α₁-Antitrypsin deficiency, chronic obstructive pulmonary disease and the serpinopathies

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ABSTRACT

α₁-Antitrypsin is the prototypical member of the serine proteinase inhibitor or serpin superfamily of proteins. The family includes α₁-antichymotrypsin, C1 inhibitor, antithrombin and neuroserpin, which are all linked by a common molecular structure and the same suicidal mechanism for inhibiting their target enzymes. Point mutations result in an aberrant conformational transition and the formation of polymers that are retained within the cell of synthesis. The intracellular accumulation of polymers of mutant α₁-antitrypsin and neuroserpin results in a toxic gain-of-function phenotype associated with cirrhosis and dementia respectively. The lack of important inhibitors results in overactivity of proteolytic cascades and diseases such as COPD (chronic obstructive pulmonary disease) (α₁-antitrypsin and α₁-antichymotrypsin), thrombosis (antithrombin) and angio-oedema (C1 inhibitor). We have grouped these conditions that share the same underlying disease mechanism together as the serpinopathies. In the present review, the molecular and pathophysiological basis of α₁-antitrypsin deficiency and other serpinopathies are considered, and we show how understanding this unusual mechanism of disease has resulted in the development of novel therapeutic strategies.

EPIDEMIOLOGY AND CLINICAL FEATURES OF α₁-ANTITRYPSIN DEFICIENCY

α₁-Antitrypsin is a 394-amino-acid acute-phase serine protease inhibitor that is synthesized primarily in the liver and, to a lesser extent, in peripheral blood monocytes, alveolar macrophages and epithelial cells of the bronchial and gastrointestinal mucosa [1–3]. It is present in the plasma at a concentration of 1.5–3.5 g/l and has the primary role of inhibiting neutrophil elastase. α₁-Antitrypsin deficiency was reported in an Alaskan girl who died 800 years ago [4], and may have accounted for the premature death of Frederic Chopin in 1849 [5,6]. It was first described as a clinical entity in 1963 by Laurell and Eriksson [7], who noted an absence of the α₁ band on serum protein electrophoresis (Figure 1). Since then, over 100 variants of α₁-antitrypsin have been reported resulting from mutations in the SERPINA1

Key words: α₁-antitrypsin, chronic obstructive pulmonary disease (COPD), endoplasmic reticulum, proteolytic cascade, serine protease inhibitor, serpinopathy.

Abbreviations: COPD, chronic obstructive pulmonary disease; ER, endoplasmic reticulum; ERAD, ER-associated degradation; FENIB, familial encephalopathy with neuroserpin inclusion bodies; FEV₁, forced expiratory volume in 1 s; NF-κB, nuclear factor κB; PAI-1, plasminogen activator inhibitor-1; UPR, unfolded protein response.

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Deficiency of α1-antitrypsin and others

The first case of α1-antitrypsin deficiency was identified by the deficiency of the α1 band (circled) on serum protein electrophoresis. A control sample is shown on the right. Reproduced from What we owe to α1-antitrypsin and to Carl-Bertil Laurell, Robin W. Carrell, COPD: Journal of Chronic Obstructive Pulmonary Disease, December 2004, reprinted by permission of Taylor & Francis (Taylor & Francis Group, http://www.informaworld.com).

gene at chromosome 14q31-31.2 [8,9]. The majority of individuals carry two copies of the normal allele and are designated as M homozygotes. Variants have historically been classified on the proteinase inhibitor (PI) nomenclature if they migrate faster (A–L) or slower (N–Z) than M α1-antitrypsin in isoelectric focusing analysis [10].

The most common severe deficiency allele in Europe is the Z allele (Glu342Lys), with 4 % of individuals being heterozygous (PI*MZ) for this variant and approx. 1 in 1700 being homozygotes (PI*Z) [11]. The Z mutation results in the retention of 85–90 % of the synthesized α1-antitrypsin in hepatocytes. Accumulation of mutant α1-antitrypsin starts in utero [12] with 1 in 10 infants developing cholestatic jaundice in the first few months of life. Symptoms resolve in the majority of children, but approx. 15 % of those with cholestatic jaundice progress to juvenile cirrhosis [13,14]. The overall risk of death from liver disease in PI*Z children during childhood is 2–3 % [15,16], whereas nearly a half of α1-antitrypsin-deficient adults over the age of 50 have pathological features of liver cirrhosis and occasionally hepatocellular carcinoma [17,18]. The predilection for hepatocellular carcinoma in homozygotes for the Z allele is higher than that attributable to cirrhosis alone [19]. Risk factors for cirrhosis in adult severe α1-antitrypsin deficiency include male gender and obesity, but not alcohol or viral hepatitis [20].

