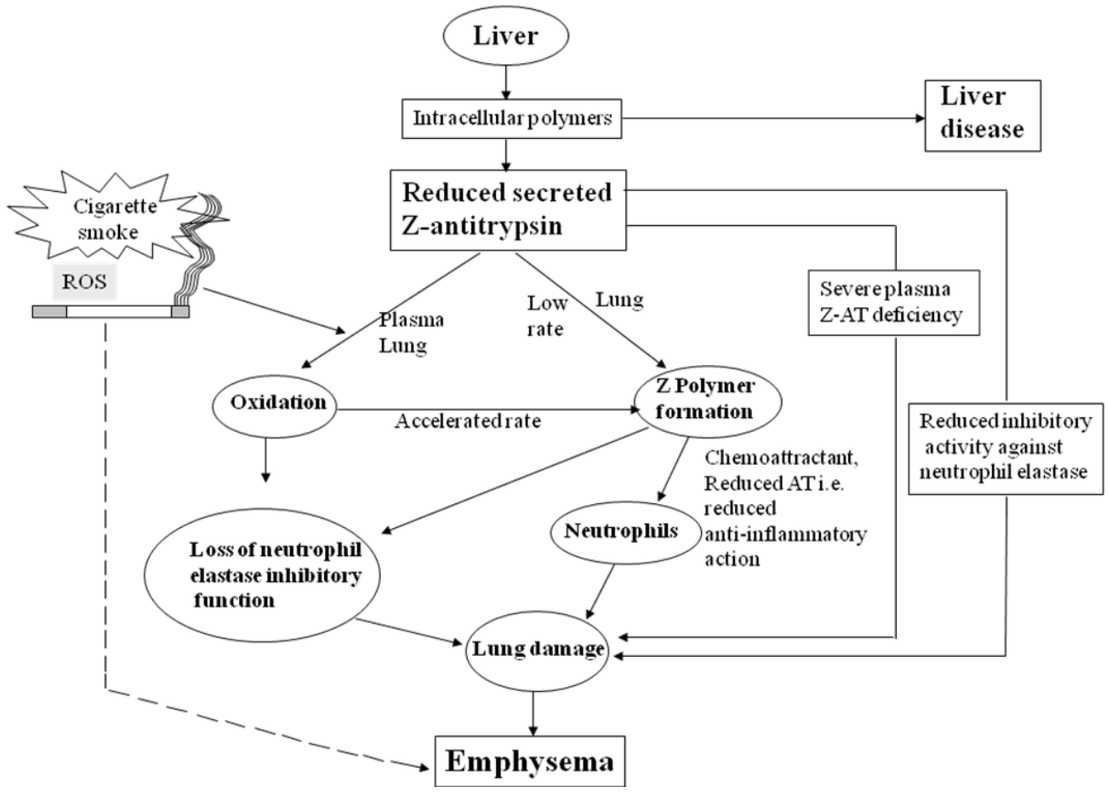
individuals tends to have more polymorpholeucocytes compared with M-AT emphysema (Morrison et al., 1987).

The main mechanism contributing to the development of emphysema in individuals with Z-AT is the imbalance of AT-elastase, in favour of elastase caused by severe AT deficiency. It is now well established that the conformational changes originating from this mutation predispose Z-AT molecules to irreversible polymerization, with consequent accumulation within the ER of hepatocytes (Lomas et al., 1992; Mahadeva et al., 2002). As a consequence, only approximately 15% of the molecules produced reach the circulation. In addition, in the presence of cigarette smoking a major portion of these secreted proteins has been shown to be either oxidized monomeric AT or in its polymeric form (Alam et al., 2011), which are inactive as proteinase inhibitors. Z-AT also has a reduced activity against elastase (Oakeshhott et al., 1985; Lomas et al., 2003). The inactivation of AT as in M-AT related emphysema can also occur by cleavage by non-target proteinases. The end result of these processes is a further reduction in the quantity of functional AT.

