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REVIEW

Alpha-1 Antitrypsin: A Potent Anti-Inflammatory and Potential Novel Therapeutic Agent

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Abstract Alpha-1 antitrypsin (AAT) has long been thought of as an important anti-protease in the lung where it is known to decrease the destructive effects of major proteases such as neutrophil elastase. In recent years, the perception of this protein in this simple one dimensional capacity as an anti-protease has evolved and it is now recognised that AAT has significant anti-inflammatory properties affecting a wide range of inflammatory cells, leading to its potential therapeutic use in a number of important diseases. This present review aims to discuss the described anti-inflammatory actions of AAT in modulating key immune cell functions, delineate known signalling pathways and specifically to identify the models of disease in which AAT has been shown to be effective as a therapy.

Keywords Alpha-1 antitrypsin · Innate immune cells · Alpha-1 antitrypsin deficiency · Replacement therapy

Introduction

Alpha-1 antitrypsin (AAT) is a prototypic member of the Serpin (serine protease inhibitors) family of protease inhibitors, and is one of the most abundant active anti-protease found in the human circulatory system. AAT is one of many plasma proteins that comprise the "acute phase proteins" but AAT also demonstrates unique anti-inflammatory properties. Plasma levels of AAT have been demonstrated to increase within hours post inflammation or infection (Perlmutter 2004). This rich source of circulating AAT is primarily produced by hepatocytes within the liver (Rogers et al. 1983); however, other cells, such as epithelial cells (Cichy et al. 1997), monocytes (Carroll et al. 2010), macrophages and neutrophils (Bergin et al. 2010; du Bois et al. 1991), intestinal epithelial cells (Molmenti et al. 1993), alpha and delta cells of human pancreatic islets (Bosco et al. 2005) and cancer cells (Chen et al. 2010) have also been shown to produce smaller quantities of the protein. The AAT molecule is synthesised as a single polypeptide chain that is post-translationally modified by glycosylation through the addition of *N*-glycosidically linked oligosaccharides (Kolarich et al. 2006a, b) that exist as di-, tri- or tetra-antennary structures (Kolarich et al. 2006b) (Fig. 1). The primary role of AAT is to function as a serine protease inhibitor and it has been shown to inhibit a range of proteases derived from degranulating neutrophils including neutrophil elastase (NE), cathepsin G and proteinase 3 (PR3) (Duranton and Bieth, 2003). Moreover, the described anti-protease activity of AAT has been observed for other serine proteases including mast-cell-derived tryptase and chymase (Chen and He 2004; He et al. 2004), lymphocyte-derived granzyme B (Mahrus et al. 2004) and the epithelial-cell-membrane-bound protease matriptase (Janciauskiene et al. 2008). Other serine proteases associated with coagulation (Gallimore 1975; Mast et al. 1991), digestive enzymes (Beatty et al. 1980), kalkriens derived from serum and tissue (Luo and Jiang 2006; Patston et al. 1990) and urokinase (Clemmensen and Christensen 1976) are also inhibited by AAT. In addition, recent evidence has emerged on the ability of AAT to inhibit other classes of proteases including neutral- and aspartic-cysteine proteases (Al-Omari et al. 2011; Petrache et al. 2006) and the matrix metalloprotease, ADAM-17 (Bergin et al. 2010).

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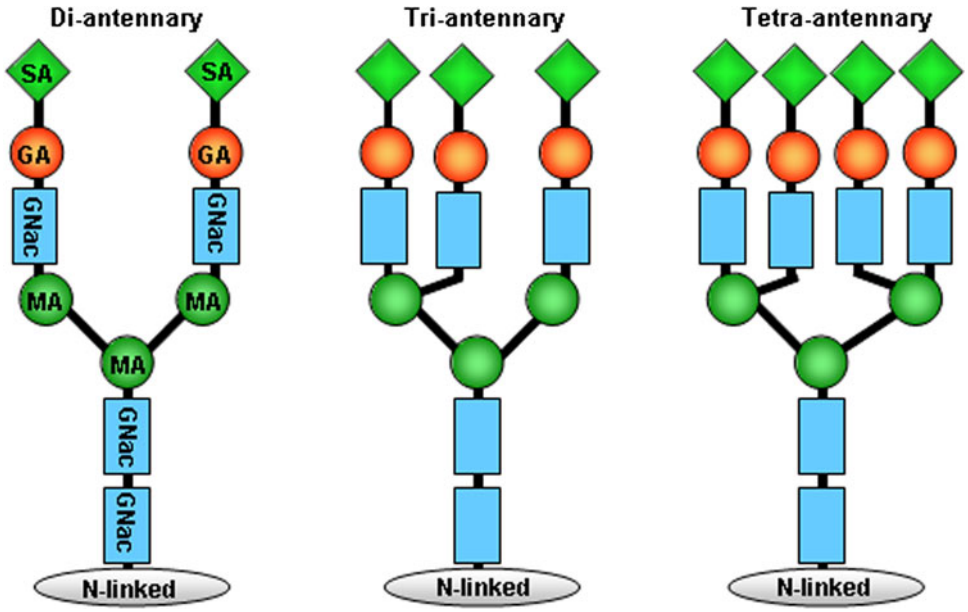

Fig. 1 N-linked glycan structures associated with native AAT. *N*-glycan is composed of sialic acids (*SA*), galactose (*GA*), *N*-acetyl-glucosamine (*GNac*) and mannose (*MA*). *N*-glycans exist in di-, tri- or tetra-antennary structures on serum purified native AAT protein

Of the serine protease inhibitors found in human plasma including alpha-1 antichymotrypsin and alpha-2 macroglobulin, the importance of AAT in maintaining tissue protease:anti-protease homeostasis is demonstrated by the genetic disorder of AAT deficiency (AATD) in which patients are predisposed to the development of lung disease. However, our understanding of the pathophysiology of AATD is currently undergoing a significant reassessment and several lines of evidence have converged to prompt a shift towards emphasis on the anti-inflammatory properties of AAT which impact upon myeloid and lymphoid immune cell function. The role of AAT in maintaining the balance of proteases and anti-proteases is critical to protecting lung tissues and has been extensively studied and reviewed (Greene et al. 2008; Reeves et al. 2010; Tuder et al. 2010). Instead in this review we consolidated the literature in order to discuss the prospective role of AAT as a key anti-inflammatory with the potential of AAT to be utilised as a therapeutic agent in the treatment of a variety of human diseases. Our review of the literature was carried out using the MEDLINE database (from 1966 to 2011), Google Scholar and The Cochrane Library databases using several appropriate generic terms.

The Use of AAT Augmentation Therapy in Treatment of Lung Disease

AAT Deficiency and AAT Augmentation Therapy

AAT deficiency results in AAT serum levels below a putative protective threshold level of 11 μmol/L (Crystal 1990). Clinical presentation of individuals diagnosed with AATD involves early onset emphysema, liver disease (Eriksson et al. 1986; Perlmutter 2002), panniculitis (McElvaney NG 1997; Warter et al. 1972) and anti-neutrophil cytoplasmic antibody associated vasculitis (e.g. Wegener's granulomatosis) (Fig. 2). The most common variants associated with both lung and liver diseases are the Z (Glu342Lys) and S (Glu264Val) mutations (Curiel et al. 1989; Lomas et al. 1992). The Z allele causes the most severe plasma deficiency and occurs in >95% of individuals with AATD, whilst the S allele causes a milder plasma deficiency (Brantly et al. 1988). It has been estimated that in 69 countries with 4.6 billion people, there are almost 1 million individuals who have either the SZ or ZZ phenotype (de Serres et al. 2007). An additional class of *SERPINA1* variants include the rare "null" mutations that results in a complete absence of AAT synthesis, and although not associated with liver disease, this mutation does confer a high risk of devloping emphysema (Fregonese et al. 2008). A common feature associated with the AATD phenotypes, is AAT serum levels below the putative protective threshold level (11 μmol/L).

