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Characterization of Pleiotropic Activities of alpha1-Antitrypsin

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Characterization of Pleiotropic Activities of α_1-Antitrypsin

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Doctoral Thesis

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Till mina nära och kära…

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Do native or polymeric alpha-1-antitrypsin activate human neutrophils *in vitro*?
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II Zhenjun Li, Jicun Wang, **Caroline S. Sandström**, Sabina Janciauskiene and Ravi Mahadeva
Oxidized α_1-antitrypsin stimulates release of monocyte chemotactic protein from lung epithelial cells: potential role in emphysema
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III **Caroline S. Sandström**, Eeva Piitulainen and Sabina Janciauskiene.
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IV **Caroline S. Sandström**, Natalia Novoradovskaya, Corrado M. Cilio, Eeva Piitulainen, Tomas Sveger and Sabina Janciauskiene.
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V **Caroline S. Sandström**, Bodil Ohlsson, Olle Melander, Ulla Westin, Ravi Mahadeva and Sabina Janciauskiene.
A possible link between diabetes type II and α_1-antitrypsin deficiency.
Submitted

ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) is a respiratory disease characterized by chronic inflammatory response in the airways and lung parenchyma that involves the influx of inflammatory, structural cells and mediators. The inflammatory process resulting in lung tissue destruction, emphysema, includes the excess of uninhibited proteases released from inflammatory cells.

α_1-antitrypsin (AAT), an acute phase protein, is the prototypic member of the serpin super family and a major inhibitor of serine proteases such as neutrophil elastase. The clinical importance of AAT is highlighted in individuals with inherited AAT deficiency who exhibits an increased susceptibility to develop chronic inflammatory conditions including COPD. Both native (inhibitory) and modified (non-inhibitory) molecular forms of AAT, such as oxidized, nitrated, polymerized, cleaved and complexed with other proteins, have been detected *in vivo*. Recently it has become apparent that various forms of AAT express biological properties which are independent of protease inhibition. Studies characterizing these new biological activities of AAT are incomplete, particularly with regard to understanding the involvement of signalling mechanisms.

We hypothesize that novel biological activities of native AAT and its by-products may play an important role in the pathological processes characterized by chronic inflammation. We therefore examined; a) the effects of polymerized AAT on neutrophil activation, *in vitro*; b) the role of oxidized AAT on monocyte/macrophage activation both *in vitro* and *in vivo*; c) the effects of augmentation therapy on levels of plasma inflammatory markers and the properties of blood neutrophils obtained from a severe AAT-deficient COPD subject; d) the relationship between AAT levels and endotoxin receptor CD14 expression in young AAT-deficient subjects, and because low plasma AAT levels have been linked to diabetes type 1 we; e) measured plasma AAT levels in diabetes type 2 subjects.

Our findings show that:

1. Some of the reported pro-inflammatory activities of polymerized AAT may be due to bacterial or other contaminants and that endotoxin-free polymerized AAT does not exhibit pro-inflammatory activity.

2. Oxidized AAT induces a pro-inflammatory activation of both structural and inflammatory cells and may be involved in the development of COPD.

3. Blood neutrophils from a COPD patient with severe PiZZ AAT deficiency isolated after augmentation therapy release significantly lower levels of IL-8 in response to zymosan as compared to neutrophils obtained before therapy. Our results support the idea that augmentation therapy with human AAT has anti-inflammatory effects.

4. Blood monocytes from clinically healthy subjects with severe AAT-deficiency show significantly higher expression of CD14, an endotoxin receptor, than age and gender matched non-deficient controls. This finding in part explains why reduced AAT levels are related to increased susceptibility to infections and the development of chronic inflammation.

5. A possible link may exist between low plasma levels of AAT and the development of diabetes type 2.

To summarize, the findings from our studies further improve our understanding of the biological activities of AAT and highlight the potentially broader modulatory role of AAT in inflammatory diseases.

ABBREVIATIONS

AAT	α_1-antitrypsin
AATD	α_1-antitrypsin deficiency
ACT	α_1-antichymotrypsin
ANCA	anti-neutrophilic cytoplasmic antibodies
BAL	bronchoalveolar lavage
COPD	chronic obstructive pulmonary disease
CRP	c-reactive protein
GM-CSF	granulocyte-macrophage colony-stimulating factor
CT	computed tomography
CTSG	cathepsin G
GPI	glycophosphatidylinositol
FEV_1	forced expiratory volume in one second
ICAM-1	intercellular adhesion molecule 1
IFNγ	interferon γ
IL-	interleukin-
IEF	isoelectric focusing
LBP	lipopolysaccharide binding protein
LDL	low density lipoprotein
LPS	lipopolysaccharide
LRP	low density lipoprotein receptor-related protein
LTB_4	leukotriene B4
LVRS	lung volume reduction surgery
MCP-1	monocyte chemoattractant protein 1
MMP	matrix metalloproteinase
NADPH	nicotinamide adenine dinucleotide phosphate
NE	neutrophil elastase
NFκB	nuclear factor κB
NOD	nonobese diabetic
Pi	protease inhibitor
PR3	proteinase 3
RCL	reactive centre loop
SEC	serpin-enzyme complex
SERPIN	serine protease inhibitor
SLPI	secretory leukocyte protease inhibitor
s-TIC	serum trypsin inhibitory activity
TGFβ	transforming growth factor β
TLR_4	toll like receptor 4
TNFα	tumor necrosis factor-α
VCAM-1	vascular cell adhesion molecule 1
VEGF	vascular endothelial growth factor

INTRODUCTION

INFLAMMATION, TISSUE DAMAGE AND EMPHYSEMA

Inflammation is a prominent feature of respiratory tract diseases including asthma, acute bronchitis, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease (COPD) and emphysema (de Benedictis, del Giudice et al. 2001; Hurst, Kuchai et al. 2006).

COPD is associated with a chronic inflammatory response, predominantly in the small airways and lung parenchyma, which is characterized by the influx of inflammatory cells such as macrophages, neutrophils and T-lymphocytes and mediators, including inflammatory peptides, reactive oxygen and nitrogen species, lipid mediators, chemokines, cytokines and growth factors. These mediators are derived not only from activated inflammatory cells that are recruited to the airways and lungs but also from structural cells in the respiratory tract, including epithelial and endothelial cells and fibroblasts.

The structural cells, in combination with the signaling molecules from the inflammatory cells, contribute to the additional recruitment of inflammatory cells from the circulation, broncho-constriction, vascular damage and mucus secretion resulting in small airway fibrosis and alveolar destruction. This process involves various proteases that are responsible for the destruction of elastin fibres in the lung parenchyma, which is the hallmark of emphysema (Barnes 2004) (Figure 1).
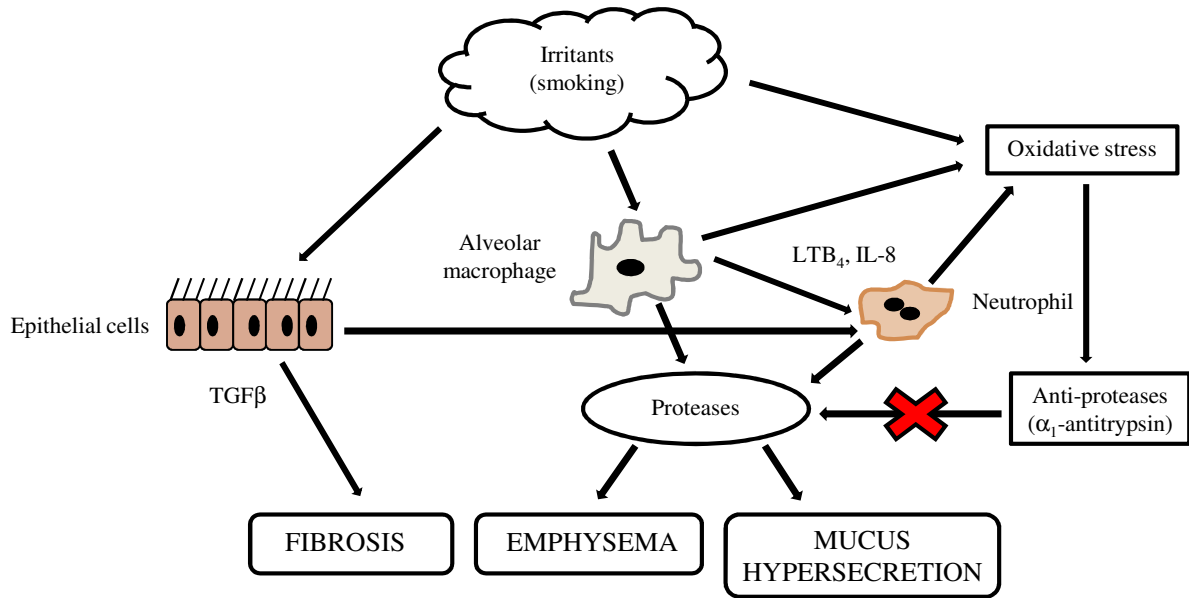
The figure is a modified version of several illustrations included in Barnes PJ, 2004.

Figure 1. Inflammatory mechanisms in COPD.

Inflammatory cells

Monocytes & Macrophages

The circulating blood monocytes arise in the bone marrow from pluripotent stem cells and in less than a day after formation they enter the blood stream. In the peripheral blood stream, monocytes constitute about 4-8% of the total leukocyte population (Hume, Ross et al. 2002). After circulation (8-71 hours), monocytes migrate through the walls of the blood vessels into various organs where they differentiate into macrophages (Whitelaw 1966). Since the population of macrophages is not constant, the influx of monocytes from the blood stream contribute to the cell renewal (Linden, Rasmussen et al. 1993; Kuschner, D'Alessandro et al. 1996). Macrophages are reactive cells that respond to various endogenous and exogenous stimuli and are proactive by producing mediators that modulate the behaviour of surrounding cells (Tetley 2002). Normally, alveolar macrophages are the predominant long-lived effector cell within the lung and under conditions associated with chronic inflammation, macrophages account for the majority of inflammatory cells recovered by airway lavage (Linden, Rasmussen et al. 1993; Kuschner, D'Alessandro et al.

1996). The cells release compounds that include reactive oxygen species, chemotactic factors, inflammatory cytokines, smooth muscle constrictors, mucus gland activators, extracellular matrix proteins, and an array of matrix metalloproteinase (MMP) enzymes (O'Donnell, Breen et al. 2006). In addition, they play a critical role in the clearance of apoptotic neutrophils (Tetley 2002).

Despite the fact that macrophages do not transcribe the neutrophil elastase (NE) gene, their ability to internalise the enzyme has led to the proposal that the macrophage-released NE can add further to the proteolytic potential of the cell (Finlay, O'Driscoll et al. 1997). Macrophages can also indirectly induce mucus hypersecretion e.g via leukotriene B4 (LTB$_4$) and interleukin (IL)-1. Though, most studies have focused on the possible role of macrophages in emphysema, particularly by the production of MMPs like MMP-2 (gelatinase A), MMP-9 (gelatinase B) and MMP-12 (macrophage elastase). These MMPs can, when combined, degrade a similar spectrum of proteins like enzymes released from activated neutrophils. MMPs are also believed to facilitate leukocyte migration and infiltration into injured tissues (Kumagai, Ohno et al. 1999; O'Donnell, Breen et al. 2006). Further, recent evidence suggests that macrophage-derived MMPs can induce neutrophil influx probably via the release of the pro-inflammatory cytokine tumor necrosis factor-α (TNFα) with the subsequent up regulation of adhesion molecules. In turn, this event can cause endothelial activation and the recruitment of inflammatory cells resulting in tissue breakdown (Churg, Wang et al. 2003). In support of this, Barnes demonstrated a direct relationship between the density of alveolar macrophages in the parenchyma and the severity of lung destruction in emphysematous human lung tissue (Barnes 2004).