The retention of Z α1-antitrypsin within hepatocytes causes plasma deficiency of an important protease inhibitor. The lungs are thus exposed to proteolytic attack with the consequent risk of early-onset emphysema. Deficiency of α1-antitrypsin is the most important genetic factor in the development of COPD (chronic obstructive pulmonary disease) and is found in 1–2 % of affected individuals [21]. Symptomatic COPD is unusual before the third decade in smokers, and the symptoms of breathlessness, cough and sputum production are similar to those of ‘usual’ COPD [22]. FEV1 (forced expiratory volume in 1 s) [23] and thoracic computer tomography densitometry [24] are the most important predictors of survival, with more rapid deterioration being associated with current smoking, age between 30 and 44 years, male gender, FEV1 between 35 and 60 % predicted, asthmatic features, chronic bronchitis and previous episodes of pneumonia [23,25]. Median life expectancy for smokers is between 40–49 years and 65–69 years for never-smokers, with respiratory failure from emphysema and cirrhosis accounting for the excess mortality [25]. Cirrhosis is more commonly the underlying cause of death in never-smokers [23–26].

Although the Z allele is the commonest severe deficiency allele worldwide, other mutations can also cause similar clinical features. Mmalton (ΔPhe52) is the commonest severe deficiency variant in Sardinia, whereas the Šiíyama (Ser53Phe) allele is the commonest, albeit rare, deficiency variant in Japan [27–29]. A total lack of circulating protein can occur sporadically from null alleles that truncate the α1-antitrypsin protein [30]. Other mutations cause milder plasma deficiency. The S variant of α1-antitrypsin (Glu264Val) is common in Southern Europe, where 28 % are heterozygous (PI*MS) and up to 1 % are homozygous [11,31]. It results in S homozygotes having plasma α1-antitrypsin levels that are 60 % of the M allele [32]. PI*Z prevalence in North America ranges from 1 in 3000 to 1 in 5000 and is infrequent in Asian, African and Middle Eastern populations [33].

The structure of α1-antitrypsin is composed of three β-sheets (A–C), nine α-helices (A–I) and an exposed mobile reactive loop that presents a peptide sequence as a pseudosubstrate for the target proteinase [34–36]. The PI–P1′ residues of the reactive loop define the inhibitory specificity of α1-antitrypsin for neutrophil elastase [37–41]. After docking with elastase, the enzyme cleaves the PI–P1′ peptide bond of the reactive loop and the proteinase is swung 70 Å (1 Å = 0.1 nm) from the upper to the lower pole of the protein in association with the insertion of the reactive loop as an extra strand into β-sheet A (Figure 2a) [38,42]. The resulting covalently bound serpin then inactivates the protease by distortion of the catalytic triad at the active site [42–45]. The stable complex is cleared from the circulation by the serpin–enzyme complex receptor on hepatocytes [46]. This unique conformational transition is central to the inhibitory activity of the serpins, but is subverted by point mutations to cause deficiency and disease.
The effect of point mutations is best characterized for the Z mutation which lies at the head of strand 5 of $\beta$-sheet A and the base of the mobile reactive loop (Figure 2b). The loop can dynamically move in and out of the upper pole part of $\beta$-sheet A. However, with raised temperature or the presence of chaotropic agents, the N-terminus of the reactive loop is released from strand 1C [47] and helix F unwinds [48,49]. This causes $\beta$-sheet A to ‘unzip’ and adopt the receptive or $M^*$ conformation [50–52]. The patent $\beta$-sheet A then accepts the reactive loop of another $\alpha_1$-antitrypsin molecule to form a dimer [53]. Extension of this dimer results in loop–sheet polymers in which the reactive centre loop of one $\alpha_1$-antitrypsin molecule sequentially inserts into the accessible $\beta$-sheet A of another [54–58]. A recent hypothesis has proposed a novel structure for the intermolecular linkage between monomeric components of the polymer chain [59]. It suggests that the conformational event underlying the formation of $M^*$ is release of strand 5 from $\beta$-sheet A of one monomer to form an extensive 51-residue loop with strand 4 that is available for domain swap and insertion into $\beta$-sheet A of another [59]. The physiological importance of this surprising structural variant requires further study. Nevertheless, whatever the mechanism, polymers are retained within hepatocytes to cause the liver diseases described above. Spectroscopic analysis has demonstrated that oligomers of $\alpha_1$-antitrypsin form during an initial lag phase before condensing to form a heterogenous mixture of extending polymers [50,58] (Figure 2c).