Therapeutic approaches for AATD include AAT augmentation therapy (Abboud et al. 2001), administration of either natural (elafin (Simpson et al. 2001) or secretory leukoprotease inhibitor (SLPI) (Gibbons et al. 2009)) or synthetic (Sivelestat) anti-proteases (Iba et al. 2006; Yasui et al. 1995) or inhibition of AAT polymerization (Bjork et al. 1992; Chang et al. 1996; Fitton et al. 1997)) and gene therapy (Liqun Wang et al. 2009). Augmentation therapy involves infusion of purified human plasma AAT (60 mg per kilogram of body weight per week) to achieve serum

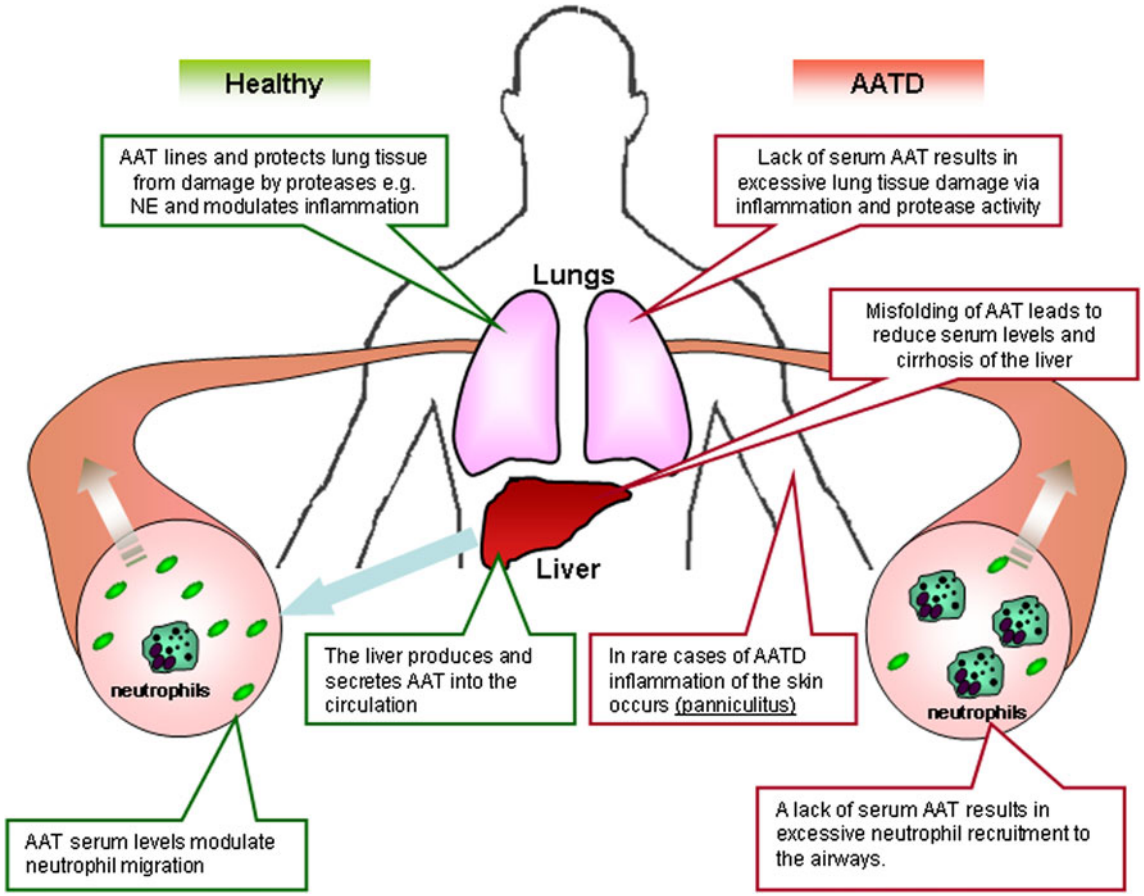

Fig. 2 The importance of AAT and the clinical manifestations of AATD. In a healthy individual AAT is produced primarily by the liver and released into the circulation. It can play an important role in modulating key immune cell activities and protecting the lungs against damage caused by proteases and inflammation. In AATD, the mutation in AAT results in misfolding and retention of the protein in the liver. This causes reduced serum levels of AAT and excessive mobilisation of neutrophils into the airways and extensive lung tissue damage

levels $\geq$11 μmol/L. Augmentation therapy was FDA approved and is now widely used in Europe and North America in the treatment of AATD individuals with lung disease (Abboud et al. 2001). Documented studies on the clinical efficiency of infused AAT augmentation therapy have reported on patient outcome measures including the rate of decline in forced expiratory volume in one second (FEV_1) (Seersholm et al. 1997; Wencker et al. 2001), the incidence of acute exacerbations (Campos et al. 2009) and the change in lung density (Dirksen et al. 1999) calculated by computed tomography scanning (Dirksen et al. 2009).

One of the first studies examining the effect of AAT augmentation therapy compared the ΔFEV_1 of 97 ex-smokers from a Danish AATD register to a German group of 198 AATD (ZZ phenotype) patients receiving AAT augmentation therapy (60 mg/kg). Overall, the ΔFEV_1 in the treated group was significantly lower than in the untreated group, with annual declines of 53 and 75 mL yr^{-1}, respectively ($p = 0.02$). However, there was no beneficial effect of augmentation therapy in 103 patients with initial FEV_1 $\leq$30 or in the 25 patients with FEV_1 >65% (Seersholm et al. 1997). In a further report, one of the main observational studies ($n = 1129$) centred on the NHLBI Registry for individuals with severe AATD (The Alpha-1 Antitrypsin Deficiency Registry Study Group 1998) and showed no overall difference in FEV_1 decline between the augmentation-therapy treated and untreated groups. However, in a subgroup with FEV_1 between 39 and 45% predicted there was a significant difference. The risk ratio for death in augmentation therapy recipients was 0.64, significantly lower than non-recipients ($p = 0.02$) and the risk ratio for individuals receiving augmentation therapy with stage II COPD was 0.21 ($p < 0.001$).

More recently, a meta-analysis suggested that infusion of AAT augmentation therapy could slow lung function decline (Chapman et al. 2009); however, the clinical significance and cost-effectiveness of AAT therapy remains controversial (McCarthy and Dimitrov 2010). Reduced exacerbations or lung infections is an important endpoint in clinical studies of pulmonary disease and recent data from

a German AATD registry (455 with the ZZ and SZ genotype) suggests that a reduction of exacerbations is directly associated with health-related quality of life of patients (Koczulla et al. 2008). Clinicians have attempted to address this important endpoint (Campos et al. 2009; Lieberman 2000; Needham and Stockley 2005) with Lieberman (2000) conducting an internet survey of patient-reported respiratory infections in individuals with the ZZ phenotype, which were markedly decreased after the introduction of augmentation therapy (Lieberman 2000). Post hoc analysis of the EXACTLE study, adjusted for exacerbation severity showed that the treatment group had fewer severe exacerbations than the control group.