Neutrophils

Neutrophils are the front-line defensive cells of the immune system and a source of reactive oxygen metabolites, inflammatory cytokines, lipid mediators, antibacterial peptides and tissue damaging enzymes (Dubravec, Spriggs et al. 1990; Tetley 1993; Hiemstra, van Wetering et al. 1998)

Neutrophils are short-lived (up to 48 hours) and transient cells that are produced in the bone marrow. The development of mature neutrophils from stem cells occurs via three

stages (myeloblast, promyelocyte and metamyalocyte) and the cells are then released into the blood stream (Bainton, Ullyot et al. 1971). Neutrophils are usually recruited to the airways from the circulation as part of the lung's secondary defences in response to the presence and release of chemoattractants, where they are widely accepted to be a central effector cell (Sampson 2000). The passage through the interstitial space is a rapid event and the cells are usually found in the circulation or in the airways (Stockley 2002).

The primary role of neutrophils is to kill and eliminate both endogenous and exogenous stimuli by phagocytosis (Stockley 2002). The cells store high levels of serine proteases like NE, cathepsin G (CTSG) and proteinase 3 (PR3) within azurophil granules. Following neutrophil activation, the granules undergo exocytosis whereby the enzymes diffuse, contributing to the degradation of extracellular matrix components (Borregaard and Cowland 1997).

Now it is becoming clearer that there are several factors influencing the migration of the neutrophils into the airways. Among them and the most studied today is the chemoattractant CXC chemokine, IL-8, which is found to be increased in bronchoalveolar lavage fluid (BAL) from patients with interstitial lung disease, with adult respiratory distress syndrome or with stable COPD (McCrea, Ensor et al. 1994; Drost, Skwarski et al. 2005). In support of this, current smokers with chronic bronchitis had significantly elevated sputum IL-8 levels compared to ex-smokers (Hill, Bayley et al. 2000).

Other investigators suggest that macrophage-derived LTB_4 is the major neutrophil chemoattractant in the peripheral airways of COPD subjects. Elevated levels of elastase, released from activated neutrophils, can stimulate macrophages to secrete LTB_4. In turn, this may lead to an amplification of neutrophil recruitment and lung tissue damage (Hubbard, Fells et al. 1991). In addition, low levels of endogenous inhibitors such as α_1-antitrypsin (AAT) can further contribute to the connective tissue destruction observed during this recruitment process (Stockley 1999) (Figure 2). Moreover, chronic bronchitis patients with genetically deficient AAT plasma levels demonstrated greater inflammation in the larger airways with significantly increased LTB_4 and neutrophil elastase activity (Hill, Bayley et al. 2000).
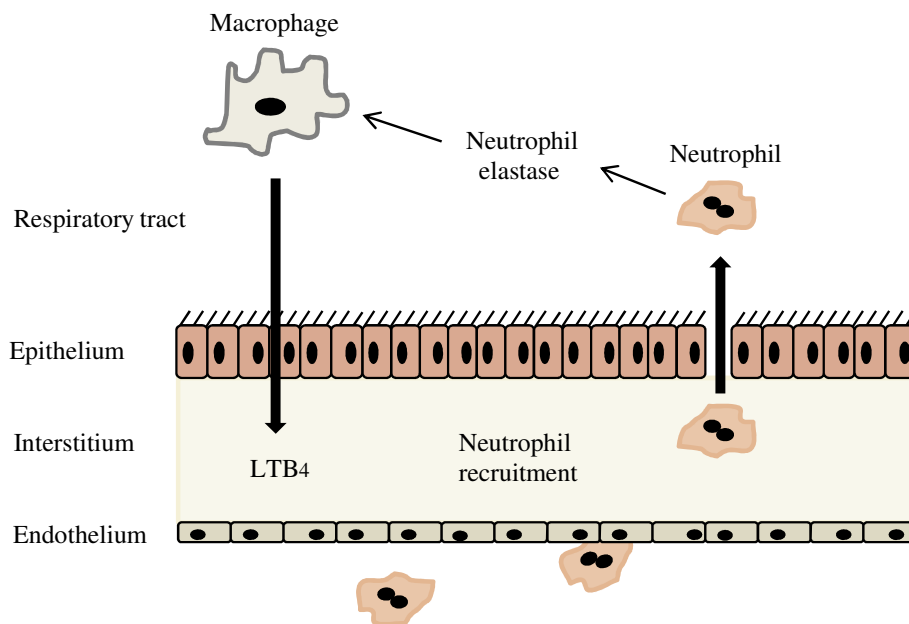
Figure 2. Recruited neutrophils release elastase, which produce an area of connective tissue destruction in the airways. In conditions such as α_1-antitrypsin deficiency, inadequately inhibited neutrophil elastase stimulates the release of LTB_4, which may result in amplifying the inflammation by further neutrophil recruitment.

Structural cells

Epithelium

In addition to its role as a mechanical barrier, the airway epithelium functions as a regulator of tissue responses to external stimuli such as cigarette smoke, inhaled particulate pollutions and toxic substances. In COPD, airway and alveolar epithelial cells are an important source of inflammatory mediators and proteases. When activated by, for example, cigarette smoke, epithelial cells produce mediators such as TNF□, IL-1□, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8 and intercellular adhesion molecule 1 (ICAM-1) (Mio, Romberger et al. 1997; Hellermann, Nagy et al. 2002; Floreani, Wyatt et al. 2003). Both TNF□ and IL-1□ can induce fibroblast proliferation, and IL-1β can increase synthesis of fibronectin and collagen, and thereby contribute to tissue remodelling. GM-CSF accelerates the proliferation of neutrophil maturation and the release of mature cells into the circulation, which

increases the circulating neutrophil count (Lord, Bronchud et al. 1989; Chung 2001). Further, IL-8 released from the epithelium is a neutrophil chemoattractant that can activate neutrophils by inducing a transient change in shape, a rise in and exocytosis from intracellular storage organelles leading to respiratory burst (Chung 2001).

It has been demonstrated that epithelial cells in the small airways can induce fibrosis by secreting transforming growth factor β (TGFβ) (Takizawa, Tanaka et al. 2001). Airway epithelial cells are also important in the defence of the airways (Adler and Li 2001). Goblet cells in the epithelium are a part of the innate defence system by secreting mucus containing defensins and other cationic peptides with antimicrobial effects but are also involved in tissue repair processes (Aarbiou, Rabe et al. 2002).

Finally, the epithelium has also been shown to secrete antioxidants as well as antiproteases, such as secretory leukocyte protease inhibitor (SLPI) (Barnes, Shapiro et al. 2003).

In chronic bronchitis and COPD, the airway epithelium often shows squamous metaplasia, which may result from increased proliferation of airway epithelial cells. In smokers, studies have demonstrated that increased proliferation of basal airway epithelial cells correlated with tobacco consumption and in patients with chronic bronchitis, with the degree of squamous metaplasia (Demoly, Simony-Lafontaine et al. 1994). The nature of the growth factors involved in epithelial cell proliferation, cell cycle, and differentiation in COPD are not yet known. However, epithelial growth factor receptors show increased expression in airway epithelial cells of smokers, which may contribute to basal cell proliferation (Franklin, Veve et al. 2002).

Endothelium

The lung vascular endothelium compromises approximately one-third of the body endothelium and in combination with the lining fluid, endothelial cells (ECs) are a major defence against inhaled xenobiotics in the lungs (Danilov, Gavrilyuk et al. 2001). Next to the liver cells, vascular ECs are the most metabolically active cells when it comes to detoxifying xenobiotics, irrespective of whether they come via the blood stream or from inhaled cigarette smoke (Voelkel 2003).

At the sites of inflammation, the activation of the endothelium allows macrophages and neutrophils to attach and then pass through the vascular wall. In turn, the tissue leukocytes may then release cytokines such as TNFα and other factors resulting in chronic inflammation (Subramaniyam, Virtala et al. 2007).

As the airway disease progresses, the lung vascular ECs become dysfunctional, undergo apoptosis, ischemia, proteolysis and fibrosis, and eventually remodelling of the airways takes place (Voelkel 2003).

Activation of ECs and intima thickening, found in pulmonary arteries, may lead to pulmonary hypertension which is a characteristic feature of COPD (Peinado, Barbera et al. 1998; Santos, Peinado et al. 2002). Vascular endothelial growth factor (VEGF), initially linked to the structural maintenance and growth of the endothelium has been shown to be important in the protection against endothelial apoptosis. In an animal emphysema model, blocking of the VEGF-receptor results in emphysema in the absence of inflammatory cells (Kasahara, Tuder et al. 2000).

It has been proposed that lung vascular endothelium becomes more thrombogenic causing thromboembolic complications in COPD patients under the influence of oxidative stress and in inflamed tissue (Lesser, Leeper et al. 1992).

ECs are reported to bind the two serine protease inhibitors α_1-antitrypsin (AAT) and α_1-antichymotrypsin (ACT) and it has been suggested that these inhibitors might protect them against proteases released from neutrophils (Forsyth, Talbot et al. 1994).

SERINE PROTEASE INHIBITOR (SERPIN)

One important requirement in the maintenance of homeostasis in tissues is the regulation of proteolytic enzymes by endogenous inhibitors. The majority of these inhibitors are present in human blood plasma and account for more than 10% of the total protein content. In plasma, there are several classes of protease inhibitors but the vast majority are targeted towards the serine proteases and are therefore referred to as serpins (serine protease inhibitors) (Carrell and Travis 1985). Several hundred serpins have been identified both intracellularly and extracellularly in a wide variety of viruses, plants and higher eukaryotes. Members of the serpin family have been proposed to play a role in blood pressure regulation, hormone transport, inflammation,

complement activation, angiogenesis, apoptosis, blood coagulation etc (Potempa, Korzus et al. 1994; Irving, Pike et al. 2000) (Figure 3).
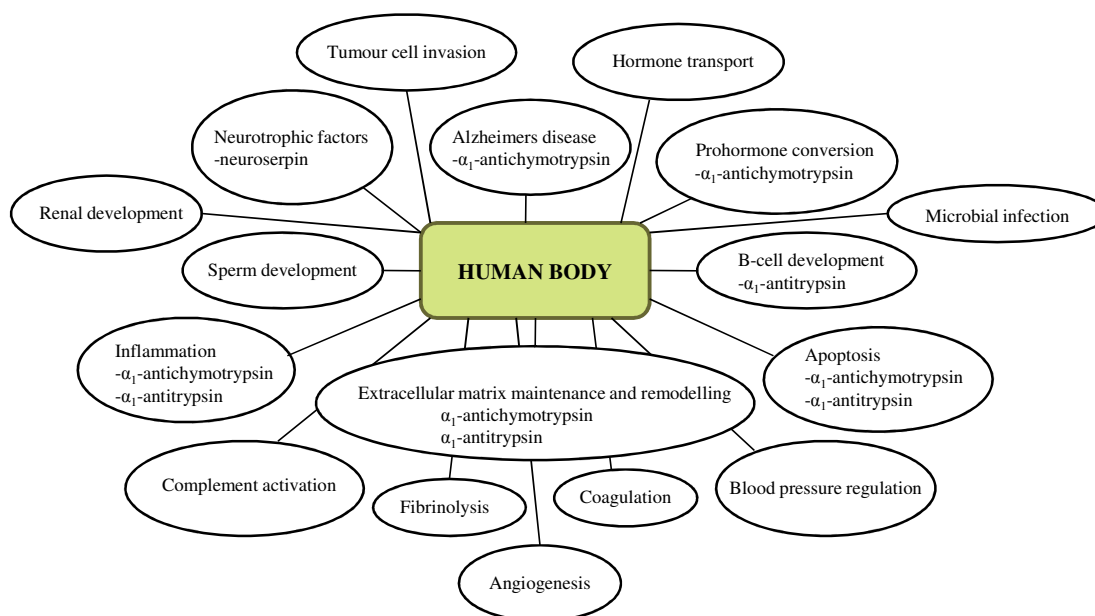
Picture modified from Silverman et al, 2001.