Polymeric conformation of Z-AT has also been found in Z-AT emphysematous lungs in association with neutrophils (Mahadeva et al., 2005). Polymers of Z-AT are also thought to contribute the inflammation and lung damage in emphysema. Polymers of Z-AT are thought to be produced locally within the lung, however, a recent study reported finding of polymers of Z-AT not only in the lung, but also in the serum of transgenic mice expressing human Z-AT that had been exposed to cigarette smoke (Alam et al., 2011) (Section 3.4). Formation of Z-AT polymers may be accelerated by local inflammation e.g. bacterial infection. The polymers are themselves chemotactic for human neutrophils *in vitro* and *in vivo* and are co-localized with neutrophils in the alveoli of individuals with Z-AT-related emphysema (Elliott et al., 1998a; Parmar et al., 2002; Mulgrew et al., 2004). The transition of native Z-AT to polymers inactivates its anti-proteinase and anti-inflammatory function, and also converts it to a pro-inflammatory stimulus and may explain the excess numbers of neutrophils in bronchoalveolar lavage fluid (BALF) and lung tissue from Z-AT homozygotes (Morrison et al., 1987; Mahadeva et al., 2005) and in transgenic Z-AT mice (Alam et al., 2011). The presence of polymers may also contribute to the progression of PiZ lung disease after smoking cessation.

3.5 Cigarette smoking and emphysema

Z-AT related emphysema is potentiated by cigarette smoking, characteristically occurring in the third to fourth decade compared with fifth to sixth decade in non-smokers (Luisetti and Seersholme, 2004; Evald et al., 1990). The mechanism of accelerated decline in smokers with Z-AT is in part due to the independent effects of cigarette smoke, but also due to oxidation of Z-AT which promotes polymerization (production of oxidized polymers) of Z-AT (Fig. 2) (Alam et al., 2011). Polymers are inactive as an anti-elastase, and are not only unable to perform their normal anti-inflammatory role, but are also chemotactic for neutrophils (Alam et al., 2011; Mulgrew et al., 2004; Morrison et al., 1987; Parmar et al., 2002; Bergin et al., 2010; Al-Omari et al., 2011). The acceleration of COPD by cigarette smoke in Z-AT individuals exemplifies the critical importance of gene-environmental interactions to the development of COPD. This provides a molecular explanation for the striking association of premature emphysema in ZZ homozygotes who smoke.



Proposed model for the pathogenesis of emphysema in patients with Z-AT deficiency. Cigarette smoke induces oxidation of Z-AT, which accelerates Z-AT polymerization. Plasma deficiency and reduced inhibitory activity of Z-AT would be exacerbated by the oxidation and polymerization of AT within the lungs, thereby further reducing the antiproteinase screen. Conversion from a monomer to a polymer results in a loss of anti-inflammatory effect. Z-AT polymers also act as a pro-inflammatory stimulus to attract and activate neutrophils, resulting in further increase in neutrophils and liberation of NE thereby imbalance of AT-elastase in the favour of NE leading to tissue damage and subsequently causing emphysema. Adapted from Alam et al., 2011.

Fig. 2. Schematic diagram depicting the role of conformations of α1-antitrypsin and the interaction with cigarette smoke in the development of emphysema.

4. Prognosis and review of current treatments

4.1 Treatment of Z α_1-antitrypsin-associated lung disease

The major goals in the management of patients with Z-AT related emphysema are to prevent lung disease, and to reduce progression of the disease. Smoking cessation and standard management for COPD with normal AT levels is of crucial importance once the diagnosis has been made. Repeated respiratory infections can lead to permanent lung injury in patients with Z-AT deficiency. Thus, reducing exacerbation rate is also essential.

Purified plasma AT (half-life of 4.5 day) and recombinant AT (half-life of a few hours) are both commercially available. Currently four different preparations of purified plasma AT are available worldwide; Prolastin®, Zemaira™, Aralast™ and Trypsone® and have been approved for use by the regulatory agencies in several countries (Table 2). The former three preparations are available in the United States at an estimated cost of $60,000 to $150,000 per

year (Gildea et al., 2003). There is no definitive evidence to suggest superiority (specific functional inhibitor activity) of any one of the formulation comparing them to Prolastin. Prolastin was the first approved human purified plasma AT, which is usually administrated intravenously at 60 mg/kg weekly. This dose increased the serum AT level and remained above the putative protective threshold level of 11μM/L after 3 weeks of infusion. However only 2% of the infused purified plasma AT drug reaches to the lung and therefore administration *via* aerosol has also been assessed (Wencker et al., 1998; Hubbard et al., 1989; Smith et al., 1989). Some positive effects of augmentation therapy have been observed in those with moderately impaired lung function (FEV_1 30-65%) (Alpha-1-antitrypsin-Deficiency-Study-Group, 1998; Abusriwil and Stockley, 2006), and some studies have also demonstrated that the treatment reduces airway LTB4, which plays important role in exacerbations (Stockley et al., 2002). However, the therapeutic effect of augmentation therapy is debated due to the lack of a randomized controlled clinical trial (Burrows, 1983; Wewers and Gadek, 1987). There are however problems with conducting such studies: in particular the large numbers of patients with this rare disease required for placebo-controlled and randomized clinical trials; the length of follow-up required to assess efficacy and the limited supply and cost of the treatment (Abusriwil and Stockley, 2006).