Adding to the published reports on the effectiveness of AAT augmentation therapy in severe AATD, a study on urine desmosine levels, a marker of elastin breakdown, showed no change after 8 weeks of augmentation therapy ($p = 0.85$) (Gottlieb et al. 2000). Moreover, in 2009, Dirksen et al. carried out a randomised, double-blind, placebo-controlled, parallel-group study conducted at three European centres. The difference in the decline of lung density between treated and placebo groups suggested a trend towards a beneficial effect for treatment, with p values for treatment ranging from 0.049 to a non-significant value of 0.084 depending on the outcome algorithm chosen. Furthermore, a randomized study by Tonelli et al. examined the decline in FEV_1 in patients enrolled in the Alpha-1 Foundation DNA and Tissue Bank (Tonelli et al. 2009). When adjusted by age at baseline, sex, smoking status, baseline FEV_1% of predicted, the mean overall change in FEV_1 was 47.6 mL/year, favouring the augmented group (ΔFEV_1 10.6 ± 21.4 mL/year) in comparison with the non-augmented group ($\Delta FEV1$ -36.96 ± 12.1 mL/year) ($p = 0.05$). Whilst still not conclusive, the evidence for the clinical efficiency of infused AAT augmentation therapy is continuing to emerge, which in turn lends credence to the concept of AAT as a therapeutic agent in the treatment of a variety of other diseases.

In addition, AAT can be aerosolized directly into the lungs of AATD individuals and a recent study that examined bronchoalveolar lavage (BAL) fluid from PiZZ AATD patients receiving aerosolized AAT therapy demonstrated reduced cathepsin B and matrix metalloproteases (MMP)-2 activity and higher levels of SLPI and lactoferrin (Geraghty et al. 2008). An important question that is only now being addressed is whether the establishment of protective threshold serum levels of AAT (11 μmol/L or 50 mg/dL) in PiZZ AATD patients (Turino et al. 1996), influences inflammatory cells including circulating neutrophils (Bergin et al. 2010) and monocytes (Carroll et al. 2010) known to be important in the development of lung disease.

Studies have demonstrated that AATD individuals who are either homozygous or heterozygous for the Z allele (Rouhani et al. 2000) illustrate increased neutrophil influx into the airways (Malerba et al. 2006). By way of explanation, Mulgrew et al. (2004) demonstrated that polymers of the Z-AAT protein present within the airways can act as a potent chemoattractant, comparable to that of interleukin (IL)-8 in vitro (Mulgrew et al. 2004). In addition, it has been shown that M-AAT polymers can induce significant neutrophil migration, similar to that observed with C5a (Parmar et al. 2002). In contrast, the inhibitory effect of native M-AAT on neutrophil chemotaxis was first documented in 1973, when Ward and Tallemo stated that AATD sera lacked an "inactivator" of neutrophil chemotaxis (Ward and Talamo 1973). More recent evidence has shown that M-AAT is a potent inactivator of fMLP induced neutrophil chemotaxis (Stockley et al. 1990), in part thought to be due to its ability to inhibit protease involvement and thus effecting downstream events, such as cytoskeletal change and polarisation (Aoshiba et al. 1991). This latter phenomenon is further supported by a recent study illustrating the ability of AAT to inactivate calpain-1 activity thereby inhibiting neutrophil directional migration (Al-Omari et al. 2011). In addition, increased neutrophil numbers in the lungs of patients with AATD is thought in part due to a chemotactic process involving the alveolar macrophage. As there is a reduced anti-protease screen present in the AATD airways, NE is free to bind to alveolar macrophages resulting in the production and release of the potent neutrophil chemoattractant, leukotriene B_4 (LTB_4). Indeed it has been shown that free NE causes the same release of LTB_4 by healthy control alveolar macrophages (Hubbard et al. 1991). Moreover, in agreement with the ability of AAT to inhibit LTB_4 release by NE mediated macrophage activation (Hubbard et al. 1991), treatment of patients with AATD (ZZ phenotype) with AAT augmentation therapy has been shown to reduce the levels of LTB_4 in the airways of individuals with AATD (Stockley et al. 2002).

It has also been demonstrated that plasma AAT binds circulating neutrophils and AAT is localised predominantly on the outer surface of the cell membrane (Fig. 3). Localization to the cell membrane may facilitate the ability of AAT to regulate key neutrophil inflammatory responses including cell chemotaxis (Bergin et al. 2010). Moreover, the ability of infused AAT to bind circulating neutrophils supports the use of AAT augmentation therapy and in vivo animal models have been employed to reinforce the ability of AAT to modulate neutrophil migration to the airways. In a hamster model of fibrosis induced by bleomycin, AAT reduced neutrophil and lymphocyte migration after 7 days, with a further decrease in cell counts after 30 days (Nagai et al. 1992). Moreover, in animal models of smoke-induced emphysema AAT significantly reduced the number of infiltrating neutrophils (Churg et al. 2003; Daemen et al. 2000). An additional in vivo model has demonstrated that

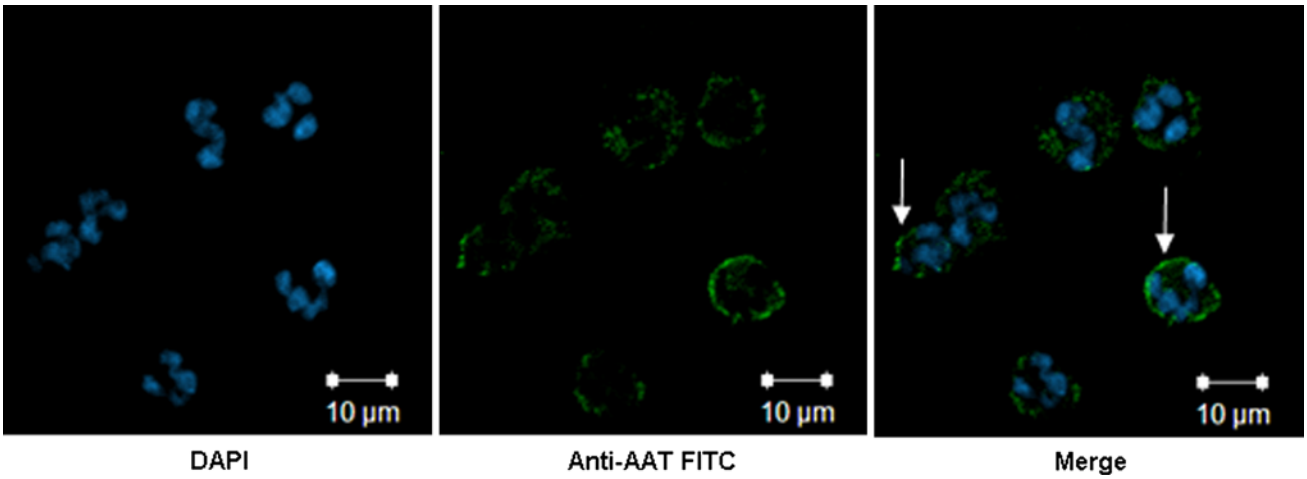

Fig. 3 AAT is associated with the human neutrophil. Confocal immunofluorescence of purified circulating neutrophils. Nuclei are stained with 4′,6-diamidino-2-phenylindole (*DAPI*) which binds to DNA. The localisation of AAT was visualised by a fluorescein isothiocyanate (*FITC*) labelled antibody against AAT. The merged image on right indicates localisation of AAT primarily to the neutrophil membrane (as indicated by *arrows*)

low-dose total-body irradiation inhibited IL-8-induced hematopoietic stem and progenitor cell mobilisation, a phenomenon thought associated with the induction of AAT within the bone marrow (van Pel et al. 2006).