The Siyama and Mmalton alleles [27–29] similarly favour polymerization. They do so by disrupting the hydrogen-bond network in the shutter domain (Figure 2b) that underlies the bifurcation of strands 3 and 5 of $\beta$-sheet A, allowing it to open and become receptive for the formation of loop–sheet polymers [60–63]. The S mutation also perturbs the shutter domain, but causes significantly less disruption to $\beta$-sheet A thereby causing a slower rate of polymer formation, less hepatic retention and a milder plasma deficiency [64].

It is striking that the severity of accumulation of mutant $\alpha_1$-antitrypsin correlates directly with the rate of polymer formation. Variants that cause the most rapid polymerization cause the most accumulation of $\alpha_1$-antitrypsin within the liver and are associated with both a greater risk of liver disease and a more severe plasma deficiency. The rate of polymer formation for S $\alpha_1$-antitrypsin is much slower than that of Z $\alpha_1$-antitrypsin,

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**Figure 2** Conformational transitions of $\alpha_1$-antitrypsin

(a) Neutrophil elastase (top grey) docks (left-hand panel) and covalently binds the reactive centre loop (red) before being inactivated by the translocation from the upper to the lower pole of the protein (right-hand panel). The reactive loop is inserted as an extra strand into $\beta$-sheet A (green). This Figure was reproduced from [107] with permission. © (2002) Nature Review Genetics. (b) Mechanism of polymer formation. Native $\alpha_1$-antitrypsin (M) in a folded metastable conformation. Variants (Z mutation indicated by the arrow and shutter domain by blue circle) allow the opening of $\beta$-sheet A and predispose to the formation of an unstable intermediate ($M^*$) by partial loop insertion. The patent $\beta$-sheet A accepts the loop of another molecule to form a dimer (D), which propagates to form polymers (P). Siyama (Ser53Phe) and Mmalton (D.Phe52) cause rapid polymerization and severe plasma deficiency by perturbing the shutter domain. The S mutation (Glu264Val) has less effect on the shutter domain and so causes less polymer formation in association with a milder clinical phenotype. Reproduced from [108] with permission. (c) Electron microscopy demonstrates the flexibility of $\alpha_1$-antitrypsin polymers. Reproduced from [62] with permission. © (1993) American Society for Biochemistry and Molecular Biology. (d) Structures of serpins with an exogenous reactive loop peptide (yellow) in s4A (left-hand panel). The hydrophobic pocket that is the target of rational drug design is shown in purple in the right-hand panel. Reproduced from [178] with permission. © (1998) Elsevier.
resulting in the lack of a clinical phenotype [50,64]. However, if the mild slowly polymerizing S α1-antitrypsin is inherited with a rapidly polymerizing Z variant, then the two can potentially interact to form heteropolymers within hepatocytes, leading to inclusions and eventually cirrhosis [65–67]. The severity of retention of mutants of α1-antitrypsin within hepatocytes and the consequent plasma deficiency can thus be explained by the rate of polymer formation.

**α1-ANTITRYPSIN DEFICIENCY AND COPD**

COPD in α1-antitrypsin-deficient individuals is due to the loss of protective mechanisms attributed to α1-antitrypsin. The protease/antiprotease hypothesis suggests that abnormally low levels of α1-antitrypsin leads to unopposed neutrophil elastase activity and destruction of the elastin matrix within the lung [68]. The lack of a circulating protease inhibitor is exacerbated by other important mechanisms in predisposing to early-onset lung disease in severe α1-antitrypsin deficiency. Oxidation of the key P1 methionine residue by H2O2 and other oxidants in cigarette smoke causes the loss of anti-elastase activity and also renders the protein liable to reactive loop cleavage [69]. Moreover Z α1-antitrypsin has a 5-fold reduction in association rate kinetics with neutrophil elastase compared with the normal protein [70–73]. Degradation of other extracellular matrix components by MMPs (matrix metalloproteases) is also potentially important in the pathogenesis of COPD [74]. Their role is amplified by their ability to cleave and degrade α1-antitrypsin [68,75].