Drug	Manufacturer	Method of preparation (viral inactivation)	Minimum specific functional inhibitor activity per mg total protein	Countries approved for use
Prolastin®	Talecris Biotherapeutics, Research Traingle Park, NC	Pasteurization	≥ 0.35 mg	Argentina, Austria, Bahamas, Barbados, Belgium, Bermuda, Canada, Denmark, Finland, Germany, Greece, Guam, Ireland, Italy, Netherlands, Norway, Oman, Poland, Portugal, Puerto Rico, Qatar, Spain, Sweden, Switzerland, US
Zemaira™	CSL Behring, King of Prussia, PA	Pasteurization	≥ 0.7 mg	US
Aralast™ (which was initially called Respitin)	Baxter, Deerfield, IL	Solvent detergent and nanofiltration	≥ 0.55 mg	US
Trypsone®	Grifols, SA.	Solvent detergent and nanofiltration	≥ 0.7 mg	Argentina, Brazil, Chile, Mexico, Spain,

Table 2. Preparations of purified human plasma antitrypsin are available world wide Modrykamien and Stoller, 2009; Stockley et al., 2010; Louie et al., 2005; Barker et al., 1997.

A recent study analyzed results from two randomized, double-blind, placebo-controlled trials to date; a 2-center Danish-Dutch study (n = 54) and the 3-center EXAcerbations and CT scan as Lung Endpoints (EXACTLE) study (n = 65) (Stockley et al., 2010). The study investigated the efficacy of IV AT augmentation therapy on emphysema progression using CT densitometry over an average mean follow-up of about 2.5 years. The study confirmed that IV augmentation therapy significantly reduces the decline in lung density. Decline from baseline to last CT scan was -4.082 g/L versus -6.379 g/L for placebo, with the treatment difference of 2.297 (95% CI, 0.669 to 3.926; p=0.006), the corresponding annual declines were -1.73 and -2.74 g/L/yr, respectively) and may therefore reduce the future risk of mortality in patients with AT deficiency related emphysema, in favour of IV AT augmentation therapy.

There is no evidence that IV augmentation therapy with purified plasma AT preparations is effective in MZ genotypes. MZ patients are at risk for accelerated airflow obstruction/lung disease as mentioned above and that augmentation therapy in MZ patients can be associated with side effects (Stoller et al., 2003/2009). The Medical and Scientific Advisory Committee of the Alpha-1 Foundation (Sandhaus et al., 2008) concluded that augmentation therapy for MZ phenotypes should be avoided.

4.2 Alpha-1 antitrypsin deficiency and Lung volume reduction surgery

Recently lung volume reduction surgery (LVRS) has been proposed as a treatment for severe emphysema. Over the years studies reported both in favour and against LVRS in AT deficient patients (Dauriat et al., 2006; Tutic et al., 2004). Because LVRS offers only short-term benefits for most AT deficient patients LVRS should not be recommended in these patients pending additional studies (ATS–ERS, 2003). This data is further supported by landmark studies from the National Emphysema Treatment Trial (NETT) (Fishman et al 2003; Stoller et al 2007) that included 1218 randomized subjects and 10 who were randomized had severe Z-AT deficiency and underwent LVRS. Deficient individuals had a shorter duration in FEV_1 rise, smaller increase in exercise capacity at 6 months, and higher mortality (20% vs. 0% compared with medical treatment) after 2 years. Although these conclusions are inherently limited by the small number of patients analyzed, LVRS cannot clearly be recommended for this population based on the above data (Stoller et al 2007). In addition, most patients with Z-AT deficiency have lower lobe predominant emphysema, which showed the least surgical benefit in NETT (leading to worse outcomes in good exercise capacity patients) (Stoller et al 2007). Although LVRS has small functional gains and a shorter-lasting effect in AT deficient patients than in patients with normal AT emphysema, it could potentially serve as a bridging procedure that postpones the need for lung transplantation (Dauriat et al., 2006; Tutic et al., 2004). Emerging techniques for bronchoscopic lung volume reduction are covered in another chapter.