Whilst previous reports suggest that AAT can modulate neutrophil migration via inhibition of serine protease activity, a more recent study revealed that AAT can modulate both IL-8 and soluble immune complex (sIC) induced chemotaxis by divergent pathways (Bergin et al. 2010). This study demonstrated that neutrophil chemotaxis is dependent on opposing gradient concentrations of both IL-8 and glycosylated AAT (Fig. 4) and that AAT:IL-8 complex formation determined CXCR1 engagement, thus negatively impacting upon downstream CXCR1 signalling events including calcium flux, cytoskeleton rearrangements and F-actin formation. This binding capacity of AAT to immune molecules has been previously demonstrated as AAT possess the ability to bind alpha defensins (Panyutich et al. 1995; Spencer et al. 2004; Wencker and Brantly 2005). Furthermore, AAT was found in association with neutrophil membrane lipid rafts, interacting with the glycosylphosphatidyl-inositol linked membrane protein FcγRIIIb. The rationale for this localization was revealed as AAT was shown to control immune complex mediated neutrophil chemotaxis by inhibiting ADAM-17 activity and preventing release of FcγRIIIb (CD16b) from the cell membrane. This AAT-induced modulatory effect was also observed in vivo, in AATD individuals receiving AAT augmentation therapy. Within the study by Bergin et al. (2010), increased serum levels of AAT post augmentation therapy in ZZ-AATD patients was found to bind FcγRIIIb-expressing neutrophils and decrease AATD neutrophil chemotactic responses to that of normal levels. Moreover, circulating neutrophils isolated after augmentation therapy have been shown to release lower levels of IL-8 when compared to neutrophils isolated before therapy (Sandstrom et al. 2008a). This latter case study further supports the potential of AAT augmentation therapy to impact upon neutrophil activity and suggests that infused AAT may attain an anti-inflammatory role in vivo.

An additional key neutrophil function involves production of reactive oxygen species (ROS) by the well-characterised NADPH oxidase multi-component enzyme complex (Fig. 5). Whilst the ability to produce ROS is required for successful microbial killing within the confines of the phagocytic vacuole, uncontrolled extracellular release of these radicals can result in extensive lung tissue damage. The anti-inflammatory effects of AAT on the circulating neutrophil are further consolidated by the fact that AAT was shown to abrogate ROS production elicited by a variety of stimulants including serum-coated zymosan and concanavalin A combined with cytochalasin E from *Aspergillus clavatus* (Bucurenci et al. 1992). A possible mechanism by which AAT may modulate ROS level is by acting as a direct ROS scavenger (Taggart et al. 2000), or by acting on a component of the NADPH oxidase complex as seen for other serum anti-proteases (Kilpatrick et al. 1992) or thirdly, by inhibition of integral membrane-bound proteases involved in ROS generation (Bucurenci et al. 1992) (Fig. 5). Furthermore, the ability of AAT to inhibit anti-proteinase 3 (PR3) antibody-induced ROS production associated with the pathogenesis of Wegener's granulomatosis has been shown to involve a mechanism whereby AAT prevents PR3 antibody cross linking with FcγRIIa on the neutrophil membrane (Rooney et al. 2001).

The immunomodulatory function of AAT on the eosinophil may suggest a possible role for AAT in the

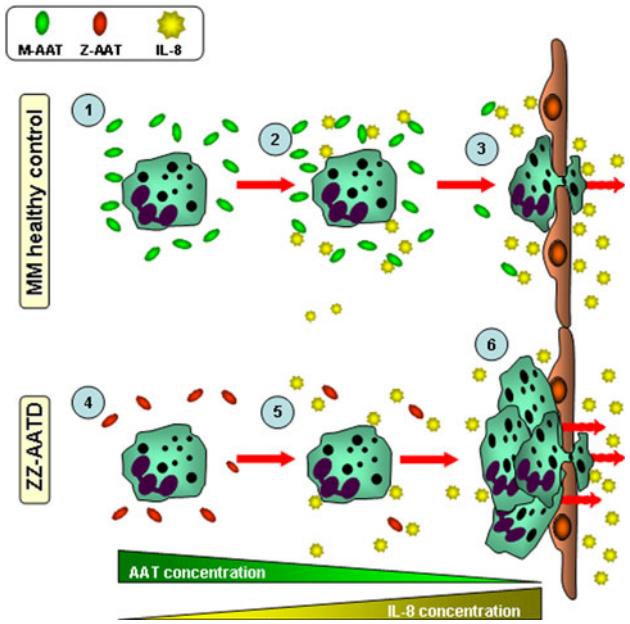

Fig. 4 Inhibition of neutrophil chemotaxis by AAT. Step *1*: in a healthy individual (*MM*), the resting neutrophil is surrounded by high levels of M-AAT. Step *2*: during exposure to increasing levels of IL-8 the cell moves down a concentration gradient of AAT and up a gradient of IL-8 (see Barnett et al. 1999), thus leading to neutrophil migration to the area of inflammation (step *3*). Step *4*: In AATD individuals homozygous for the Z allele, low levels of circulating Z-AAT surrounds the neutrophil. The described AAT gradient is grossly disrupted (step *5*), resulting in increased chemotactic responsiveness of neutrophils (step *6*)

treatment of atopic asthma and other allergic diseases. A number of studies have looked at the prevalence of asthma in homozygous (ZZ) or heterozygous (SZ) AATD individuals, with rates reported between 20 and 50% (Eden et al. 2003, 2006; Palma-Carlos and Palma-Carlos 2007). Many of these studies are problematic and used patient questionnaires on reported wheeze or physician diagnosed asthma, without validating the method of diagnosis. This could certainly lead to over reporting of the prevalence of asthma in this patient group. Moreover, AAT has also been shown to inhibit IgE-dependent and calcium ionophore-induced histamine release from mast cells (He et al. 2004), suggesting that AAT most likely plays a protective role in mast-cell-associated diseases including allergy and asthma.

Whilst case reports exist of AAT augmentation therapy in resistant asthma in individuals with AATD who are homozygous and heterozygote for the Z allele (Blanco et al. 2008), no study has been performed to validate its efficacy. Indeed, given the high price of AAT and the lack of suitably powered studies published to date, the use of AAT in individuals with obstructive lung disease who are not homozygous for the disease is not recommended (Sandhaus et al. 2008).