The recognition that Z α1-antitrypsin formed polymers within hepatocytes raised the question of whether polymers could also form within the lung. Z α1-antitrypsin diffuses into the lung from the circulation. Bronchial epithelial cells and macrophages also produce α1-antitrypsin locally. In all cases it retains the ability to form polymers. Indeed, polymers have been detected in lung lavage and in tissue sections from individuals with α1-antitrypsin deficiency [76–78]. These polymers are inactive as protease inhibitors and so amplify the local deficiency of α1-antitrypsin. Moreover α1-antitrypsin polymers are chemotactic for neutrophils with an effect size that is similar to that of known chemotactants such as C5a and IL-8 (interleukin-8) [78,79]. The effect has been supported in vivo, as the instillation of α1-antitrypsin polymers into mouse lung has been demonstrated to cause a neutrophil influx [77]. Polymers also induce neutrophil adhesion, shape change and enzyme release [79]. Their pro-inflammatory properties combine with the effects of cytokines, reactive-loop-cleaved α1-antitrypsin [80], matrix breakdown products [81] and cigarette smoke to explain the excessive numbers of neutrophils in bronchoalveolar lavage [82] and tissue sections [77] from individuals with α1-antitrypsin deficiency [83].

Alveolar cell apoptosis, a central element in the pathobiology of COPD [84,85], offers a novel area of interest in α1-antitrypsin deficiency. α1-Antitrypsin has been shown to exert a protective pro-survival effect against ischaemia/reperfusion injury [86], serum-withdrawal-induced apoptosis [87] and to prolong islet allograft survival in mice [88]. Furthermore, α1-antitrypsin supplementation attenuates the development of apoptosis-dependent emphysema and oxidative stress injury in mice, possibly via direct inhibition of caspase 3 [89,90], a cysteine protease intimately involved in the apoptotic cascade. The anti-apoptotic activity of α1-antitrypsin mutants is unknown, but polymers and other conformers of α1-antitrypsin do not exhibit this protection against apoptosis, as an intact reactive loop appears to be essential [89]. Emphysema associated with Z α1-antitrypsin deficiency may thus result from a combination of loss-of-function of α1-antitrypsin, toxic gain-of-function as a result of intra-alveolar polymer formation and possibly reduced pro-survival activity of mutant protein (Figure 3).

**POLYMERS, INFLAMMATION AND VASCULITIS**

The observation that polymers are pro-inflammatory may also explain the association of Z α1-antitrypsin with other inflammatory conditions. A variety of anecdotal and epidemiological studies have linked Z α1-antitrypsin deficiency (or the Z allele) to panniculitis (or Christian–Weber syndrome) [91], Wegener’s granulomatosis [92,93], glomerulonephritis [94], asthma [95,96], pancreatitis [97] and possibly bronchiectasis [98–100]. Given that polymers are pro-inflammatory for neutrophils (and possibly other cells of the inflammatory response), it is feasible that they underlie this exuberant inflammation in different organs; however, to date, there have been no studies investigating the role of polymers in any of these conditions.

**POLYMERS AND INFLAMMATION: THE SURVIVAL ADVANTAGE OF Z α1-ANTITRYPSIN**

The high α1-antitrypsin gene mutation frequency suggests that α1-antitrypsin-deficiency alleles confers a selective advantage in affected individuals. This may be explained by their effect on the inflammatory response [101]. The major cause of death in the pre-antibiotic era was infectious disease, such as pneumonia and gastroenteritis. Infection generally leads to an increase in the secretion of the acute-phase protein Z α1-antitrypsin by hepatocytes, which then concentrates at the site of inflammation. The lower pH at the site of inflammation and the associated fever coupled with the
relatively high concentration of mutant $\alpha_1$-antitrypsin predisposes to polymer formation [30,54,72]. Polymers in turn enhance the recruitment of protective neutrophils that amplify the inflammatory response thereby repelling invading organisms [101]. Over the past 100 years, the use of antibiotics and improved living standards has reduced the risk of death from infectious disease. Moreover the increased longevity and widespread adoption of cigarette smoking, the main cause of lung inflammation, has converted a protective gene to one that now causes disease [101].