Figure 3. Biological functions of human serpins.

Crystal structures have shown that members within the serpin family have a remarkable structural homology, more than 30% amino acid sequence similarity and a conserved tertiary structure (Carrell and Travis 1985). The conformation required for the inhibitory activity consists of the conserved secondary structure comprised of β-sheets A, B and C and at least 7 □-helices (the most typical members have 9). The mobile reactive centre loop (RCL) contains an amino acid sequence that is presented and functions as a pseudo substrate for the target protease (Kaslik, Kardos et al. 1997). In 1983 Löbermann et al first recognized that serpins undergo dramatic conformational changes when cleaved by the protease that they inhibit (Whisstock 1998). Serpins inhibit their target protease by means of a unique suicide substrate mechanism.

First, the metastable inhibitor presents its RCL as an ideal substrate and upon interaction with the protease, the flexible RCL is cleaved at the P_1-P'_1 bond and inserted into the middle of the β-sheet A. Upon complete loop insertion, the bound

protease is inactivated and translocated by a "mousetrap" action from the upper to the lower pole of the serpin which forms a stable, irreversible complex with the inhibitor (Kaslik, Kardos et al. 1997) (Figure 4).
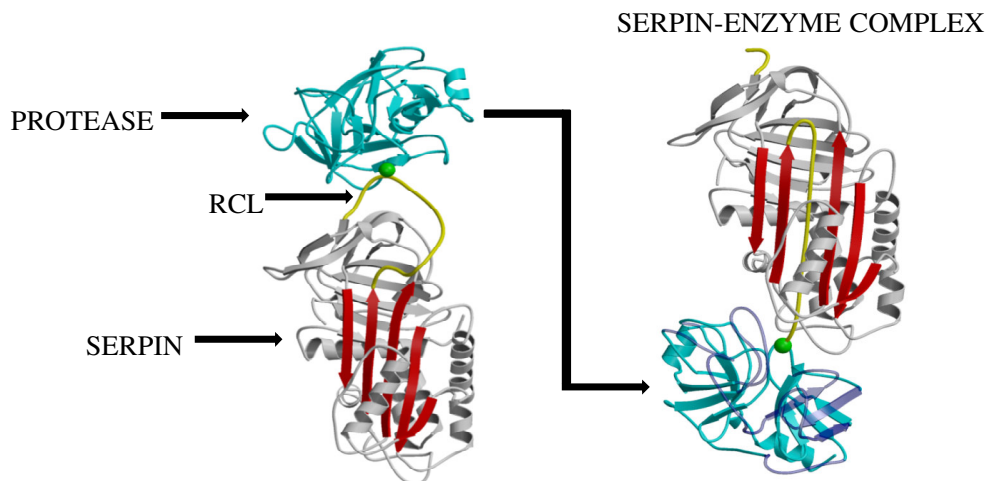
The picture is a modified version of illustrations provided by courtesy of Dr Robin Carell (Carrell and Lomas 2002).

Figure 4. The unique substrate suicide mechanism of the serpins is illustrated by the interaction of α_1-antitrypsin and its target protease, neutrophil elastase.

The requirements for the effective inhibition of the serpins include the length of the critical RCL and appropriate residues within the loop that are associated with a rapid and favourable insertion into β-sheet A (Gettins, Patston et al. 1993). The "mouse trap" action of the serpins is critical in maintaining homeostasis but it is also considered to be the "Achilles heel", since any change, such as oxidation and genetic aberrations in structure and/or secretion, can reduce the functional activity of the serpin molecule, resulting in substantial pathological problems (Potempa, Korzus et al. 1994; Lomas, Belorgey et al. 2004).

α₁-antitrypsin (AAT)

α_1-antitrypsin (AAT), is an archetypal member of the serpin family, which was first isolated and described as an inhibitor of trypsin in 1955 (Schultze, Heide et al. 1962). The protein is relatively small with a molecular weight of 52kD and composed of 394 amino acid residues (Huber and Carrell 1989) (Figure 5).
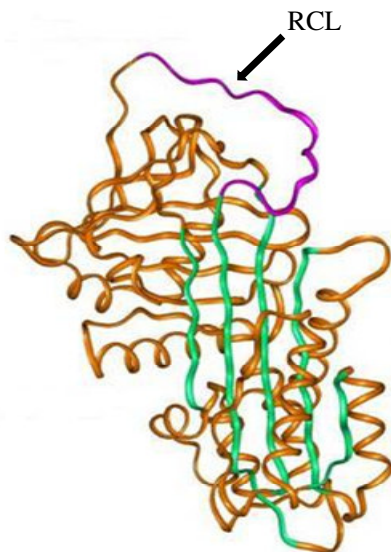
Native α₁-antitrypsin

The picture is an adapted version of a picture included in Janciauskiene, 2001.

Figure 5. The structure of the native α_1-antitrypsin molecule.

Originally, AAT was named because of its ability to inhibit pancreatic trypsin but today it is also known to irreversibly bind both PR3 and CTSG, though the main target protease is predominately NE (Schultze, Heide et al. 1962; Beatty, Bieth et al. 1980; Rao, Wehner et al. 1991).

AAT is an acute phase protein with rapidly increasing plasma concentrations, up to three- to four- fold above normal, in response to inflammation (Morgan and Kalsheker 1997). The normal concentration of AAT in plasma is 20-53µM (Travis 1988). It is mainly synthesised by hepatocytes and, to a lesser extent, by macrophages and intestinal and bronchial epithelial cells (Eriksson, Alm et al. 1978; Mornex, Chytil-Weir et al. 1986; Perlmutter, Daniels et al. 1989; Cichy, Potempa et al. 1997). After synthesis, AAT is secreted into the blood stream from where it readily enters tissues in

the lung and protects from proteolytic attack by forming complexes with the target protease, NE (Carrell and Lomas 2002). As mentioned above, AAT traps the protease by presenting its reactive centre loop (RCL), having a methionine residue situated at position 358.

Once formed, it is suggested that the complex is removed from the circulation either by the serpin-enzyme (SEC) receptor expressed on hepatocytes as well as in mononuclear cells, or by the hepatic low density lipoprotein receptor-related protein (LRP) (Perlmutter, Glover et al. 1990; Kounnas, Church et al. 1996).

AAT can be detected in various biological fluids such as tears, semen, milk, saliva and bile (Ordonez, Manning et al. 1983; Aroni, Kittas et al. 1984). In addition, AAT expression has been identified in a variety of human tissues including kidney, stomach, small intestine, spleen, thymus, adrenal glands, ovaries, testes and pancreas (Mornex, Chytil-Weir et al. 1986; Carlson, Rogers et al. 1988).

AAT synthesis can be up-regulated by pro-inflammatory cytokines like TNFα, IL-6, IL-1 and directly or indirectly by TGFβ. Knoell et al demonstrated that LPS, IL-1 and TNF up-regulate both AAT protein and mRNA, thereby indicating an increase in AAT synthesis (Knoell, Ralston et al. 1998). Moreover, IL-6 has been recognized as the main regulator of the AAT synthesis in hepatic cells (Baumann and Gauldie 1994). Perlmutter et al indicated in their study that IL-6 can regulate AAT gene expression both in extrahepatic tissue such as blood monocytes and in hepatic cell types (Perlmutter, May et al. 1989). Oncostatin M, a member of the IL-6 family, is a potent stimulator of AAT synthesis in a human epithelial cell line. This synthesis, at both the protein and mRNA level, can be either down-regulated by interferon (IFN) or up-regulated by TGF (Boutten, Venembre et al. 1998).

When considering that monocyte AAT production is augmented by neutrophil elastase and LPS, local regulation of AAT may be an important front-line defence mechanism particularly in the microenvironment of local inflammation (Knoell, Ralston et al. 1998).

<u>Native AAT</u>

AAT is not just an inhibitor of NE, increasing evidence today suggests that AAT might have other functions such as up-regulation of β-cell differentiation (Jeannin, Lecoanet-Henchoz et al. 1998), and contribute to the host defence against bacterial pathogens by binding to and neutralizing pathogenic proteins secreted from *E.coli* (Knappstein, Ide et al. 2004).

In addition to anti-inflammatory effects on monocytes and neutrophils, *in vitro* (Janciauskiene, Larsson et al. 2004; Janciauskiene, Zelvyte et al. 2004), AAT can also inhibit the production of neutrophil superoxide (Bucurenci 1992) and induce the release of monocyte-derived IL-1 receptor antagonist (Tilg, Vannier et al. 1993). AAT protein has also been shown to inhibit TNFα-induced self expression in a cell line of human lung ECs, *in vitro* (Subramaniyam, Virtala et al. 2007).

When smoke-exposed transgenic mice, expressing extremely low levels of AAT, were treated with exogenous AAT, both plasma AAT levels and elastase inhibitory capacity were increased. Moreover, smoke-induced elevations in lavage neutrophils and matrix breakdown products were abolished. Treatment with AAT for 6 months provided partial protection against emphysema and abolished smoke-mediated increases in TNFα (Churg, Wang et al. 2003). It has also been shown that pre-treating rabbits with AAT infusions can attenuate endotoxin-induced acute lung injury (Jie, Cai et al. 2003). Previous *in vitro* studies have demonstrated that AAT can protect both lung ECs, vascular smooth muscle cells and β-cells against apoptosis possibly through the inhibition of caspase-3 activity (Ikari, Mulvihill et al. 2001; Petrache, Fijalkowska et al. 2006; Zhang, Lu et al. 2007). *In vivo* findings show that over-expressing AAT by using gene delivery with recombinant adeno-associated virus, significantly reduced insulitis and prevented the development of overt hyperglycemia in nonobese diabetic (NOD) mice (Song, Goudy et al. 2004; Lu, Tang et al. 2006). Also, administration of clinical-grade human AAT prolongs pancreatic islet allograft survival and exhibits islet-related cytoprotective effects (Lewis, Shapiro et al. 2005).

Oxidized AAT

Oxidized AAT, a modified form of AAT, is found in inflammatory exudates at levels of about 5-10% of total AAT (Wong and Travis 1980; Ueda, Mashiba et al. 2002). For example, oxidation of the AAT protein can be achieved *in vitro* by incubating the protein with myeloperoxidase, stimulated phagocytes or N-chlorosuccinimide (Johnson and Travis 1978; Wallaert, Gressier et al. 1991; Van Der Vliet, Nguyen et al. 2000). *In vivo*, oxidized AAT has been found in inflammatory synovial fluid obtained from rheumatoid joints and in BAL fluid from patients with acute or chronic bronchitis (Maier, Leuschel et al. 1992; Zhang, Farrell et al. 1993).