Cystic Fibrosis Airway Disease

Cystic fibrosis (CF) is caused by mutations to the CF transmembrane conductance regulator (CFTR) gene resulting in defective regulation of chloride transport by epithelial cells. Cystic fibrosis is a common disorder with 1 in 20 Caucasians being heterozygotic carriers of a mutated CFTR gene, resulting in an incidence of 1 in 3,000 live births. Cystic fibrosis is generally diagnosed early in childhood and is characterised by sustained neutrophil recruitment and neutrophil dominated inflammation from a very young age (Birrer et al. 1994; McElvaney et al. 1992). Several studies have investigated the usefulness of AAT in balancing the protease:anti-protease inequity within the CF airways and have explored effective aerosol delivery systems (Brand et al. 2009; Geller and Kesser 2010). Initial clinical studies illustrated the ability of AAT to suppress NE activity within respiratory epithelial lining fluid (ELF) and also reverse the inhibitory effect of CF ELF on neutrophil-mediated killing of *Pseudomonas aeruginosa* (McElvaney et al. 1991). These early studies suggested that AAT may augment host defences in CF and this theory was supported by a recent study by Griese et al. (2007), who showed a decrease in sputum NE activity, neutrophil numbers and levels of pro-inflammatory cytokines (IL-8, IL-1beta, tumour necrosis factor alpha (TNF-alpha) and LTB_4) after 2 and 4 weeks aerosolized AAT treatment compared to baseline measurements. Moreover, a phase II trial to assess the clinical efficacy of aerosolized transgenic AAT showed a trend towards significant improvement in time to first pulmonary exacerbation and total number of exacerbations compared to placebo. Of note, no significant differences in adverse events were reported in this trial and the treatment was well tolerated (Martin et al. 2006). Of interest, none of the described studies found a significant improvement of AAT treatment on lung function. This may be due to the extent of irreversible disease occurring in an older population and the extent of the mucous plugging blocking drug dispersion within the lung, which combined may contribute to the loss of potential anti-inflammatory properties in the adult population (Brennan 2007). Recent trials have also suffered from a lack of a control group and short duration (all inhaled AAT trials have been $\leq$4 weeks) which could impair their ability to adequately assess efficacy. The potential anti-inflammatory effects may be best realised after efficient drug administration to children with early and less-severe lung disease over a longer trial period (Brennan 2007).

An additional point to make concerning aerosolized AAT in treatment of CF relates to the glycosylation state of the protein employed. In the study by Martin et al. (2006) the authors report on the use of non-glycosylated recombinant AAT (rAAT) which was found to be safe and well

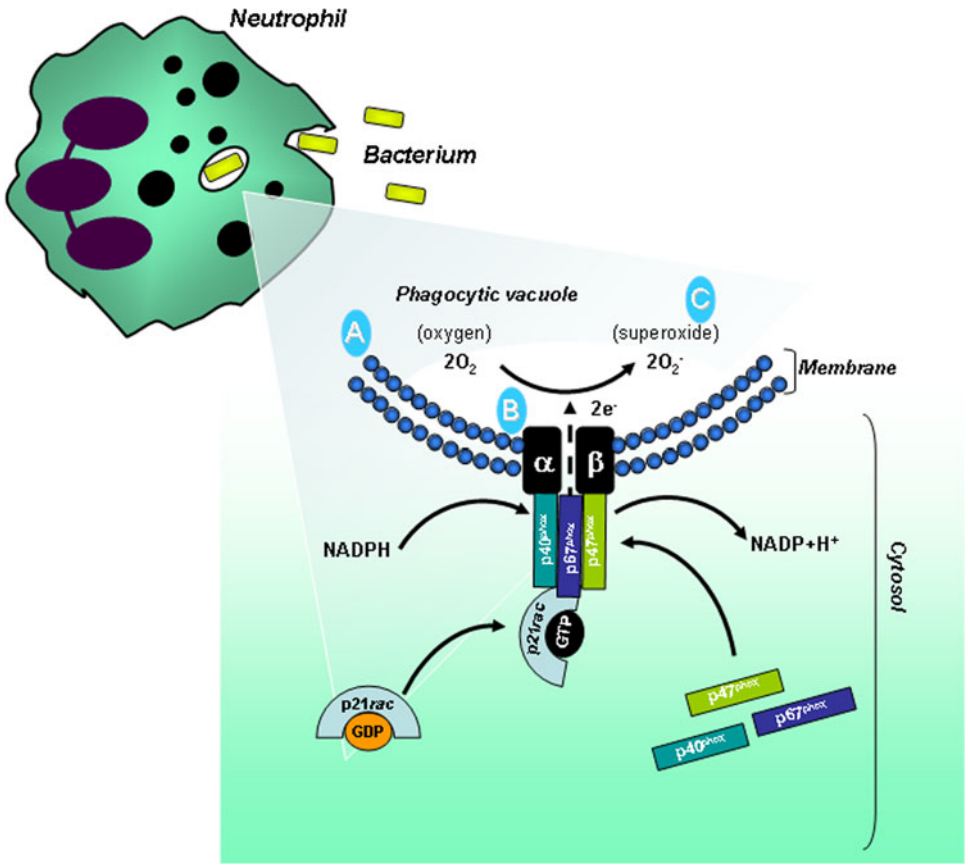

Fig. 5 An overview of the NADPH oxidase complex involved in the production of superoxide by neutrophils in response to the phagocytosis of bacteria. Neutrophils phagocytose bacteria resulting in the formation of the phagocytic vacuole. Upon forming this phagosome the NADPH oxidase multi-enzyme complex is activated. This involves translocation of the cytosolic factors ($p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and p21rac in the GTP bound form) to the membrane of the vacuole to associate with the α and β subunits of the membrane flavocytochrome b_{558}. NADPH donates electrons to generate the first ROS, namely superoxide (O_2^-) within the vacuolar lumen. AAT may inhibit ROS production by inhibition of membrane proteases (*A*), directly acting on components of the enzyme complex (*B*) or by scavenging generated ROS (*C*)

tolerated by patients with CF enrolled within the study. However, overall their data demonstrated a limited effect on inflammation and NE activity (Martin et al. 2006). Conversely however, a study employing aerosolized glycosylated native AAT (nAAT) demonstrated a decrease in NE activity, neutrophil numbers, *Pseudomonas* colonies and reduced levels of pro-inflammatory mediators (IL-8 and TNF-alpha) (Griese et al. 2007). This latter study suggests the importance of glycosylation in augmenting AAT potential and is further supported by results that have demonstrated the ability of glycosylated nAAT to bind IL-8 and inhibit signalling through CXCR1, an effect not observed with non-glycosylated rAAT protein (Bergin et al. 2010). Advances in this field have also shown that hyperglycosylation of AAT, which involves addition of sialic acid residues to nAAT, to increase the inhibitory activity of disialylated AAT against NE and increase the serum half-life of the protein within an animal model (Lindhout et al. 2011).

Chronic Obstructive Pulmonary Disease

A number of studies have shown that both macrophage-derived MMPs and NE are the critical mediators of smoke-induced COPD and emphysema through breakdown of connective tissue within the lung. In a mouse model of emphysema, cigarette smoke was shown to induce a dose-dependent increase in lavage neutrophils, desmosine and hydroxyproline (a marker of collagen breakdown). However, pre-treatment with AAT completely eliminated the acute infiltration of neutrophils and connective tissue breakdown within the airways (Dhami et al. 2000).

Within a cigarette smoke-induced murine model of emphysema or smoke-exposed alveolar macrophage cultures, the anti-inflammatory properties of AAT have been demonstrated to include inhibition of plasma TNF-alpha levels (Churg et al. 2003) and amelioration of MMP-12 production, respectively (Churg et al. 2007). The authors suggested that one possible mechanism for increased MMP-12 production by the macrophage was through activation of proteinase-activated receptor-1 (PAR-1) by serum constituents, such as plasmin/plasminogen and thrombin, which leak into the lung after smoke exposure. The authors proposed that MMP-12 can regulate TNF-alpha release by functioning as a TNF-alpha converting enzyme, releasing the active form of the molecule as reported for other MMPs (Gearing et al. 1994). Of interest, the authors suggest that AAT prevents MMP-12 and TNF-alpha release by inhibiting activity of both thrombin and plasmin. An earlier study on the ability of AAT to inhibit these serine protease revealed association rate constants of

4.8×10^1, 1.9×10^2 and 6.5×10^7 M^{-1} s^{-1} for thrombin, plasmin and NE, respectively (Beatty et al. 1980).