Enhanced fertility caused by proteinase inhibitor deficiency was suggested previously as the mechanism of selective advantage in affected individuals [102]. Sperm requires the enzyme acrosin to penetrate the zona pellucida of the ovum during fertilization, so the deficiency of $\alpha_1$-antitrypsin may favour penetration of sperm and therefore increase fertility. It is however doubtful that this interaction is crucial, as the association rate constant of acrosin with $\alpha_1$-antitrypsin is very low [103]. Twin studies have suggested that Z variant antitrypsin increase the chance of ovulation rate and so enhance the success of multiple pregnancies [102,104], although parallel studies have not shown any increase in family size in individuals with $\alpha_1$-antitrypsin deficiency.

**$\alpha_1$-ANTITRYPSIN DEFICIENCY PROVIDES A PARADIGM FOR THE SERPINOPATHIES**

The serpin superfamily is characterized by more than 30% sequence homology with $\alpha_1$-antitrypsin and conservation of tertiary structure. Other members include C1 inhibitor, antithrombin and PAI-1 (plasminogen activator inhibitor-1), which play crucial roles in the regulation of proteinases involved in the complement, coagulation and fibrinolytic cascades respectively [105]. Point mutations cause defective conformational transitions by the same mechanism as described for $\alpha_1$-antitrypsin. This results in the retention of the serpin within the cell of synthesis [106–108], and gives rise to clinical conditions that result from toxic gain-of-function, with intracellular protein overload and death of the cell in which the serpin is synthesized. This is most striking in the dementia FENIB (familial encephalopathy with neuroserpin inclusion bodies) [109] (see below). The polymerization of mutants of other members of the family, antithrombin, C1-inhibitor and $\alpha_1$-antichymotrypsin, also result in the formation of polymers that are retained within the cell of synthesis. This results in plasma deficiency and overactivity of important proteolytic cascades that are associated with thrombosis, angio-oedema and emphysema respectively. The same process had been reported in a mutant of heparin co-factor II [110]. This also causes plasma deficiency, but this has not been associated with a clinical phenotype. These diseases all share a common mechanism and so we have grouped them together as a new class of disease: the serpinopathies [111].

**FENIB**

Neuroserpin is secreted from axonal growth cones of the developing and adult nervous system, where it inhibits tissue plasminogen activator and is implicated in regulating axonal growth, emotional behaviour and memory, reducing seizure activity and limiting damage in cerebral infarction [112–119]. The autosomal-dominant dementia FENIB is characterized by inclusions of mutant neuroserpin within cortical and subcortical neurons. Six families with FENIB have been identified worldwide as a result of five different mutations in neuroserpin [120,121]. The five mutations lead to different combinations...
of dementia, tremor, seizures, progressive myoclonic epilepsy and dysarthria [120]. Comparison of the severity of disease, the number of neuronal inclusions and the age of onset of dementia associated with each mutation confirmed that FENIB displays a genotype–phenotype relationship based on propensity of the mutant to polymerize (Table 1). Affected members in the original family with Ser49Pro neuroserpin (neuroserpin Syracuse) have diffuse small intraneuronal inclusions of neuroserpin with an onset of dementia between the ages of 45 and 63 years [109,122,123]. A second family, with a conformationally more severe mutation (neuroserpin Portland; Ser52Arg), had larger inclusions and an onset of dementia in early adulthood, whereas a third family, with yet another mutation (His338Arg), had even more inclusions and the onset of dementia in adolescence. The most severe disease is caused by mutations of Gly392, a conserved residue in the shutter region. Replacement with glutamic acid in the Gly392Glu mutation results in large inclusions with affected family members dying by 20 years of age [120]. More recently, a fifth mutation has been reported [121]. The Gly392Arg mutation results in severe dementia and electrical status epilepticus of slow-wave sleep in a child of 8 years of age.