The oxidized form of AAT has lost its inhibitory activity on free and membrane-bound neutrophil elastase which was demonstrated in a study by Korkmaz et al (Korkmaz, Attucci et al. 2005). In addition, Moraga et al showed that monocytes stimulated, *in vitro*, with oxidized AAT released significantly higher levels of monocyte chemoattractant protein 1 (MCP-1), IL-6, and TNF□, and increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity (Moraga and Janciauskiene 2000).

Polymerized AAT

The AAT molecule is also vulnerable to conformational changes that allow inter-molecular linkage leading to the formation of polymers. The formation of AAT polymers might involve the generation of an unstable intermediate, which in turn can generate polymers or latent protein (Dunstone, Dai et al. 2000). Under conditions of optimal pH, temperature and concentration, native AAT is reported to form polymers *in vitro* (Devlin, Chow et al. 2002; Purkayastha, Klemke et al. 2005).

In 2002, staining of ECs with a specific monoclonal antibody raised against hepatic AAT from severe AAT deficient subjects (PiZZ), showed that AAT was attached to the cells in a polymerized form in subjects with both normal (PiMM) and genetically deficient AAT plasma levels (Janciauskiene, Dominaitiene et al. 2002). Moreover, the polymerized Z protein was also detected in the lung epithelial lining fluid (Mulgrew, Taggart et al. 2004). Followed by these findings, both pro and anti-inflammatory properties of polymeric AAT have previously been published.

First, it was shown that polymers induced neutrophil shape change and stimulated myeloperoxidase release and neutrophil adhesion (Parmar, Mahadeva et al. 2002). Second, both normal M AAT polymers formed by heating and mutant Z AAT polymers have been demonstrated to stimulate neutrophil adhesion and chemotaxis (Mulgrew, Taggart et al. 2004). In parallel, Aldonyte et al demonstrated concentration-dependent effects of polymerized AAT on human monocytes, *in vitro*. In contrast to higher concentrations, lower concentrations of polymerized AAT significantly increased expression of TNF□, IL-6, IL-8, MCP-1 and nuclear factor κB (NFκB) p50 activation (Aldonyte, Jansson et al. 2004).

In addition, Mahadeva et al showed that especially Z polymers co-localize with neutrophils in emphysematous alveoli and that they are chemotactic in a mouse model (Mahadeva, Atkinson et al. 2005). Though, *in vitro*, Persson et al failed to demonstrate similar pro-inflammatory effects of polymeric AAT on human neutrophils suggesting that the observed effects might be critically dependent on the presence of other cell activators, bacterial or otherwise (Persson, Subramaniyam et al. 2006).

AAT DEFICIENCY (AATD)

In 1963 Laurell and Eriksson reported, for the first time, the absence of the AAT band on protein electrophoresis of serum taken from a patient with obstructive lung disease (Laurell CB 1963). Later, in the 1970's it was shown that neutrophil elastase, introduced into the respiratory tract of animals, induced emphysema. This finding then contributed to the formulation of the protease-antiprotease hypothesis (Senior, Tegner et al. 1977).

AATD is a hereditary disorder characterized by a severe diminution in plasma AAT levels. Mutations in the AAT gene can result in the failure of processing and secretion due to misfolding of the protein and intracellular accumulation (Mulgrew, Taggart et al. 2007).

Epidemiology

AAT deficiency is a relatively common but under-recognized genetic condition (1997). It is more common in Caucasians but increasing evidence demonstrates that AATD affects all major races worldwide (de Serres, Chest 2002, de Serres, Genet Med 2005).

The Z allele, causing a severe plasma deficiency, is most prevalent in the southern Scandinavia and the north-west European seaboard, with reducing gene frequencies towards the south and east of the continent (Blanco, de Serres et al. 2006).

Depending on where studies have been conducted, the prevalence reported for the Z phenotype varies between one in 5097 and one in 1600 (O'Brien, Buist et al. 1978; Sveger 1978).

After the divergence of races, 66 generations or 2000 years ago, the Z allele was believed to arise from a single origin and the high frequency in southern Scandinavia implies that the mutation arose in the Viking population. The time of origin suggests that the allele arose when the Vikings populated mid- or southern Europe and before the migration to Scandinavia (Cox, Woo et al. 1985; Blanco, Fernandez et al. 2001). Between year 800 and 1100, it is likely that the Z allele of AAT was distributed across northern Europe by the Viking raiders, then to the United States and during the past 200 years it has been spread over the rest of the world during migration (Blanco, Fernandez et al. 2001). Today, more than 90% of the clinical AATD cases are caused by the PiZZ mutation (Coakley, Taggart et al. 2001)

The heterozygote state (PiMZ) occurs in about 4% of the Caucasian population and is more prevalent in northern than in southern Europe (Blanco, de Serres et al. 2006).

The S allele, in contrast, causes only a mild plasma deficiency and is most common in southern Europe, becoming less frequent moving north-east. The S allele is more common in the United States than in northern Europe compared to the frequencies of the Z allele, that in the United States are similar to the lowest numbers in Europe. AATD is infrequent in Asia, in Africa, in the Middle East and Japan, but, when present in Japan, it is usually the result of the Siiyama mutation (Seyama, Nukiwa et al. 1995; de Serres 2002). Also, in Sardinia, a genetically isolated island, the most

common cause of severe AATD is the Mmalton mutation (Ferrarotti, Baccheschi et al. 2005).

The date of origin of the S allele appears to have arisen in the north of the Iberian Peninsula and then similarly introduced into North America by mass migration (Blanco, Fernandez et al. 2001). The MS heterozygote state occurs in about 4-11% of the white population in Europe, highest in the Iberian Peninsula and lowest in Scandinavia (Hutchison 1998; Blanco, de Serres et al. 2006).

Genetics

The AAT protein is encoded by the protease inhibitor (Pi) locus located on the q arm on chromosome 14. The Pi locus is a 12.2-kilo base pair gene, composed of seven exons, 4 coding (II, III, IV, V), 3 non-coding exons (IA, IB, IC) and six introns; with exon V containing the region coding for the reactive centre loop (Lai EC, Kao FT, Law ML, Woo SL. 1983) (Figure 5).

Non-coding exons IA, IB and IC   Coding exons II-V
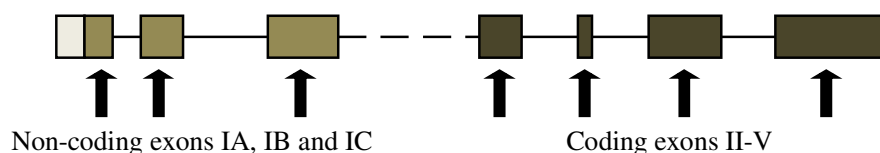
Picture adapted from Wood et al, 2007.

Figure 5. The structure of the AAT gene.

After translation, the 418-amino acid protein, including a signal peptide, is post-translationally modified in the endoplasmatic reticulum and its carbohydrate side chains are modified in the cis-Golgi apparatus before packaging and release of the 52kDa glycosylated protein (Carrell 1986).

Genotypes

AATD is inherited as an autosomal co-dominant disorder, indicating that both AAT alleles are expressed in cells. Mutations of the AAT gene have been categorized into four groups: base substitutions, in-frame deletions, frame-shift mutations, and exon

deletions. More than 100 alleles have been reported and classified by using the Pi nomenclature, which assesses AAT mobility using pH gradient isoelectric focusing (IEF) analysis (Hutchison 1990; de Serres 2002). Traditionally, each variant is then identified by its speed of migration on the IEF gel electrophoresis, the most common forms being F (fast), M (medium), S (slow) and Z (very slow) (Fagerhol and Laurell 1970). It is the changes in the amino acid composition that contribute to the alteration in the speed of movement through the gel, explained by the variation in protein charge (Fagerhol and Laurell 1967). Diagnosis relies on nephelometry (protein concentration determination), IEF phenotyping and polymerase chain reaction genotyping findings (Mulgrew, Taggart et al. 2007).

Individuals that are homozygous for the normal M allele are designated PiMM genotype and a heterozygote for the Z gene is PiMZ. Finally, mutations that cause severe deficiency of AAT occur in those who are homozygous for the Z allele (PiZZ). Variants may also be classified by their effect on AAT level and function categorizing AAT alleles into four groups: Normal, Null, Deficiency and Dysfunctional.

Normal M AAT alleles are characterized by a serum AAT concentration of 20-53μM. Alteration in gene expression, translation or abnormal intracellular processing might interrupt synthesis and contribute to serum/plasma deficiency or as in the Null variant, total absence of AAT protein in serum/plasma (Q variant). The genetic mutation associated with the Null variant is characterized by the deletion of a single base which forms a stop codon within the AAT gene (Brantly, Nukiwa et al. 1988).

Deficient alleles typically lead to a serum AAT concentration of less than 20 μM, and for some variants (Z), decreased functional activity of AAT. The most common deficiency variants are the S and the Z mutation and the homozygous Z and S variants are associated with serum level of about 10-15% and 60%, respectively, of the normal MM (Brantly 1996) (Table 1). Both the Z and the S mutation result from a single nucleotide polymorphism. In the S variant there is a substitution of a valine residue for a glutamate at position 264. The glutamate residue forms a stabilizing salt bridge resulting in a misfolding and increased turnover of the molecule leading to lower plasma levels (Curiel, Chytil et al. 1989; Elliott, Stein et al. 1996).

A mutation in the Z allele is characterized by the substitution of one lysine for glutamic acid at position 342 which causes a conformational change in the reactive loop opening up β-sheet A, allowing the insertion of a reactive loop of a second molecule, contributing to the formation of a chain of AAT molecules or polymers. The Z abnormality differs from the S in that it is rather a problem of secretion than synthesis, demonstrated by the fact that the gene is normally translated but 85% of the produced protein is retained within the endoplasmatic reticulum. Recent evidence has even indicated the possibility of polymer formation outside the hepatocyte (Lomas, Evans et al. 1992; Elliott, Bilton et al. 1998; Janciauskiene, Dominaitiene et al. 2002). Dysfunctional alleles (F variant) code for an abnormal form of the protein, which, whilst present at detectable level, does not function normally because of reduced binding to NE (Stoller and Aboussouan 2005).

Pi genotype	Serum level, range (μM)
MM	20-53
MS	18-52
SS	20-48
MZ	15-42
SZ	10-23
ZZ	3-7

Table adapted from Mulgrew et al, 2007.

Table 1. Common AAT genotypes and serum levels.

Clinical features

Pulmonary manifestations

The most common clinical manifestation of severe (PiZZ) AATD in smokers is the development of early onset (by the age of 40-50 years) emphysema with severe airflow obstruction (Abboud 2006). Smoking has been estimated to shorten the lifespan of AATD individuals by up to 20 years and 75-80% of the subjects develop COPD (Larsson 1978).

Target screening studies have revealed that approximately 1-4.5% of patients with COPD have underlying severe AATD (2003).

COPD, defined as chronic irreversible airflow limitation, is characterized by a slowly progressive and irreversible deterioration in lung function. The destruction of the lung parenchyma, emphysema, and the inflammation in the central and peripheral airways (bronchioles) are pathological hallmarks of COPD (1987; 1997; Pauwels 2001). Individuals with the very rare homozygous null variant are at greatest risk of developing emphysema (Luft 1999; Perlmutter 2002). Moreover, it is still not entirely clear whether heterozygotes are predisposed to lung disease but abundant evidence suggest that PiMZ subjects are at little or no risk of emphysema, even though their plasma AAT levels are about 57% of normal (Bruce, Cohen et al. 1984; Dahl, Tybjaerg-Hansen et al. 2002; Hersh, Dahl et al. 2004).