Alveolar epithelial cells are known to produce AAT which may contribute to both the anti-protease defences and anti-inflammatory effects within the COPD and normal lung (Venembre et al. 1994). This local production has been shown to increase in response to inflammatory mediators including oncostatin M, IL-1-beta and treatment with dexamethasone (Boutten et al. 1998; Cichy et al. 1997; Kalsheker et al. 2002). Oncostatin M, a member of the IL-6 family was shown to induce AAT production by human bronchial epithelial cells. This effect of oncostatin M was in turn modulated by TGF-beta and IFN-gamma at both the protein and mRNA level. IFN-gamma decreased oncostatin M-induced AAT production whilst TGF-beta induced a significant and synergistic up-regulation of AAT that was not observed in a hepatocyte cell line (Boutten et al. 1998). The authors of this study postulated that in vivo, production of AAT by airway epithelial cells may play a pivotal role in protecting the lung from acute inflammation through its local production. Pulmonary endothelial cells also play a significant role in inflammation and cell dysfunction participates in the pathogenesis of COPD and in related secondary pulmonary hypertension (Cella et al. 2001; Karoli et al. 2004). A study by Subramaniyam et al. (2008), examined the expression of pro-inflammatory genes in endothelial cells post AAT treatment. Results revealed that neither native nor oxidised forms of AAT induced pro-inflammatory gene expression and that both types of AAT inhibited TNF-alpha-induced self expression. Significantly the authors found that 25% of genes up-regulated by TNF-alpha were inhibited by co-administration of AAT (Subramaniyam et al. 2008).

The production of AAT by both monocytes and macrophages within the airways may be up-regulated in response to lipolysaccharide (LPS), IL-6, IL-1beta, NE and TNF-alpha (Knoell et al. 1998; Perlmutter et al. 1988, 1989). Newly produced AAT or aerosolized AAT may in turn modulate key cellular inflammatory signalling pathways such as the cyclic adenosine monophosphate (cAMP)-dependent pathway. This essential intracellular signal transductor has long been known to effect leukocyte function and its elevation in leukocytes inhibits pro-inflammatory cellular signalling (Bourne et al. 1974). Activators of cAMP, forskolin and an inhibitor of its catabolism, the phosphodiesterase inhibitor rolipram, have been shown to reduce the release of TNF-alpha and increase the release of the anti-inflammatory cytokine IL-10 in response to LPS (Kunkel et al. 1988; Ollivier et al. 1996; Ouagued et al. 2005). AAT has been found to have a similar effect on purified monocytes, by increasing cellular cAMP by activation of adenylate cyclase in both unstimulated and LPS-stimulated cells (Janciauskiene et al. 2007). Importantly this anti-inflammatory action of AAT was found to be independent of its anti-protease activity as both oxidised and heat-inactivated forms of AAT displayed similar inhibitory results (Janciauskiene et al. 2004). Moreover, AAT has previously been shown to inhibit the lethal response to TNF-alpha or LPS in mice (Libert et al. 1996). Conversely however, in an in vitro experimental model of LPS-induced airway inflammation the exposure of monocytes and neutrophils to LPS followed by AAT 2 h later, resulted in a more pronounced increase in TNF-alpha, IL-6 and IL-8 levels when compared to LPS alone (Subramaniyam et al. 2010). This effect of increased cytokine expression was also seen in a mouse model of LPS-stimulated inflammation. The authors concluded that AAT's enhancement of endotoxin-induced cytokine and chemokine production may provide an insight into the role of AAT as an acute phase protein, contributing to the initial amplification and ultimate resolution of the inflammatory response (Subramaniyam et al. 2010). Moreover, monocytes recognise LPS by the membrane-bound or soluble form of the glycoprotein CD14 and its associated signal transducer, toll-like receptor 4 (TLR-4). In vitro, Nita et al. (2007) demonstrated that AAT could regulate the expression and release of CD14 suggesting that AAT may function to prevent excessive activation of monocytes in vivo thereby preventing an exaggerated pathogenic immune response. Authors have attempted to explain these seemingly contradictory roles of AAT as an inhibitor and enhancer of the LPS response in monocytes, macrophage and indeed neutrophils by explaining that the timing or setting of LPS stimulation appears to be the differentiating factor. Short-term treatment with LPS (4 h) appears to enhance the cytokine response, but in the longer term (18 h) it plays an inhibitory role (Subramaniyam et al. 2010). This theory has some grounding in the wider literature concerning acute phase proteins as C-reactive protein and alpha1-acid glycoprotein have been shown to exhibit both pro- and anti-inflammatory functions thereby amplifying, regulating and resolving inflammation (Ballou and Lozanski 1992; Boutten et al. 1992; Dobrinich and Spagnuolo 1991; Laine et al. 1990).

Although quantitatively and qualitatively COPD and acute lung injury are distinct clinical entities, in a model of acute lung injury a number of important anti-inflammatory and anti-protease effects of AAT are postulated to be useful in treatment. Initial studies demonstrated that NE inhibitors could prevent progression of acute lung injury in various animal models (Ahn et al. 1993; Gossage et al. 1993; Kawabata et al. 2000). A more recent study in an animal model of endotoxin-induced lung injury, found that treatment with AAT post LPS insult attenuated lung injury, in part due to the reduction of inflammatory mediators (IL-8 and TNF-alpha) involved in neutrophil chemotaxis (Jie et al. 2003).

The Use of AAT Augmentation Therapy in Treatment of Panniculitis and Other AATD-Associated Diseases

Several case studies have revealed that infusion of a higher dose of AAT augmentation therapy compared to that required for the treatment of lung disease, successfully managed and reduced inflammation associated with severe panniculitis in AATD individuals with the ZZ phenotype (Al-Niaimi and Lyon 2011; Blanco et al. 2011; Chowdhury et al. 2002; Dowd et al. 1995; Gross et al. 2009). The prevalence of panniculitis as a complication of AATD is infrequent, with an occurrence of approximately 0.1%. Clinical manifestations include nodular lesions involving degenerative changes of the dermal collagen (Fernandez-Torres et al. 2009) and the pathophysiology possibly relates to consistent neutrophilic inflammation and unopposed NE activity. In addition, it has been proposed that Z-type AAT polymers in the skin, supports the inflammatory pathogenesis of panniculitis (Gross et al. 2009), although this latter inference requires further verification. Moreover, a number of case studies have shown AAT's effectiveness in treating a range of other diseases associated with severe AATD including fibromyalgia, leukocytoclastic vasculitis and bronchial asthma (Blanco et al. 2004, 2008, 2010b, 2011).