The role of polymerization in disease is supported in vitro by the demonstration that recombinant Ser49Pro neuroserpin has a greatly accelerated rate of polymerization when compared with the wild-type protein [115,124] and that Ser52Arg, which causes a more severe clinical phenotype, polymerizes even more rapidly [125]. Cell models of FENIB show that mutant neuroserpin is retained as intracellular inclusions within the ER (endoplasmic reticulum) [126,127] (Figure 4a). These inclusions are composed of polymers similar to the loop–sheet polymers of mutant neuroserpin that are isolated from the brains of individuals with FENIB [126,127]. When mutants of neuroserpin are expressed in cell cultures, the striking genotype–phenotype correlation observed in FENIB is still seen. With an increasing propensity to polymerization, there is an increasing number of cells with polymer inclusions and a decreasing secretion of neuroserpin into the extracellular medium (Figures 4b and 4c) [126,127]. Moreover, the overexpression of mutants of neuroserpin in fly brain demonstrates a direct correlation between the intracellular retention of mutant neuroserpin, polymer formation and locomotor phenotype [127] (Figure 4d). Thus the accumulation of polymers is toxic to neurons in vivo [127].

### CELLULAR HANDLING OF MUTANT PROTEINS IN THE SERPINOPATHIES

The liver disease associated with α1-antitrypsin deficiency shows variation in onset and severity, which implies that environmental modifiers and genetic polymorphisms may play a crucial role in the pathogenesis of hepatocyte damage [128]. The subgroup of α1-antitrypsin-deficient individuals that develop significant liver disease may result from inefficient ER degradation of accumulated mutant α1-antitrypsin [129]. The ER usually responds to the accumulation of misfolded protein within its lumen by activating the UPR (unfolded protein response), a signal transduction pathway that allows for an increase in the capacity for protein folding and an increase in the degradation of misfolded proteins [130]. If misfolded protein is not sufficiently cleared by this process, the UPR is capable of signalling for cell apoptosis [130]. In contrast, the accumulation of polymers of α1-antitrypsin does not elicit the UPR, probably due to their ordered structure, but can activate the ER-overload response, generating a distinct signalling profile from the UPR which includes activation of NF-κB (nuclear factor κB) (Figure 5) ([131–133], and M.J. Davies, E. Miranda, R.J. Kaufman, S.J. Marciniak and Lomas, D.A., unpublished work). Two major pathways are responsible for the degradation of mutant glycosylated secretory proteins that accrue in the ER: ERAD (ER–associated degradation) and autophagy [135–139]. The precise mechanism by which the cell monitors glycoprotein folding within the ER and targets terminally misfolded client proteins for degradation remains unclear; however, enzymatic trimming of

### Table 1 Genotype–phenotype relationship in FENIB

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Rate of polymerization</th>
<th>Number of inclusions</th>
<th>Disease onset (years)</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser49Pro</td>
<td>+</td>
<td>+</td>
<td>45–63</td>
<td>Dementia and seizures</td>
</tr>
<tr>
<td>Ser52Arg</td>
<td>++</td>
<td>++</td>
<td>20–40</td>
<td>Dementia and myoclonus</td>
</tr>
<tr>
<td>His338Arg</td>
<td>+++</td>
<td>+++</td>
<td>15</td>
<td>Progressive myoclonus and epilepsy</td>
</tr>
<tr>
<td>Gly392Glu</td>
<td>++++</td>
<td>++++</td>
<td>13</td>
<td>Progressive myoclonus, epilepsy and chorea</td>
</tr>
<tr>
<td>Gly392Arg</td>
<td>++++</td>
<td>++++</td>
<td>8</td>
<td>Severe dementia and status epilepticus</td>
</tr>
</tbody>
</table>

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carbohydrate side chains [138] and the extraction of mutant proteins from the calnexin/calreticulin cycle mediated by factors such as EDEM (ER degradation-enhancing α-mannosidase-like protein) [140,141] allows their delivery from the ER to the cytoplasm for ubiquitinization and destruction by the proteasome [139].