Between 4 to 35% of AATD subjects have a history of reactive airway disease including pulmonary symptoms such as dyspnea, wheeze, cough, sputum production and lower respiratory tract infections (Larsson 1978). Since many of the symptoms of AATD are non-specific this might contribute to the delay in diagnosis, which is 5.6 years on average (Stoller, Sandhaus et al. 2005).

In severe AATD, smoke exposure is likely to be the most important determinant of lung function decline. Even at 18 years of age detectable differences can be seen between smokers and non-smokers and, in adults, a significant relationship exists between smoking and decline in lung function (Piitulainen and Sveger 1998; Piitulainen and Eriksson 1999). In addition, other determinants of more rapid decline in lung function include low body mass index, male sex, frequent exacerbations and the severity of upper zone emphysema (Dawkins PA 2006).

In general, pulmonary function, determined by lung function tests or spirometry, is maintained in most AATD individuals until the third to fourth decade of life, though with the exception of a possible tendency to develop reactive airways disease. In never smokers, pulmonary function can be maintained until the fifth or sixth decade of life (Wall, Moe et al. 1990). The variable yearly reduction of forced expiratory volume in one second (FEV_1) in smokers with severe AATD is 80-150ml although some studies suggest even more accelerated rates (Buist, Burrows et al. 1983; Janus, Phillips et al.

1985; Wu and Eriksson 1988; Cook, Janus et al. 1994). In contrast, healthy individuals and non-smokers demonstrate a more modest lung function decline of 15-20ml and 60-100ml, respectively (Mulgrew, Taggart et al. 2007).

Today, several mechanisms are recognized as contributing to the pulmonary damage in the Z AATD homozygote. First, low plasma/serum AAT levels and an excess of neutrophil elastase, released from azurophilic granules of active polymorphonuclear leukocytes, results in an imbalance between the protease and its inhibitor. As aforementioned, the excess of neutrophil elastase originates from increased neutrophil recruitment to the lungs, probably due to the release of various chemoattractants such as LTB_4 (Stockley 2000).

Second, it has recently been recognized that the Z mutation also favours the spontaneous formation of AAT loop-sheet polymers within the lungs. The existing inflammatory mileu within the lungs of severe AATD subjects might accelerate the formation of polymers which is increased at low pH level due to, for example, cigarette smoke (Lomas and Mahadeva 2002). In turn, these polymers have been suggested to contribute additionally to the destruction of lung tissue by stimulating neutrophil adhesion and chemotaxis (Mulgrew, Taggart et al. 2004).

Furthermore, the role of *in vivo* inactivation of AAT is still a controversial issue but it remains possible that this mechanism might be of importance. The available Z protein is 50-80% less effective at inhibiting neutrophil elastase than normal M AAT (Perlmutter 2000). In addition to the already less effective inhibitory capacity of the Z AAT protein, exogenous (cigarette smoke) or endogenous (phagocytes) sources can decrease the rate of association of the inhibitor with neutrophil elastase by converting the methionine residues at or near the RCL of the AAT molecule. An alternative inactivation mechanism, proteolytic cleavage, can be produced by enzymes from either endogenous (tissue metalloproteinase's) or exogenous (proteases released by pollens or microorganisms) sources. The two inactivating mechanisms might interact, since oxidation of the AAT molecule rapidly makes it more susceptible to proteolytic cleavage by non-target proteases (Sveger, Thelin et al. 1997; Campbell 2000; de Serres 2002; Khan, Salman et al. 2002; Luisetti, Miravitlles et al. 2002).

Hepatic disease

For individuals with phenotypes Z, Mmalton and Siiyama alleles, characterized by intra-hepatocyte polymerization, liver disease, (including hepatitis, cirrhosis, and hepatoma) represents another clinical manifestation of AATD (Stoller and Aboussouan 2005). The intra-hepatocyte polymerization can be explained by a conformational rearrangement of the molecule forming chains of several AAT molecules, polymers, within the endoplasmatic reticulum causing liver disease (Stoller and Aboussouan 2005).

AAT deficiency is the most common genetic cause of liver disease in children and it predisposes adults to chronic liver disease and hepatocellular carcinoma (Eriksson, Carlson et al. 1986; Teckman, Qu et al. 1996). In children, AATD is the most frequent metabolic disease leading to liver transplantation. In the PiZZ population, approximately 10-15% develop liver disease and 5% of them will require liver transplantation within the first 4 years of life (Francavilla, Castellaneta et al. 2000; Carrell and Lomas 2002). Individuals that carry a heterozygote variant of the PiZ type have an increased risk for chronic liver disease and if the disease develops, the clinically relevancy of this genetic defect will become pertinent only in middle-aged or elderly adults. Sveger et al carried out a nationwide prospective screening study in Sweden showing that only 10-15% of subjects within the PiZZ population developed clinically significant liver disease over the first 20 years of life (Sveger, Thelin et al. 1997).

Other clinical manifestations

Disequilibrium between proteolytic enzymes and their inhibitor AAT might contribute to other clinical manifestations like diabetes, arterial aneurysm, systemic necrotizing vasculitides and panniculitis. In diabetic individuals, several studies have reported altered variations in measured plasma/serum AAT levels compared to controls. For example, serum AAT levels have been shown to be higher in all stages of pregnancy with diabetes type 1 compared to healthy pregnant women (Lisowska-Myjak, Sygitowicz et al. 2001). McMillian et al demonstrated a marginal increase in serum AAT concentration in adult diabetes, principally type 2 (McMillan 1989). In contrast,

other studies demonstrate a lower mean AAT concentration in combination with a lower AAT inhibitory activity in diabetic subjects compared to controls (Lisowska-Myjak, Pachecka et al. 2006; Hashemi, Naderi et al. 2007). In addition, a significant inverse correlation between serum trypsin inhibitory activity (s-TIC) and the duration of diabetes suggested that diabetes is associated with plasma s-TIC (Hashemi, Naderi et al. 2007).

Further, an abdominal aortic aneurysm, characterized by dilation of the aorta involving expansion and thinning of the layers of the arterial wall, can be caused by the protease-antiprotease imbalance which might lead to structural abnormalities of the extracellular matrix (Elzouki and Eriksson 1994; Dahl, Tybjaerg-Hansen et al. 2003; Vila, Millan et al. 2003). Despite a growing number of examples, like the eightfold higher frequency of the Z allele in the London intracranial aneurysm data, between AATD and the development of aneurysm, no significant relationship has been found (Elzouki and Eriksson 1994).

Systemic necrotizing vasculitides is a heterogeneous group of conditions associated with widespread focal and segmental inflammation combined with acute infiltrates of polymorphonuclear leukocytes, and later monocytes, and fibrinoid necrosis in blood walls and perivascular interstitial spaces (Mazodier, Elzouki et al. 1996).

As mentioned, AAT is a naturally occurring inhibitor of NE and PR3 which are the two target antigens of anti-neutrophil cytoplasmic antibodies (ANCA). In patients with anti-PR3 antibodies, an increased incidence of AAT phenotypes associated with dysfunctional AAT or low serum levels has been reported (Savige, Chang et al. 1995).

Though, panniculitis caused by severe AATD in adults and children is quite rare, there are around 40 cases of AATD with panniculitis reported in the English literature to date (Ortiz, Skov et al. 2005).

In addition, AAT replacement therapy has also been shown to control fibromyalgia symptoms in PiZZ deficient individuals (Blanco, Canto et al. 2004).

Treatment

Several strategies, including the correction of the primary genetic abnormality, increasing hepatic production and/or the release of AAT, and the augmentation of the pulmonary interstitial anti-protease defensive screen, have been explored in the treatment of AATD. In addition, there are numerous non-specific treatment options of AATD like bronchodilators, preventive vaccination against pneumococcus and influenza, corticosteroids, long-term oxygen therapy and nutritional therapy, etc (Mulgrew, Taggart et al. 2007). Apart from specific therapeutics measurements, it is critical that AATD subjects do not smoke, decreasing the likelihood of developing pulmonary disease (Coakley, Taggart et al. 2001).

Augmentation therapy

Since serum deficiency of AAT followed by the reduced protection against NE in the lung underlies the lung disease in AATD, a logical approach would be to treat it by trying to raise the circulating level of AAT (Turino, Barker et al. 1996). AAT enters the lung by passive diffusion which determines the concentration of AAT both within the interstitium of the lung and in the secretions lining the airways. Early physiological studies on albumin diffusion have demonstrated that the epithelium, in contrast to the relatively permeable endothelium, is a major restrictive barrier to protein movement which has an important implication when deciding the treatment dose and route of administration (Gorin and Stewart 1979) (Figure 6).

Picture adapted from Abusriwil et al, 2006.

Figure 6. Concentration in the epithelial lining fluid taken from clinical studies for the MM and ZZ genotypes. Concentrations for SZ are predicted using the same gradient (Gorin and Stewart 1979).

Epidemiological studies have demonstrated that non-smoking individuals with PiSZ phenotype, having plasma AAT levels of 11-14µM, appeared to be at lower risk for developing lung disease (Turino, Barker et al. 1996). On the basis of these observations, a minimum AAT plasma level of 11µM was likely to be protective, thereby preventing NE-mediated destruction of lung parenchyma. Early studies indicated that it was possible to infuse AAT into the plasma of deficient subjects resulting in an increase of AAT in the lung lining fluid, indicating that diffusion of plasma had taken place (Gadek, Klein et al. 1981).

In 1989, after observing that weekly infusions of human purified pooled plasma AAT (60mg/kg) were shown to achieve the desired protective threshold, augmentation therapy were approved in the USA for patients with genetic deficiency associated with the PiZZ or Pi-null genotypes (1989).

The aim of infusion therapy is to reduce the excessive decline in lung function or to slow down the progression of emphysema, but is not expected to repair emphysema or improve lung function (Abboud 2006).

As aforementioned, several studies have assessed and demonstrated the biochemical effectiveness of intravenous infusions with pooled AAT by raising serum concentration above the protective threshold in combination with increased anti-elastase activity in BAL (Wewers, Casolaro et al. 1987). To investigate clinical effectiveness, numerous outcome measurements like the rate of FEV_1 decline, change in computed tomography (CT) densitometry and frequency of exacerbations have been studied (Stoller and Aboussouan 2005). Results from a large observational cohort showed that although treatment reduced mortality rate in the recipients, no significant difference regarding rate of FEV_1 decline in the overall group was found. However, a subgroup analysis demonstrated a significant reduction in FEV_1 decline in patients with FEV_1 of 35-49% predicted (1998). In a randomised, double-blind, placebo-controlled trial of augmentation therapy Dirksen et al failed to show any difference in

FEV_1 decline between augmentation and placebo recipients, though a trend towards slower loss of lung tissue (by CT scan) was noted in augmentation recipients (Dirksen, Dijkman et al. 1999).

After more than 15 years of use of AAT augmentation therapy, the overall opinion with respect to the safety of the treatment is that it is generally well tolerated and without important side-effects (Wencker, Banik et al. 1998; Stoller, Fallat et al. 2003).