4.3 Alpha-1 antitrypsin deficiency and Lung transplantation

End stage pulmonary emphysema is the most common indication for lung transplantation worldwide. Lung transplantation is considered in patients with declining lung function or symptomatic patients with a poor quality of life after receiving all conservative treatment options including smoking cessation and rehabilitation programmes. A functional

improvement and better quality of life are clear benefits deriving from lung transplantation, while a survival advantage has not yet been proven (Marulli and Rea, 2008). Studies have shown advantage of single versus double lung transplantation for COPD or AT deficiency. However, a common cause of death PiZ post transplantation was due to pulmonary infection and bronchiolitis obliterans syndrome (BOS), and sepsis in the presence of excess NE (Meyer et al., 2001; Tanash et al., 2011; de Perrot et al., 2004). A recent study analyzed a total of 83 PiZZ patients with severe emphysema who underwent lung transplantation between 1990 and June 2010 compared to 70 age, gender, smoking history and lung function matched controls (Tanash et al., 2011). Of 83 transplanted patients, 62 (75%) underwent single-lung transplantation. During follow-up, 37 (45%) deaths occurred in transplanted patients and 45 (64%) in the non transplanted patients. In the transplanted patients, the estimated median survival time was 11 years (95% confidence interval [CI] 9 to 14 years), compared with 8 years (95% CI 4 to 6 years) for the non transplanted patients ($p = 0.006$) (Tanash et al., 2011). Constant annual death rates due to BOS and other complications result in a 50% 5-year survival (Patterson and Cooper, 1995). In addition, Mal and colleagues (Mal et al., 2004) have shown an association between cigarette smoking induced NE activity and recurrence of pulmonary emphysema in the transplanted lung of a 49 year old PiZ patient 11 years after receiving single lung transplant. Therefore, lung transplantation should only be offered to selected candidates.

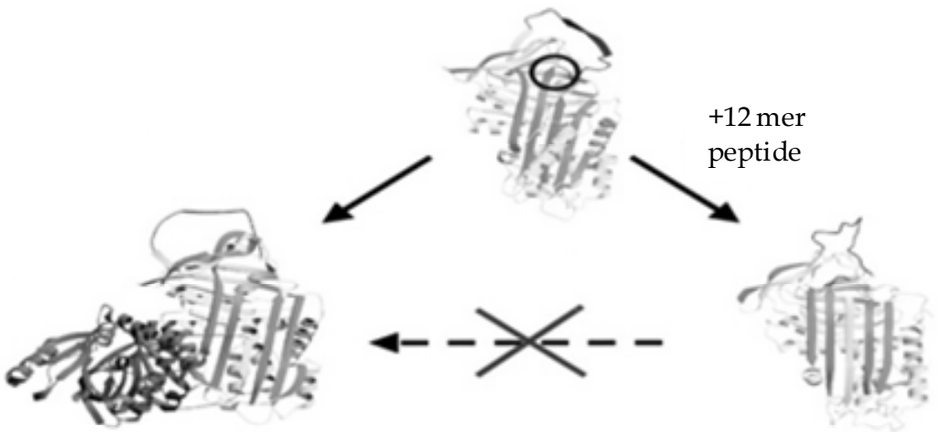
4.4 Treatment of Z α1-antitrypsin-associated liver disease

A major distinction between pathogenesis of lung and liver disease in Z-AT deficiency is loss of function and gain of function, respectively. In liver disease it relates to the intracellular accumulation of misfolded and unsecreted AT from hepatocytes rather than unopposed elastolysis in the lung due to lack of AT. Therefore, augmentation therapy does not confer protect against and not indicated for liver disease relating to severe AT deficiency. Other strategies have been assessed for treatment of Z-AT related liver disease including targeting a lateral hydrophobic cavity to prevent polymerization, and enhancing clearance of Z-AT aggregates by drugs promoting autophagy (Zhou et al., 2004; Burrows et al., 2000; Mallya et al., 2007; Hedvegi et al., 2010; Devlin et al., 2001; Kaushal et al., 2010). These methods reduce intracellular aggregation of Z-AT but do not increase the secretion Z-AT. Use of short synthetic peptides targeting β-sheet A may show therapeutic potential for Z-AT related liver and lung disease (Figure 3) (Chang et al., 2006; 2009; Alam et al., Unpublished observation).