Overexpression of mutant α₁-antitrypsin may overwhelm the ERAD pathway and lead to the accumulation of insoluble mutant protein [142]. ER-aggregated Z α₁-antitrypsin may in turn activate autophagy, a catabolic process involving the degradation of cellular organelles through the lysosomal degradation pathway [143]. Mice lacking autophagic apparatus have aggressive propagation of mutant α₁-antitrypsin aggregates, indicating that autophagy may be responsible for the degradation of α₁-antitrypsin polymers [143]. Defects in autophagy genes have also been shown to render cells vulnerable to the toxic effects of aggregation-prone proteins, suggesting that liver disease in α₁-antitrypsin deficiency ensues when the autophagic pathway is overwhelmed [144,145]. The susceptibility of the degradative mechanisms to overloading increases with age [146] and may provide
Figure 5 Model of the handling of serpin polymers
Left-hand panel, the UPR. Accumulation of misfolded protein within the ER triggers intracellular signalling pathways that are both protective and apoptotic, but with different time courses. BiP (immunoglobulin heavy-chain-binding protein), a calcium-dependent ER chaperone, controls the activation of all three transmembrane proteins that make up the components of the UPR by dissociating from their luminal domains in the presence of misfolded protein. Once activated these pathways promote cell survival by reducing misfolded protein levels. However, if homoeostasis cannot be re-established, UPR signalling eventually induces apoptosis. ATF, activating transcription factor; IRE1, inositol-requiring kinase 1; PERK, PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase; XBP1, X-box-binding protein 1. Right-hand panel, polymer-induced ER overload response. Serpin polymer aggregation does not induce the UPR, but generates a distinct signalling profile via the activation of NF-κB by the release of Ca^{2+} from the ER.

an explanation for the late development of liver disease in severe α₁-antitrypsin deficiency, although this remains controversial.

The temperature- and concentration-dependence of polymerization [50,54] may also play a role in the heterogeneity of liver disease among individuals who are homozygous for the Z mutation. The synthesis of α₁-antitrypsin increases during episodes of inflammation as part of the acute-phase response. At these times, the formation of polymers may overwhelm the degradative pathway thereby exacerbating the formation of hepatic inclusions leading to hepatocellular damage. This toxic gain-of-function mechanism of liver damage is poorly understood, but NF-κB activation and mitochondrial dysfunction are thought to play central roles [147,148]. Moreover, mitochondrial autophagy and dysfunction have been reported in liver biopsies from patients with α₁-antitrypsin deficiency [148], and elevated levels of TNF-α (tumour necrosis factor-α) have been linked to similar changes seen in patients with hepatic steatosis [149]. ER-stressed liver cells may accumulate NF-κB-associated inflammatory damage that has been linked to the pathogenesis of hepatocellular carcinoma in chronic inflammatory liver diseases [150]. NF-κB signalling pathways may also preserve the selective proliferative advantage of damaged cells and provide a paradigm for other liver diseases that predispose to development of hepatocellular carcinoma [151].

NOVEL STRATEGIES TO TREAT α₁-ANTITRYPSIN DEFICIENCY AND THE SERPINOPATHIES

Guidelines for treatment of ‘usual’ COPD apply equally to α₁-antitrypsin-deficient disease [152,153], except for lung-volume-reduction surgery, which offers only temporary benefits and is generally not recommended [154]. The most severely affected individuals should be considered for lung transplantation. Cigarette smoke and environmental pollution avoidance is critical in preventing the development of all forms of COPD [25,155]. The mainstay of treatment for severe α₁-antitrypsin deficiency in some countries is the correction of the plasma deficiency with intravenous augmentation of pooled plasma purified α₁-antitrypsin [22,156,157]. Doses are administered weekly or bimonthly to achieve a trough level above a protective threshold of 0.5 g/l [156,157]. Observational cohort studies of clinical efficacy show lower overall mortality and slower rate of FEV₁ decline only in individuals with moderate COPD [23]. In addition, α₁-antitrypsin augmentation is associated with a possible reduction in the frequency of respiratory tract infections, decline in sputum markers of inflammation and few serious side effects [158–160]. The one randomized control trial failed to show significant differences in the rate of decline of FEV₁ or in the rate of loss of lung density, as it was underpowered [161]. The
lack of evidence and cost pressures has ultimately limited the use of intravenous α₁-antitrypsin [162–164]. Understanding that Z α₁-antitrypsin polymerization underlies the liver accumulation and subsequent plasma deficiency has facilitated the development of novel strategies to attenuate polymerization and thereby treat the associated diseases. Three strategies of note include the use of the following. (i) Chemical chaperones to stabilize the unstable mutant serpin [165–167]. Trials of chemical chaperones show that certain agents stabilize intermediates on the Z α₁-antitrypsin-folding pathway and are effective in cell and animal models of disease, but human trials have been disappointing [168]. (ii) A second strategy exploits peptide analogues of reactive loop peptides that compete for binding to β-sheet A and so directly block the polymerization of Z α₁-antitrypsin [52,54,169–171] (Figure 2d). Although useful in establishing the mechanism of polymerization, these peptides are unsuitable for administration to humans. (iii) The third approach uses small molecules to target a surface cavity for allosteric blockade of the conformational transition that underlies polymer formation [172]. The allosteric cavity in α₁-antitrypsin is bounded by strand 2 of β-sheet A and helices D and E (Figure 2d). It is patent in the native protein but is filled as β-sheet A accepts an exogenous reactive loop peptide during polymerization [39,173] (Figure 2b). The importance of the cavity as a target for rational drug design was demonstrated by the introduction of the cavity-filling mutation Thr114Phe, which reduced polymer formation and increased the secretion of Z α₁-antitrypsin from a Xenopus oocyte expression system [174]. This has led to the in silico discovery of drug-like small molecules that target this cavity. The lead candidate blocks α₁-antitrypsin inhibitory function, inhibits the polymerization of Z α₁-antitrypsin in vitro and significantly reduces the aggregation of Z α₁-antitrypsin in a cell model of disease [172]. This specific approach, however, is not universally applicable to all serpins as amphipathic organoligands can bind to this region of PAI-1 to induce polymer formation [175–177], and other serpins may not have similar drug-sensitive allosteric pockets [39].