Since only a small portion (2%) of the intravenous infused AAT reaches the lung, inhaled AAT might be a more efficacious method of administration (Hubbard, Brantly et al. 1989). Inhalation of AAT has been shown to satisfactorily augment the anti-proteolytic capacity of the pulmonary epithelial lining fluid and the alveolar interstitial lining fluid (Hubbard, Brantly et al. 1989; Hubbard, McElvaney et al. 1989). In addition, after aerosol treatment, the detection of AAT in serum supports the hypothesis that AAT is capable of diffusing across the pulmonary interstitium (Smith, Traber et al. 1989). Twice daily inhalation of 100µg of either native or genetically engineered AAT has been shown to increase the average epithelial lining fluid concentration to "normal" (Hubbard and Crystal 1990). Results from a murine model of COPD supported the use of AAT by demonstrating a reduction in severity of emphysema (Pemberton, Kobayashi et al. 2006).

There are major challenges facing the inhaled AAT treatment including ensuring the delivery of AAT to both the periphery of the lungs and to the interstitium. As demonstrated by inhalation studies, many patients with AAT deficiency have abnormal lungs and patchy ventilation indicating that it is difficult to target abnormal regions of the lung with inhalation therapy (Stolk, Camps et al. 1995). Further studies are needed within this area.

Gene therapy

Since the most direct rationale for a genetic disorder such as AATD would be to replace defective or absent genes within a cell to make it function normally, a remaining important concept in increasing lung AAT is gene therapy. In animal models, studies on gene therapy have used retroviral (Kay, Baley et al. 1992), adenoviral (Rosenfeld, Siegfried et al. 1991; Kay, Graham et al. 1995), adeno-

associated viral (Song, Embury et al. 2001; Lu, Choi et al. 2006) and liposomal (Alino, Crespo et al. 1994) vectors to transfect cells. As recombinant adeno-associated viral vectors are capable of achieving therapeutic levels of AAT and are less likely to induce an inflammatory response than adenoviral vectors, so far, they have been the most successful delivery system (Song, Embury et al. 2001; Lu, Choi et al. 2006). Moreover, the specific site for incorporation into the human genome (ASV site), carried by the vector, gives the genetic material the potential for long-term expression (Wood and Stockley 2007). The transfection of airway cells with the AAT gene has been shown to increase the concentration of AAT in the lung. Studies have suggested that vectoral secretion of AAT might be a potential strategy for targeting the protein to the interstitium (Thompson, Nielson et al. 1996).

A number of difficulties are associated with gene therapy. For example, an amount of 10-20mg of AAT capable of reaching the pulmonary interstitium must be synthesized each day (Coakley, Taggart et al. 2001).

At present, the primary limiting factor is the low transfectional efficacy with a relatively limited production of AAT and when using the currently available vectors, gene transfection remains an unproven and potentially harmful procedure.

Targeting protein polymerization and secretion

AATD is often referred to as a conformational disease due to the aberrant intermolecular aggregation of protein. This makes it possible for both general as well as specific approaches to treatment like the current attempts that are aimed at blocking the formation of aggregates, or increasing the turnover of the accumulated protein (Burrows, Willis et al. 2000; Devlin, Parfrey et al. 2001). Still, blocking the formation of the aggregates does not need to be complete but sufficient to allow the degradation mechanism of the cell to cope with the aggregates. In the presence of competitive agents, the formation of serpin oligomers and polymers can be slowly reversed, though the pharmaceutical challenge is to deliver them in an effective way (Lomas, Evans et al. 1993).

Stem cells

A potential treatment for AATD is the use of stem cells, although such approaches will need further development before clinical use. It might be possible to facilitate normal production of AAT in deficient individuals by transplanting stem cells that have differentiated into liver cells and are capable of expressing AAT (Saito, Yoshikawa et al. 2006; Zhou, Huang et al. 2007). Increasing the production of normal AAT will not prevent accumulation of polymers within the liver, whereby this approach will not affect liver manifestations of the disease. Since it is possible to differentiate human embryonic stem cells into alveolar epithelial (type II) cells, targeting the lung might be an alternative approach (Wang, Haviland et al. 2007).

Lung transplantation

Currently, severe pulmonary parenchymal lung damage cannot be reversed and if supportive therapy and supplemental oxygenation/ventilation fail to achieve adequate tissue oxygen delivery, one must consider surgical approaches.

In a cohort of COPD patients, lung volume reduction surgery (LVRS) was shown to be most favourable in individuals with heterogenous, upper zone disease (1999; Koebe, Kugler et al. 2002). Although the results of LVRS in AATD subjects are less impressive, the best effects are most likely to be found in patients with heterogeneous disease (Tutic, Bloch et al. 2004).

Over the past two decades, the incidence of lung transplantation in AATD individuals has increased exponentially over time and accounts at present for 10-15% of all lung transplantations in the United States. Because the actuarial survival in patients with obstructive lung disease is better after transplantation, the increase in procedures is likely to continue to be the case also in the future. In addition, lung transplantation has been shown to increase both lung function and exercise tolerance (Coakley, Taggart et al. 2001).

The most commonly performed procedure is the single-lung transplantation (Mal, Andreassian et al. 1989). Due to the relatively young age of the candidates, a higher proportion of patients with AATD than COPD undergo double-lung transplantation,

which has been suggested to improve the outcome for emphysema subjects (Low, Trulock et al. 1992).

Liver transplantation

Since the cause of hepatic disease in AATD is due to the pathological accumulation of abnormal AAT in hepatocytes, no expected benefits with exogenous AAT, raising AAT plasma levels, are to be found. Currently, the only corrective therapy associated with good results and survival in AATD children and adults with severe liver disease is liver transplantation (Hood, Koep et al. 1980; Gartner, Zitelli et al. 1984). The hepatic transplantation should, in theory, arrest the progression of emphysema by correcting the protease/antiprotease imbalance. Although, the effect of the procedure on the AAT plasma levels and lung deterioration is less well characterized (Coakley, Taggart et al. 2001). Also, another potential beneficial effect is the possibility to seed lymphoreticular cells from the transplanted liver to the native lungs. The production of AAT from these cells might contribute to the anti-protease defensive screen in the lung (Starzl, Demetris et al. 1992).

THE PRESENT INVESTIGATION

HYPOTHESIS

The serine protease inhibitor AAT is an important part of the regulatory mechanism in inflammation by inhibiting the activity of serine proteases, particularly neutrophil elastase, derived from inflammatory cells and tissue. Inherited AAT deficiency, characterized by low AAT plasma levels due to intracellular polymerization and blocking of secretion, is clinically linked to the development of COPD and liver disease. In addition, the AAT protein in severe AATD subjects has been demonstrated to be a less efficient protease inhibitor than the normal AAT protein.

Recent studies have demonstrated that AAT plays an important role in limiting ongoing inflammatory processes in our body, not only as an inhibitor of serine proteases but also as an immune-regulator. The properties of the AAT molecular structure that confers protease inhibitory activity makes it sensitive to mutations and environmental factors that might lead to the transformation of the inhibitory form into non-inhibitory by-products. This acquired deficiency, due to decreased levels of functional circulating AAT protein, results in a disturbed protease/antiprotease balance contributing to disease development. On the other hand, the novel biological functions expressed by AAT by-products may play an important role in the pathological processes associated with chronic inflammation and disease progression (Figure 7).
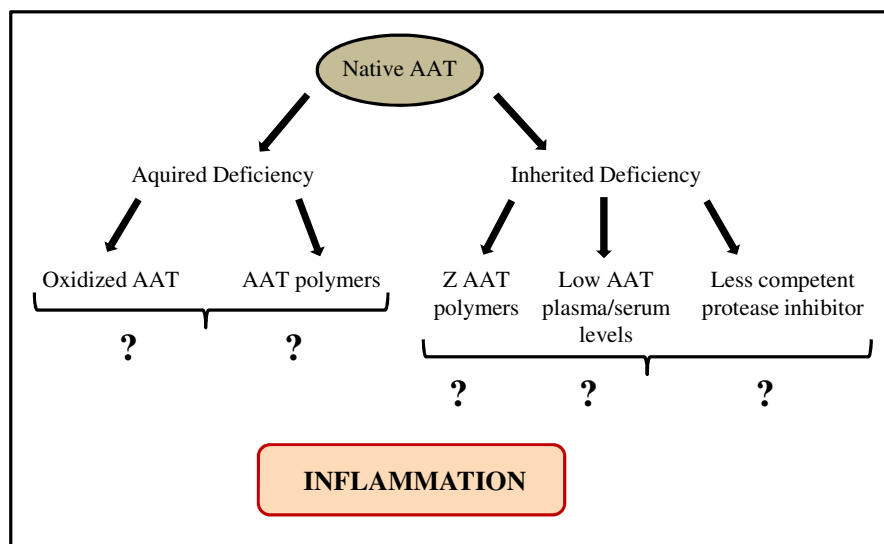
Figure 7. Hypothesis of this present investigation.

AIMS

The aims of this present investigation are as follows:

- To investigate the chemotactic effects of AAT polymers, *in vitro*. (Paper I)

- To examine, *in vitro and in vivo*, the potential role of oxidized AAT in emphysema. (Paper II)

- To demonstrate possible cellular and clinical beneficial effects of replacement therapy in a severe AAT deficient individual with COPD. (Paper III)

- To analyse for differences regarding the soluble and membrane bound form of the endotoxin receptor CD14 between AAT deficient and non-deficient subjects. (Paper IV)

- To elucidate whether there is a link between AAT deficiency and the development of diabetes type 2. (Paper V)

RESULTS AND DISCUSSION

Chemotactic properties of AAT polymers (Paper I)

Due to discrepancies in existing literature, in this first paper we have re-examined whether polymers of AAT can activate neutrophils, *in vitro*.

It is assumed that in severe AAT deficiency emphysema may develop as a result of unopposed serine protease activity in the lungs. The increased protease activity can result from low levels of AAT because of spontaneous polymerization of the Z-AAT, cellular retention and failure of secretion (Brantly 1996; Carrell, Lomas et al. 1996; Janciauskiene 2001; Carrell and Lomas 2002). Now, this assumption is strengthened by the finding that AAT polymers have been found in the lungs and circulation of Z-AAT deficient subjects (Elliott, Bilton et al. 1998; Janciauskiene, Dominaitiene et al. 2002).

Previous *in vitro* studies in our laboratory have demonstrated anti-inflammatory effects of AAT polymers on LPS-induced human monocyte activation (Janciauskiene, Larsson et al. 2004). In support of this, our group also found anti-inflammatory effects of polymerized M-AAT (concentrations up to 0.5mg/ml) on human neutrophils, *in vitro* (Janciauskiene, Zelvyte et al. 2004). In contrast, others have shown that M-AAT polymers can stimulate neutrophil adhesion and that both Z and M-AAT polymers can generate neutrophil chemotaxis, *in vitro* (Parmar, Mahadeva et al. 2002; Mulgrew, Taggart et al. 2004). Recently, Mahadeva *et al* demonstrated that polymeric AAT can cause an influx of neutrophils when instilled into murine lungs and that they co-localize with neutrophils in the alveoli of Z-AAT deficient subjects (Mahadeva, Atkinson et al. 2005).