The Potential Impact of AAT Therapy on Viral Infection and Microbial Pathogenicity

Studies have investigated AAT's potential as a therapeutic agent in the control of infection and pathogenicity of microbes. Research includes the ability of AAT to inhibit enterpathogenic *Escherichia coli* induced red blood cell hemolysis (Knappstein et al. 2004). Moreover, AAT has been demonstrated to inhibit cytotoxins from *Entamoeba histolytica*, a serious parasitic infection of humans (Lushbaugh et al. 1981) and excystation of *Cryptosporidium parvum*, a parasitic disease of the mammalian intestinal tract (Forney et al. 1997a, b). The bacteriostatic properties of AAT against rapidly growing mycobacterias have recently been documented (Chan et al. 2007). Post *S*-nitrosylation of AAT at sites of infection, AAT exhibits strong growth inhibition on various Gram-positive and Gram-negative bacteria (Miyamoto et al. 2000a, b).

Whilst literature discussed thus far has demonstrated that AAT is a potent inhibitor of neutrophil migration and ROS production, it is important to highlight that there is some evidence to demonstrate that AAT can also play an important role in preventing bacterial colonisation by preserving neutrophil function at sites of inflammation. Aerosolised AAT was found to positively impact upon neutrophil-mediated killing of *P. aeruginosa* in CF (McElvaney et al. 1991) and in an animal model of chronic *Pseudomonas* lung infection (Cantin and Woods 1999), possibly by preventing cleavage of neutrophil complement receptors by serine proteases, a previously reported unfavourable effect of NE (Berger et al. 1989). It has also been shown that neutrophil-mediated bacterial killing is hindered by CXCR1 receptor cleavage by serine protease activity within the CF lung. This later study further demonstrated that in vivo inhibition of proteases by inhalation of AAT restored CXCR1 membrane expression and improved neutrophil-mediated bacterial killing, as assessed by sputum levels of *P. aeruginosa* (Hartl et al. 2007).

Research investigating the impact of AAT on viral infections has demonstrated that HIV replication and T-cell numbers are associated with low serum levels of AAT and recent data indicate that AAT can inhibit HIV replication in T-cells as quantified by significant decreases in viral RNA and p24 epitope. This latter observation is thought in part due to the ability of AAT to enter T cell and impact upon IκBα ubiquitination (Zhou et al. 2010). A peptide derived from AAT has also been shown to display antiviral properties in response to HIV-1 infections. Münch et al. (2007) showed that the AAT viral inhibitory peptide (VIRIP) inhibited a variety of HIV-1 strains including those resistant to current antiretroviral drugs and demonstrated that VIRIP blocked HIV-1 entry by interacting with the gp41 fusion peptide.

Potential for Additional Clinical Use of AAT Augmentation Therapy in Treatment of Rheumatoid Arthritis and Vasculitis

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease, characterised by joint inflammation, destruction and deformity. Sub-populations of patients do not respond to standard therapy with non-steroidal anti-inflammatories or biological treatments including TNF-alpha or lymphocyte proliferation inhibitors. Synovial inflammation, a key component of the disease is caused by activation, infiltration and proliferation of immune cells including neutrophils, macrophages, fibroblasts, mast cells, NK cells, NKT cells, T-cells as well as plasma cells (McInnes and Schett 2007).

Early experimental animal models of human RA using collagen-induced arthritis suggested a possible role for NE in the pathogenesis of RA. Using a novel protease inhibitor (ONO-5046), researchers showed that ONO-5046 could reduce the severity and incidence of collagen-induced arthritis in both rats and mouse models. This suppression of disease severity was correlated with reduced destruction of the articular cartilage seen histologically in mice and rats compared to controls (Kakimoto et al. 1995). Later studies

investigating the feasibility of using AAT in treatment of RA used both protein and rAVV-mediated gene therapy. The study showed that human AAT (hAAT) protein therapy as well as recombinant adeno-associated-virus-mediated hAAT gene therapy delayed the onset and modified disease progression in a murine model of arthritis. Therapy with hAAT significantly reduced serum levels of B-cell-activating factor of the TNF-alpha family and autoantibodies against bovine type II collagen and mouse collagen II. This latter finding suggests that the effects of AAT are at least partially mediated via B-cells (Grimstein et al. 2010b). Combination therapy with AAT and doxycycline (a tetracycline antibiotic which demonstrates tissue-protective properties) has shown promise as an effective treatment for RA in a mouse model of collagen-induced arthritis. This therapy significantly reduced arthritis development and progression at a macroscopic and histopathological level. The combination therapy also inhibited IL-6 expression from LPS-stimulated mouse fibroblasts which may indicate a mechanism of arthritis inhibition (Grimstein et al. 2010a). Of major importance reduced levels of AAT, as associated with heterozygotes for the Z allele, was suggested to play a contributory role to the development of RA (Cox and Huber 1976) and may be a factor promoting severe joint destruction (Cox and Huber 1980).

AAT is known to be a major inhibitor of PR3, which is the main target antigen of anti-neutrophil cytoplasm antibodies (ANCA) in Wegener's granulomatosis (WG), a potentially lethal systemic vasculitis. Several studies have shown an association between AATD phenotypes and anti-PR3 positive systemic vasculitis, suggesting a pathogenic role for AATD and the occurrence of disease in a subgroup of patients with WG (Barnett et al. 1999; Esnault et al. 1993). In a study by Lhotta et al., four abnormal AAT phenotypes (two ZZ and two MZ) among 29 patients with WG were identified (Lhotta et al. 1994), whilst in a further study 15 heterozygotes (MZ, SZ and null phenotypes) were identified among 66 PR3-ANCA-positive patients with WG (Elzouki et al. 1994). This patient subgroup with deficient levels of AAT appears to have worse prognosis and screening for AATD alleles in all individuals presenting with vasculitis has been proposed (Segelmark et al. 1995). Case reports have described the successful use of AAT as a novel therapy for locally occurring or cutaneous vasculitis in individuals with AATD but as yet no large study has looked at the efficacy or safety of its use in systemic vasculitis (Dowd et al. 1995).

The Novel Use of AAT Therapy in Treatment of Diabetes Mellitus

AAT's role as a potent anti-inflammatory has extended outside the arena of pulmonary disease into other important human disease including diabetes. Type 1 diabetes results