CONCLUSION AND FUTURE DIRECTIONS

There has been considerable progress in understanding the pathobiology of α₁-antitrypsin deficiency and extending the findings from this condition to the other serpinopathies. However, more work is required to characterize the structure of the polymer that is retained with the ER of hepatocytes and to define the pathways by which these polymers cause cell death. The long-term goal must be to refine and develop strategies to block polymer formation and so treat the associated disease.

FUNDING

The authors’ work is funded by the Medical Research Council [project grant number G0500306 (to D.A.L.) and G070099 (to D.C.C.)]. U.I.G. is a recipient of a Medical Research Council (MRC) Clinical Research Fellowship [grant number G0601403] S.J.M. is the recipient of an MRC Clinician Scientist Award (G0601840), I.M. is the recipient of an MRC Studentship (G0501381), and B.G. is the recipient of a Wellcome Trust Intermediate Clinical Fellowship. P.H. is funded by the Wennergren Foundation (Sweden) and the Swedish Society for Medical Research (SSMF).

REFERENCES

37 Elliott, P. R., Abrahams, J. P. and Lomas, D. A. (1998) Molecular
topography of a 2.0 Å structure of α1-antitrypsin reveals targets for rational drug design to prevent
congenital deficiency. J. Mol. Biol. 275, 419–425
38 Elliott, P. R., Lomas, D. A., Carrell, R. W. and Abrahams,
J. P. (1996) Inhibitory function of the reactive loop of α1-
39 Elliott, P. R., Pei, X. Y., Dafforn, T. R. and Lomas, D. A.
(2000) Topography of a 2.0 Å structure of α1-antitrypsin reveals targets for rational drug design to prevent
congenital deficiency. Protein Sci. 9, 1274–1281
α1-antitrypsin shows variability of the reactive centre and other loops. J. Mol. Biol. 316, 109–119
41 Ryu, S. E., Choi, H. J., Kwon, K. S., Lee, K. N. and Yu,
M. H. (1996) The native strains in the hydrophobic core and flexible reactive loop of a serine protease inhibitor:
crystal structure of an uncleaved α1-antitrypsin at 2.7 Å. Structure 4, 1181–1192
46 Perlmutter, D. H., Glover, G. I., Rivetna, M., Schateen,
47 Chang, W. S., Whistock, J., Hopkins, P. C., Lesk, A. M.,
48 Cabrita, L. D., Whistock, J. C. and Bottomley, S. P.
49 Gooptu, B., Nobeli, I., Purkiss, A., Phillips, R., Mally,
molbi.2009.01.069
50 Dafforn, T. R., Mahadeva, R., Elliott, P. R., Sivasothy, P.
51 Gooptu, B., Hazes, B., Chang, W. S. W., Dafforn, T. R.,
52 Mahadeva, R., Dafforn, T. R., Carrell, R. W. and Lomas,
55 Jancauskiené, S., Dominatiene, R., Sterbny, N. H.,
56 Sivasothy, P., Dafforn, T. R., Gooptu, P. G. W. and Lomas,
58 Puskaryatha, P., Klemke, J. W., Lavender, S., Oyola, R.,