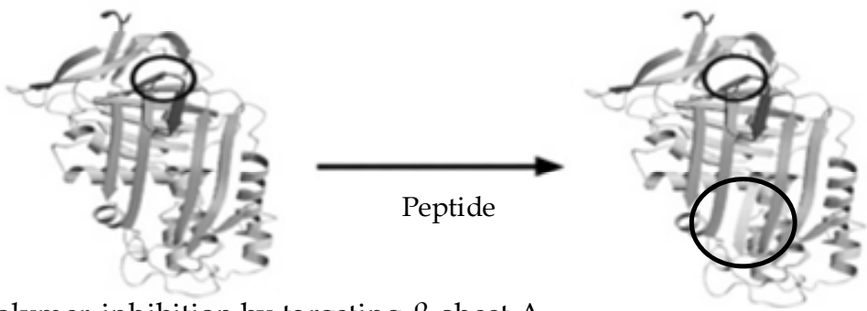
5. Novel treatments in development

5.1 Gene therapy

Future therapies for α_1-antitrypsin deficiency include gene therapy. Supplementing AT by gene delivery is an alternative way to increase the local AT in lung. Preclinical studies have shown that the Adeno-associated viral vector is capable in increasing the AT concentration to over 11μM in the lung. The safety and efficiency of this approach is under evaluation (Flotte, 2002; Flotte et al., 2004; Stecenko and Brigham, 2003; Flotte and Mueller, 2011).



A. Polymer inhibition by 12 mer peptide



B. Polymer inhibition by targeting β-sheet A

A. Z mutation (E342K) perturbs the structure of AT to allow opening of β-sheet A, which then accepts the RCL of another molecule to form a dimer (*left*) that can extend to form chains of polymers as depicted in Fig. 1. 12-mer peptide can anneal to β-sheet A thereby preventing polymer formation (*right*). B. Z mutation allows partial insertion of the RCL. This opens the lower part of β-sheet A thereby favouring polymerization (*left*). Understanding the configuration of the reactive loop and interacting with β-sheet A prompted the hypothesis that a 6-mer with homology to $P_{7\text{-}2}$ of the RCL would specifically bind to Z-AT and so prevent polymerization and explained why the 12-mer peptide preferentially bound to M-AT (*right*). Reproduced and adapted from Lomas and Mahadeva, 2002; Mahadeva et al., 2002; Chang et al., 2009.

Fig. 3. Representation Z α1-antitrypsin polymerization and the design of a selective inhibitor.

6. Summary

Alpha-1-antitrypsin is the most important proteinase inhibitor in maintaining the proteinase/antiproteinase balance within the lung. The recognition of the association between plasma deficiency of α1-antitrypsin and emphysema over 40 years ago has led to the proteinase-antiproteinase hypothesis of lung disease which remains central to understanding lung biology. In the last 20 years there has been significant progress in our understanding of α1-antitrypsin. Alpha-1 antitrypsin may modulate other biological processes such as apoptosis and inflammatory cell recruitment. Z α1-antitrypsin

polymerizes within the liver and this accounts for its severe plasma deficiency, and α1-antitrypsin polymers may have a role in the progression of emphysema, but this requires further investigation. Recent and ongoing studies should clarify the role of augmentation therapy and lung volume reduction in subgroups of PiZZ homozygotes, and the understanding of polymer formation has raised the exciting prospect of developing new therapeutic strategies for the liver and lung disease associated with Z α1-antitrypsin.

7. References

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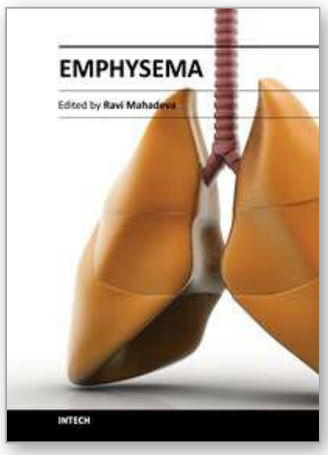

Emphysema

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Chronic Obstructive pulmonary disease (COPD) is an important cause of morbidity and mortality world-wide. The most common cause is chronic cigarette smoke inhalation which results in a chronic progressive debilitating lung disease with systemic involvement. COPD poses considerable challenges to health care resources, both in the chronic phase and as a result of acute exacerbations which can often require hospital admission. At the current time it is vital that scientific resources are channeled towards understanding the pathogenesis and natural history of the disease, to direct new treatment strategies for rigorous evaluation. This book encompasses some emerging concepts and new treatment modalities which hopefully will lead to better outcomes for this devastating disease.

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