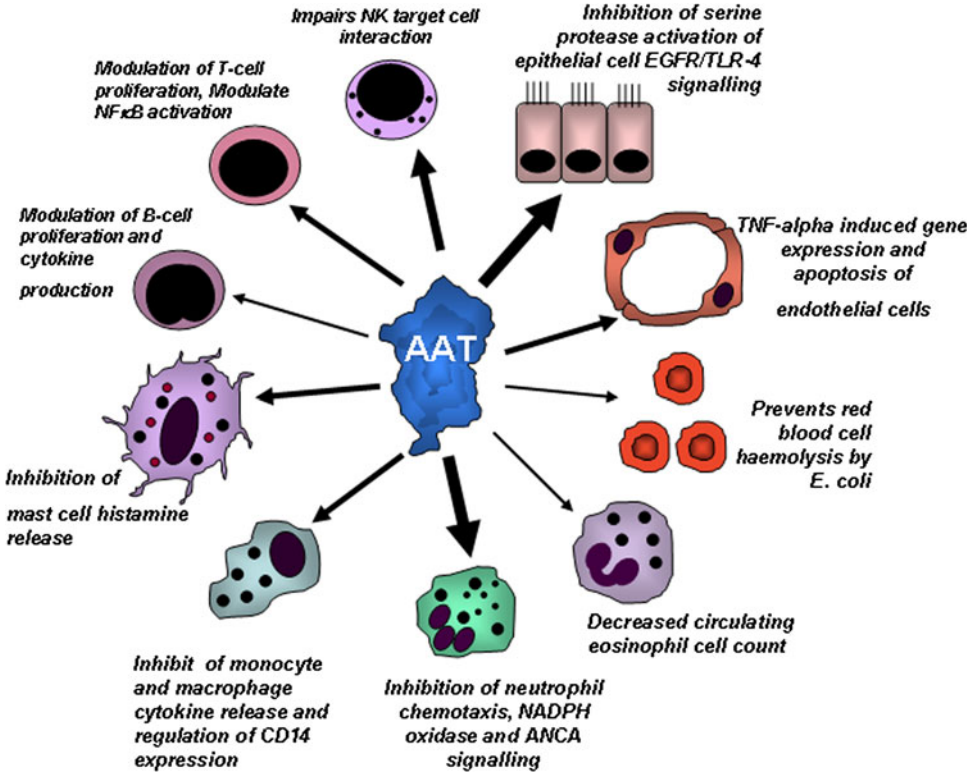

Fig. 6 AAT modulation of different cell types during inflammation. AAT is primarily produced in high quantities by hepatocytes and maintains anti-inflammatory effects on several cell types and modulates inflammation caused by host and microbial factors. *Arrow thickness* indicates the depth of data confirming the ability of AAT to modulate a particular cell, with the thickest indicating the greatest level of knowledge

Table 1 Potential use of AAT therapy in the treatment of human diseases

Disease Model	Cell type	Potential mechanism(s)	Reference
Alpha-1 antitrypsin deficiency	Neutrophil	Inactivation of neutrophil chemotaxis	(Bergin et al. 2010; Stockley et al. 1990)
		Modulation of ROS production	(Bucurenci et al. 1992)
	Macrophage	Reduction of LTB_4 in the airways	(Hubbard et al. 1991)
Cystic fibrosis	Neutrophil	Reduction in *P. aeruginosa* bacterial count	(Cantin and Woods, 1999)
	Macrophage	Decreased IL-8, IL-1beta, TNF-alpha and LTB_4	(Griese et al. 2007)
Smoke-induced emphysema	Macrophage	Reduction of MMP-12 and TNF-alpha release	(Churg et al. 2007)
Asthma	Eosinophil	Associated with decreased eosinophil count	(Johansson et al. 2001)
	Mast cell	Reduced IgE induced histamine release	(He et al. 2004)
Vasculitis	Neutrophil	Formation of complex and inhibition of PR3	(Baslund et al. 1994)
Acute lung injury	No cell identified	Reduced IL-8 and TNF-alpha in BAL	(Jie et al. 2003)
Diabetes	Pancreatic islet β-cell	Prevention of TNF-alpha induced apoptosis	(Kalis et al. 2010)
Rheumatoid arthritis	B-cells	Reduced B-cell activating factor	(Grimstein et al. 2010b)
	Fibroblasts	Inhibition of IL-6 expression	(Grimstein et al. 2010a)
Panniculitis	Multiple cell types	Increased neutrophil counts and Z-type AAT polymers in the skin	(Gross et al. 2009)
Fibromyalgia	Monocytes	Increased levels of MCP-1, TNF-alpha and VEGF	(Blanco et al. 2004, 2010a)
	Mast cells	Increased tryptase and PAR2	(Blanco et al. 2010b)

ROS reactive oxygen species, *LTB_4* leukotriene B_4, *PR3* proteinase 3, *MCP-1* monocyte chemoattractant protein-1, *TNF-alpha* tumour necrosis factor alpha, *VEGF* vascular endothelial growth factor, *PAR2* proteinase-activated receptor 2

from the autoimmune destruction of the insulin-producing pancreatic islet. This process of beta-cell death is also found in type 2 diabetes and recent results by Sanström and colleagues suggests that deficiency of AAT (<1.0 mg/ml) may be associated with an increased risk of type 2 diabetes (Sandstrom et al. 2008b). Moreover, studies have shown that over expression of the AAT gene reduces oedema and the infiltration of white blood cells into pancreatic islets and prevents the development of hyperglycaemia in non-obese diabetic mice (Lu et al. 2006; Song et al. 2004). Islet cell transplantation has shown promise as a treatment for type-1 diabetes but long-term follow up studies have shown problems with islet cell viability despite immunosuppression. Of major importance it has been shown that treating mice which have undergone pancreatic islet allograft with AAT prolongs allograft survival and modulates cellular immunity (Lewis et al. 2005; Molano et al. 2008; Pileggi et al. 2008; Zhang et al. 2007). Kalis et al.'s (2010) investigation of the mechanism by which AAT protects islets cells and increases insulin production, found that AAT increased insulin secretion in a glucose dependent manner. It also potentiated the insulin secretion effects of glucagon-like peptide-1 and forskolin. AAT was shown to protect and rescue INS-1E, a diabetic cell line from apoptosis induced by TNF-alpha and to significantly reduce the level of apoptosis induced by a mixture of TNF-alpha, IL-1beta and IFN-gamma (Kalis et al. 2010).

Substantial interest has been recently shown in the ability of AAT to modulate apoptosis in several cell types including lung endothelial cells, a diabetic mouse model and cell line and in transplanted pancreatic islet cells. (Lewis et al. 2005; Molano et al. 2008; Pileggi et al. 2008; Zhang et al. 2007). Petrache and colleagues illustrated a mechanism by which AAT instigated this pro-survival function and reported internalisation of AAT (Sohrab et al. 2009) and direct inhibition of caspase-3, the primary cytoplasmic executor protein of cellular apoptosis (Petrache et al. 2006). Furthermore, in two separate models of induced beta-cell apoptosis, treatment with AAT decreased caspase-3 activity in a dose-dependent manner (Zhang et al. 2007).

Conclusions

Early studies in the field of AATD identified AAT's essential role as an anti-protease, with successful use as a therapeutic agent in pulmonary disease (Hubbard et al. 1989; McElvaney et al. 1991). However, more recent studies have revealed that AAT is a more complex multipotent protein possessing anti-inflammatory activity against key immune cells including neutrophils, lymphocytes, macrophages, monocytes, mast cells and epithelial cells (Fig. 6). Thus, the perceived role of AAT in human disease and in health has taken a paradigm shift from that

of a simple anti-protease to a multifaceted anti-inflammatory agent with key immunomodulatory functions. As a result, AAT's potential role as a potent anti-inflammatory is now being exploited in other non-pulmonary diseases which have significant mortality, morbidity and economic impact, such as diabetes and RA, with some initial success. However, the role of AAT in new treatment programs needs further investigation and could include additional autoimmune diseases including Hashimoto's thyroiditis previously shown to be associated with AATD (Nicholls and Janus 1973). The described unexpected roles of AAT may help us to elucidate the specific signalling pathways involved in its anti-inflammatory mode of action. Disease models such as RA and vasculitis are especially useful as they are not affected by the concurrent infection often characteristic of lung disease (Table 1). However, there remain a number of challenges to face in the development of AAT as a true anti-inflammatory therapeutic agent including expanding our knowledge of its mode of action, identification of the advantages of human pooled AAT over recombinant AAT and also the development and benefits of hyperglycosylates of AAT. Despite these challenges AAT holds tremendous potential as a novel anti-inflammatory which unlike many of the new arrivals to this family of therapeutic agents has a long track record of being well tolerated and safe.

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