The plasma profile of young healthy individuals and elderly COPD patients with Z-AAT deficiency was detected by using a polyclonal antibody against AAT and a monoclonal antibody (ATZ11) that is specific for polymerized or elastase-complexed AAT. No elastase-AAT complexes were detected in our plasma samples, though it is worth mentioning that we could not detect any differences in polymer fraction or profile between our healthy and sick AAT deficient subjects. Due to the relatively

similar profile of remarkable levels of polymers in both healthy and sick Z AAT deficient subjects it is unlikely that the presence of AAT polymers *per se* is a factor contributing to the increased inflammation and disease progression in Z-AAT deficient individuals. We detected a double increase in endotoxin content of both M-AAT and Z-AAT after 3h polymerization at 60ºC. Moreover, when polymerizing both M and Z polymers for 6 days at 37ºC we found they contained significantly more endotoxin than native or 3h polymerized AAT. In contrast to previous published data, the chemotactic responses of our protein preparations with low endotoxin content failed to demonstrate any significant chemotactic activity. We continued to evaluate the effects of bacterial contamination by spiking our protein preparations with either LPS or zymosan. We observed a significant stimulation of neutrophil chemotaxis in response to bacterial contaminated proteins. As expected, native and polymerized M-AAT with low endotoxin content had no effects on neutrophil IL-8 release, whereas preparations with high endotoxin content dramatically elevated neutrophil IL-8 secretion. Finally, neutrophils stimulated with zymosan released significant amounts of IL-8 which was abolished by low endotoxin preparations of native and polymeric M-AAT. By contrast, AAT preparations containing high endotoxin contamination were without effect.

Our findings suggest that AAT contaminated with bacterial products express pro-inflammatory activities that differ from the endotoxin-free protein alone.

Potential role of oxidized AAT in emphysema (Paper II)

After exploring the chemotactic activities of AAT polymers on neutrophils, *in vitro*, we continued to investigate the potential role of the oxidized form of AAT (Ox-AAT) in emphysema both *in vitro* and *in vivo*. Uncontrolled proteolytic attack in the lungs is suggested to be an important factor contributing to the development of emphysema (Barnes 2004). Oxidation of the native AAT protein by for example cigarette smoke, neutrophils and free radicals, could lead to an acquired deficiency due to the reduced ability to inhibit neutrophil elastase (Beatty, Matheson et al. 1984; Taggart, Cervantes-Laurean et al. 2000). Despite the fact that modified molecular forms of the AAT

molecule have no inhibitory activity these can exert pro-inflammatory activities and contribute to tissue damage and disease progression. For instance, the oxidized form has been shown to stimulate monocyte activation by elevating levels of MCP-1, IL-6 and TNFα expression (Moraga and Janciauskiene 2000).

In Paper II we demonstrate that treating A549 cells at different time points with different concentrations of Ox-AAT significantly induce both IL-8 and MCP-1 release in a time and dose-dependent manner compared to PBS controls. These findings were not affected by serum-complete or serum-free conditions.

Ox-AAT significantly increased NF-κB activity in A549 cells and the production of IL-8 was inhibited in the presence of a NF-κB inhibitor. Further studies demonstrated that Ox-AAT-induced IL-8 production was inhibited by a JNK inhibitor and that Ox-AAT promoted activation of JNK. Finally, in the A549 cells we investigated the effects of an antibody to low density lipoprotein receptor-related protein (LRP) on the IL-8 and MCP-1 production induced by Ox-AAT. The results revealed that the antibody only partially inhibited the IL-8 and MCP-1 production.

Since A549 is an epithelial cell line we also wanted to further explore the effects of Ox-AAT in human monocytes and macrophages. We observed that neither monocyte chemotaxis nor monocyte adhesion to lung microvascular endothelial cells was affected by Ox-AAT. A recent study demonstrated that monocytes stimulated with Ox-AAT for 24h released significantly elevated levels of MCP-1 (Moraga and Janciauskiene 2000) and therefore we additionally investigated the effects of Ox-AAT on the IL-8 production. Treatment with Ox-AAT for 4h and 24h increased IL-8 release compared to medium alone. Although, we found significant differences when stimulating monocytes with Ox-AAT, macrophages treated with increasing concentrations of Ox-AAT at several time points did not reveal any significant effects on IL-8 and MCP-1 secretion. *In vivo*, Ox-AAT significantly induced JE, the murine homolog to the human MCP-1 gene product, production in the BAL fluid and in the homogenized lung tissue at 4h compared to native AAT but no significant change was found after 24h. Moreover, no significant changes were observed in KC, a functional homolog to the human IL-8 gene product, release in BAL or lung tissue after treatment

with Ox-AAT. Finally, Ox-AAT was found to significantly induce the influx of macrophages but not neutrophils in mice 24h after receiving intratracheal Ox-AAT.

Our results suggest that the oxidized form of AAT can exert pro-inflammatory properties in contrast to the native AAT protein.

The work included in Paper II is the result of a collaboration with the group of Dr Ravi Mahadeva, University of Cambridge, UK. My contributions to the manuscript include the experimental procedures involving human monocytes and macrophages. In addition, I have actively participated in the statistical calculations, preparation of pictures and writing of the manuscript.

Benefits of augmentation therapy in COPD with severe AAT deficiency (Paper III)

In Paper III, we describe the effects of AAT augmentation therapy in a male non-smoking COPD patient with severe AATD. First, the patient was treated weekly or every second week over 8 years (from 1992 until 2000), the therapy was then re-started in summer 2002 and terminated in autumn 2004.

Since the 1980's intravenous infusions of purified pooled AAT have been used to raise serum/plasma AAT levels in individuals with severe AATD (Gadek, Klein et al. 1981). Previous studies in our laboratory have described the *in vitro* anti-inflammatory effects of purified pooled human AAT on LPS-induced TNFα and IL-8 release from human monocytes and neutrophils (Nita, Hollander et al. 2005). In addition, several reports indicate favourable outcomes like the reduction in incidence of lung infection and mortality, the inhibition of nasal LPS-induced IL-8 release and a slower decline in lung function in individuals with initially moderate lung function (1998; Lieberman 2000; Wencker, Fuhrmann et al. 2001; Nita, Hollander et al. 2005). Therefore we wanted to combine the observation of both clinical parameters and cellular effects of augmentation therapy.

First we measured the plasma level of AAT during treatment and after treatment termination which revealed that during treatment the concentration of AAT was above the value that generally is considered to be the protective threshold (11µM) (Turino, Barker et al. 1996). Then we wanted to investigate whether this biochemical "normalization" of AAT levels influences the pathogenic processes involved in COPD. Though, lung function testing, spirometry, did not demonstrate any significant changes and high-resolution computed tomography (HRCT) confirmed progressive emphysema, no exacerbations were recorded in our patient during the observed period of time. Moreover, no clinical signs of liver disease were found after analysing the results from liver enzyme measurement and from the ultrasound.

We did not notice any differences in levels of plasma inflammatory markers (TNFα, IL-1β, IL-6, IL-8, IL-10, SLPI and ACT) during treatment or after treatment termination. By using polyclonal AAT antibody or monoclonal ATZ11 antibody we characterized the molecular profile of AAT in plasma obtained during treatment, before and after infusion, and after termination of treatment. Infusions of AAT lead to a change in variability of molecular forms of plasma AAT.

Interestingly, we demonstrate that circulating neutrophils isolated immediately after AAT infusion released much lower zymosan-induced IL-8 levels compared to neutrophils isolated one week after infusion. Since neutrophils are major effector cells in the airways during inflammation because they release chemotactic factors such as IL-8, the blocking of this release might play an important role in the recruitment and activation of neutrophils in AATD.

Disease susceptibility in AAT deficiency (Paper IV)

Due to recent *in vitro* observations in our laboratory concerning the possible regulatory effect of AAT on monocyte CD14 expression (Nita, Serapinas et al. 2007), we wanted to investigate plasma levels of soluble CD14 (sCD14) and monocyte expression of membrane-bound CD14 (mCD14) in clinically healthy AAT deficient subjects and controls. CD14 is a multifunctional receptor that is expressed on the surface of cells such as monocytes and macrophages but is also present as a soluble form in plasma/serum (Ziegler-Heitbrock and Ulevitch 1993; Bas, Gauthier et al. 2004) (Figure 8).
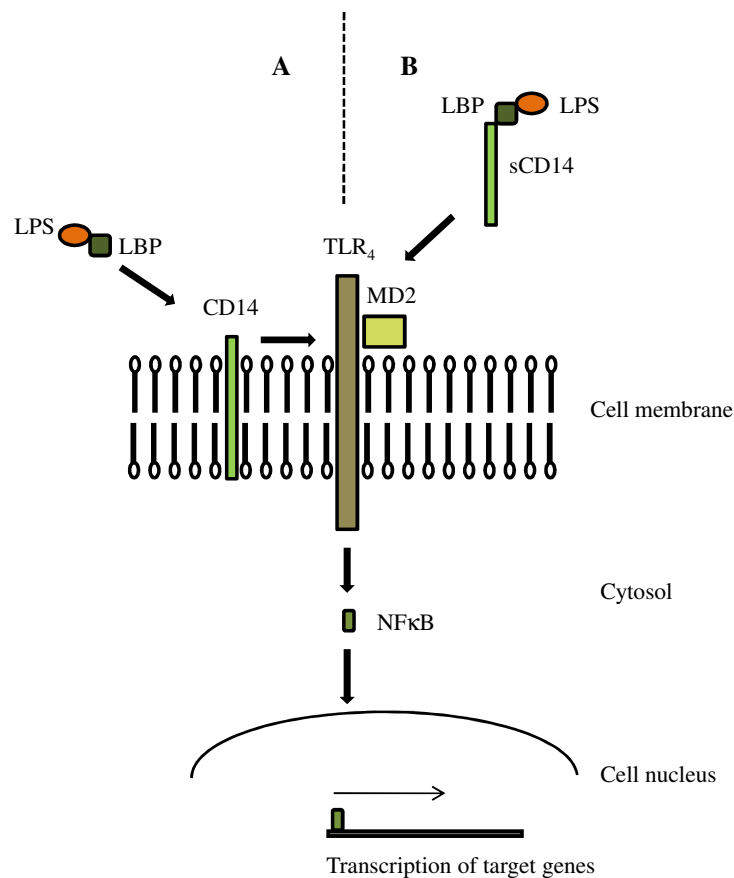
The picture is a modified version of a illustration in Ouburg et al, 2005 (Ouburg, Spaargaren et al. 2005).

Figure 8. The activation of a cell by endotoxin or lipopolysaccharide (LPS).

Panel A: In cells expressing the membrane-bound form of CD14, LPS is bound by the LPS binding protein (LBP) and the complex in turn binds to the glycosylphosphatidylinositol (GPI)-anchored receptor CD14 on the cell surface. The

tertiary complex then activates Toll-like receptor 4 (TLR$_4$) resulting in the release of NF-κB, which initiates the transcription of genes that promote immune and inflammatory responses.

Panel B: In contrast, cells that do not express the membrane-bound form of CD14 can still initiate the transcription of the target genes by the direct binding of the soluble form in complex with LPS-LBP to TLR$_4$.

After stimulation with various agents, it has been demonstrated that monocytes and macrophages release CD14 (Bazil and Strominger 1991; Durieux, Vita et al. 1994). Neutrophil elastase (NE), the main target protease of AAT, has been shown to exert a direct proteolytic effect on mCD14 which contributes to increased levels of sCD14 (Bazil and Strominger 1991; Le-Barillec, Si-Tahar et al. 1999).

Plasma levels of sCD14 were significantly higher in subjects with severe AAT deficiency (ZZ) compared to both SZ and normal MM. It is also worth mentioning that we could not detect any difference in c-reactive protein (CRP) levels between the groups. Since CD14 is predominantly expressed on the surface of monocytes, we next explored the presence of CD14 on the surface and mRNA expression level, as well as the release of sCD14 in monocytes isolated from ZZ, SZ and MM subjects.

By using flow cytometry, we could not detect any significant differences in CD14 surface expression between our three groups, however, QRT-PCR analysis revealed significantly elevated levels of CD14 mRNA expression in ZZ subjects compared to normal MM. In the last set of experiments, we observed an increased release of sCD14 from ZZ and SZ monocytes compared to MM monocytes (not statistically significant).

Taken together, our observations strongly encourage further investigations, which can give us better understanding of the factors that can contribute to the regulation and/or development of inflammation in AATD subjects.

Low plasma AAT levels, deficiency and diabetes mellitus (Paper V)

As mentioned earlier, in addition to inhibitory activity on proteases, AAT can also exert other biological functions. Recently, experimental findings have demonstrated that AAT gene therapy significantly reduced insulitis and prevented the development of overt hyperglycemia in nonobese diabetic (NOD) mice (Song, Goudy et al. 2004; Lu, Tang et al. 2006). Further, the administration of AAT prolonged pancreatic islet allograft survival and exhibited islet-related cytoprotective effects (Lewis, Shapiro et al. 2005). In support of this, a recent published study has demonstrated an association between low plasma AAT levels and trypsin inhibitory capacity (Hashemi, Naderi et al. 2007). Since AAT demonstrates preventive effects on diabetes formation, we propose that deficiencies in AAT quantity and function might increase the risk of developing not only diabetes type 1 but also type 2.

First, as expected, plasma analysis revealed that the diabetics had a significant higher level of glucose, insulin and glycosylated haemoglobin (HbA1c) compared to the controls. In addition, plasma levels of CRP, triglycerides (TG) and LDL (low density lipoprotein)/HDL (high density lipoprotein) ratio were significantly higher in the diabetics as compared to the controls. In contrast, the diabetics showed lower plasma levels of HDL. Regarding AAT levels, there were no significant difference between the diabetics and the controls. However, since lower AAT levels (<1mg/ml) might be related to inherited AAT deficiency we selected study participants with lower AAT levels than 1.0 mg/ml, for phenotyping. Genotypes were based on the results from the phenotyping, IEF. We found that among the diabetics, AAT deficiency genotypes were about 50% more abundant as compared to the non-diabetics. Moreover, in general, plasma AAT correlated with CRP levels and with parameters reflecting the metabolic syndrome. Interestingly, in contrast to the non-diabetics, we found that low (<1mg/ml) AAT levels inversely correlated with waist hip ratio in the diabetics.

In conclusion, the above mentioned clinical observations in combination with the anti-apoptotic experimental findings of AAT suggest that AAT might play a role in the development of both diabetes type 1 and 2. Our findings demonstrate a possible

increased risk of developing diabetes type 2 when individuals possess low plasma AAT levels due to, for example, inherited AAT deficiency or decreased AAT production. Future larger clinical studies are warranted to establish the link between the genotype of AAT and its function in diabetes type 2.

CONCLUDING REMARKS

Native and modified forms of the AAT molecule like polymers and oxidized forms have been shown to exert multiple roles on host responsiveness, proteolysis, inflammation etc. In Paper I, we suggest that endotoxin-contaminated AAT polymers exert pro-inflammatory activation on human neutrophils, *in vitro*. There are also other factors that might contribute to a local pro-inflammatory action of the polymers like pH, their origin, size and/or concentration of the polymers.

In Paper II, we describe both the *in vivo* and the *in vitro* effects of the oxidized form of AAT. Although, oxidized AAT failed to significantly activate human macrophages we observed pro-inflammatory effects on A549 cells, human monocytes and murine monocytes/macrophages.

In Paper III, we wanted to explore the beneficial effects of augmentation therapy in a COPD patient with severe AATD. During the observed study period, our patient had no exacerbation and the amount of plasma inflammatory makers measured was very low or non-detectable. Interestingly, we demonstrated clear evidence for the beneficial use of AAT infusion on zymosan-induced IL-8 release from blood neutrophils which may help limit the cell responsiveness at sites of inflammation.

The increased sCD14 in plasma and the elevated CD14 mRNA levels in monocytes obtained from the severe AATD subjects, suggest that although these subjects are clinically healthy they may harbour markers that reflect a susceptibility to develop disease (Paper IV).

Finally, due to a higher number of AAT deficient individuals among the diabetics included in Paper V, we propose that a possible link exists between low AAT levels, AATD and the development of diabetes type 2.

FUTURE PERSPECTIVES

- Recent studies, such as the ones included in this thesis, have markedly contributed to the further understanding of the regulatory role of AAT in inflammatory processes. Oxidized and polymerized forms of AAT have been detected in patients with chronic inflammatory diseases. Peptides liberated by proteolytic cleavage of AAT have also been isolated from human bile, atherosclerotic plaque, urine and plasma. These have been shown to regulate lipid metabolism, inflammatory cell activation and to inhibit human immunodeficiency virus 1 production. The full spectrum of activities of these various AAT forms is still not known. Therefore, it would be important to explore whether modified forms of AAT, especially cleaved forms, are involved in the pathogenesis of COPD and whether these forms can be used as inflammatory markers to monitor disease progression and patient responses to treatment.

- Since the late 1980's infusions of human-pooled AAT has been used to normalize biochemical levels of AAT in emphysema patients with severe PiZZ AAT deficiency. Augmentation therapy for AAT deficiency-associated emphysema appears to be associated with a marked reduction in the frequency and severity of lung infections. This association must be further evaluated in future studies of AAT augmentation therapy. Our laboratory has demonstrated anti-inflammatory effects of AAT on various cells including primary neutrophils, monocytes and lung microvascular endothelial cells, *in vitro*. It would be of interest to continue investigating AAT anti-inflammatory effects in cell and animal models including studies on biological fluids and cells obtained from COPD patients with and without AAT deficiency.

- In order to identify AAT deficient individuals that are more susceptible to disease it would be helpful to identify markers of inflammation or their combinations. Therefore, we would like to continue to analyse cells and biological fluids obtained from healthy and COPD subjects with and without AAT deficiency.

- Several studies have reported beneficial effects of AAT on the development of diabetes type 1, *in vitro*. Since type 1 and 2 diabetes share similarities in β-cell destruction and pathogenesis, it would be tempting to further explore the link between AAT plasma levels and the development of diabetes in a larger cohort of subjects with type 1 and 2 diabetes.

SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

α_1-antitrypsin (AAT) tillhör familjen serine protease hämmare vars primära uppgift är att skydda kroppens vävnader från nedbrytande proteiner. Proteinet AAT produceras huvudsakligen av leverceller men även av makrofager och epitelceller som täcker insidan av kärlen i tarmen och i luftvägarna. Efter syntes i levern förs proteinet med blodcirkulationen ut till vävnader där AAT kan utöva sin effekt. AAT är ett akut-fas protein, vilket innebär att koncentrationen i blodet kan öka markant under en pågående inflammation för att sedan återgå till normala nivåer.

Ärftlig AAT brist karaktäriseras av låga nivåer av proteinet i cirkulationen. En obalans mellan AAT och det nedbrytande proteinet, neutrofil elastase vilket leder till en nedsatt hämmande aktivitet i framförallt luftvägarna. Detta i sin tur resulterar i en ständigt pågående inflammation och nedbrytande process av lungvävnaden, scenarios länkat till sjukdomen kronisk obstruktiv lungsjukdom (KOL).

AAT kan, förutom dess skyddande effekt mot nedbrytande proteiner, utöva andra viktiga biologiska funktioner såsom anti-inflammatoriska egenskaper. Nyligen har det framkommit att proteinet inte bara finns i en form (naiv) i kroppen utan även som degraderade eller klyvda fragment, oxiderad form och polymerer (kedjor av den naiva formen). Dessa modifierade former förlorar sin skyddande hämmande effekt men kan även inneha, på gott och ont, andra funktioner i vår kropp.

Syftet med studierna i denna avhandling var att undersöka nya biologiska egenskaper hos den naiva och dessa icke-skyddande former och att undersöka AAT proteinets roll i sjukdomsutveckling. Eftersom vår grupp tidigare har påvisat att polymerer av AAT inhiberar den negativa responsen hos de inflammatoriska celltyperna monocyter och neutrofiler, blev vi skeptiska till att andra grupper visade att dessa polymerer istället aktiverade neutrofiler i laborativa försök. Vi upprepade deras försök men kunde inte observera deras resultat. Dessutom gjorde vi tester för att se om kontaminerade proteinpreparationer kunde vara en förklaring till deras uppvisade resultat, vilket de i vår modell kunde (studie I).

Vi fortsatte sedan våra studier med att påvisa stimulerande effekter av den oxiderade formen av AAT i olika celltyper samt i en djurmodell (studie II).

Nästföljande studie syftade till att få mer förståelse gällande vikten av att ha tillräckliga nivåer AAT i vår kropp för att uppnå en skyddande effekt. Vi hade möjligheten att undersöka en AAT-brist patient med KOL som fick behandling med injicerat AAT. Vi kunde observera att injektioner med AAT hämmade den inflammatoriska responsen hos patientens neutrofiler. Neutrofiler är den huvudsakliga källan till neutrofil elastase, som bidrar till den nedbrytande processen av lungvävnad i sjukdomen KOL. Trots att vi inte kunde finna några uppenbarliga positiva effekter på lungfunktionen så uppmättes inga exacerbationer (ett tillstånd då patienten lades in på sjukhus eller behövde en kur med perorala steroider och/eller antibiotika) eller stegringar i analyserade inflammatoriska markörer i blodet under den observerade perioden (studie III).

I studie IV har vi haft privilegiet att jobba med material från unga individer med eller utan AAT-brist. Som tidigare nämnts, är den genetiska AAT-bristen en betydande riskfaktor för utvecklandet av KOL, samt leversjukdom. AAT har föreslagits ha reglerande effekter på förekomsten utav mängden CD14 receptorer, nödvändiga för cellers svar mot endotoxiner (LPS), på ytan utav monocyter. Vi ville undersöka mängden av den cirkulerande formen av CD14 receptorn i blod, samt mängden membran bunden receptor på monocyter från unga individer med eller utan AAT-brist. Till att börja med fann vi högre nivåer av den lösliga formen av CD14 i de individer som hade svår AAT-brist jämfört med kontrollerna. Dessutom fann vi att monocyter från individer med svår AAT-brist uppvisade fler CD14-receptorer samt en högre produktion av den lösliga varianten än celler från kontrollerna. Framtida studier lär påvisa om vårt fynd gällande CD14 kan ha koppling till utveckling av KOL hos AAT-brist individer.

AAT-brist har inte bara en koppling till KOL och lever sjukdom, men även till andra inflammatoriska sjukdomar. I laboratorieförsök har AAT visats ha skyddande effekter på insulin-producerande celler vilket kan bidra till att motverka uppkomsten utav diabetes. Vår grupp ville i den sista studien undersöka om vi kunde hitta något samband mellan låga AAT nivåer i blod, AAT-brist och diabetes typ 2. Vi kunde se att bland diabetes individerna fanns dubbelt så många personer med låga AAT-nivåer och AAT-brist än bland våra undersökta kontroller.

Detta skulle kunna uppvisa ett samband mellan AAT-brist och diabetes (studie V). Sammanfattningsvis har vår grupp funnit nya biologiska egenskaper hos modifierade former av AAT, samt påvisat vikten av att ha tillräckliga mängder cirkulerande AAT i kroppen.

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