



## $\alpha_1$ -Antitrypsin deficiency - 6: New and emerging treatments for $\alpha_1$ -antitrypsin deficiency

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*Thorax* 2004 59: 904-909

doi: 10.1136/thx.2003.006551

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## REVIEW SERIES

 $\alpha_1$ -Antitrypsin deficiency · 6: New and emerging treatments for  $\alpha_1$ -antitrypsin deficiency

R A Sandhaus

*Thorax* 2004;59:904–909. doi: 10.1136/thx.2003.006551

Alpha-1-antitrypsin (AAT) deficiency is a genetic condition that increases the risk of developing lung and liver disease, as well as other associated conditions. Most treatment of affected individuals is not specifically directed at AAT deficiency but focuses on the resultant disease state. The only currently available specific therapeutic agent—namely, intravenous augmentation with plasma derived AAT protein—is marketed in a limited number of countries. Treatments aimed at correcting the underlying genetic abnormality, supplementing or modifying the gene product, and halting or reversing organ injury are now beginning to emerge. These innovative approaches may prove effective at modifying or eliminating diseases association with AAT deficiency.

are 10–15% that of individuals with the Pi M genotype.<sup>11</sup> Other genotypes associated with severe deficiency include Pi SZ, Pi Z/Null and Pi Null, as well as an array of much rarer Pi types.<sup>1–12</sup> Genotypes have been identified that lead to production of a protein that is dysfunctional as an elastase inhibitor and cause increased risk of emphysema, but are released at normal levels into the circulation.<sup>9–13</sup> It has been estimated that there are approximately 100 000 severely deficient individuals in the US and approximately 25 million carriers of at least one deficient gene for AAT.<sup>14–15</sup> Similar numbers have been suggested for the European population. It has been estimated that less than 6% of severely deficient individuals are currently identified. In general, the co-dominant expression of the AAT gene leads to intermediate circulating levels of AAT in carriers. The severe deficiency of AAT and, to a lesser degree, the carrying of a single deficient gene, lead to an increased risk of developing pulmonary emphysema,<sup>16–17</sup> liver failure in newborns and young children,<sup>6–18</sup> liver injury with cirrhosis in adults,<sup>19–20</sup> necrotising panniculitis,<sup>21–24</sup> bronchiectasis,<sup>25–26</sup> and perhaps a number of other diseases.<sup>21–27–28</sup>

**A**lpha-1 antitrypsin deficiency, also known as  $\alpha_1$ -proteinase inhibitor deficiency or, more simply, Alpha-1, is a genetic condition that increases the risk of developing a variety of diseases including pulmonary emphysema and cirrhosis of the liver. It is caused by mutations in the gene coding for the 52 kDa glycoprotein  $\alpha_1$ -antitrypsin (AAT),<sup>1–2</sup> the body's major serine proteinase inhibitor or serpin.<sup>3</sup> This gene is located in the long arm of chromosome 14 of the human genome.<sup>4</sup> When first described in 1963,<sup>5</sup> AAT deficiency was looked upon as a vanishingly rare condition that left affected individuals with precocious severe emphysema. Now it is understood to be a genetic condition with a relatively high prevalence that can have various clinical presentations, ranging from no health effects through more typical chronic lung or liver disease in the elderly to the more classic neonatal cirrhosis or precocious emphysema of young adults.<sup>6–8</sup>

Over 100 allelic variants of this gene have been identified and 34 of them have been associated with a quantitative or functional deficiency of circulating AAT.<sup>9</sup> In its classic form, an inherited mutation of the AAT gene causes the build up of abnormal AAT within the hepatocytes of the liver. The liver is the major source of circulating AAT and this transport problem leads to low levels of AAT in the blood and tissues.

The proteinase inhibitor or Pi system has been used to name the various mutations of the AAT gene.<sup>10</sup> The normal genotype is Pi M and the classic severe deficiency is associated with the Pi Z genotype. Individuals with the Pi Z genotype tend to have circulating levels of AAT that

## ROLE OF AAT

There does not appear to be a unifying mechanism for the panoply of illnesses associated with AAT deficiency. While AAT is the archetype of the serpin family and is an extremely effective inhibitor of such tissue destroying proteolytic enzymes as neutrophil elastase,<sup>29–30</sup> it has also been shown to have both anti- and pro-inflammatory properties.<sup>31–34</sup> The classic proteinase pathogenesis model of pulmonary emphysema, formulated based on animal models of emphysema and our understanding of the serpin activity of AAT, suggests that the pulmonary emphysema associated with AAT deficiency is due to the unbridled proteolytic activity of neutrophil elastase on lung connective tissue leading to alveolar destruction.<sup>29–35–37</sup> This model is still well accepted, although there is growing evidence that the pathways leading to pulmonary emphysema may be considerably more complex and serpentine.<sup>38–40</sup>

While the effectiveness of AAT as an inhibitor of serine proteinases is undisputed, there is evidence for other properties as well that may play a role in disease due to its deficiency. It has been shown that the Pi Z mutation leads to production of a protein with a propensity to polymerisation both within the hepatocyte<sup>11</sup> and in the lungs.<sup>41</sup> This polymerised AAT may be pro-inflammatory,<sup>32–42</sup> acting as (or stimulating the

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release of) a chemoattractant for phagocytic cells. In addition, oxidative inactivation of AAT appears to play a major role in the local regulation of this antiproteinase<sup>43-47</sup> and, perhaps, in the effects of cigarette smoke on the lung, even in individuals with normal AAT.<sup>48-49</sup>

While the proteinase-antiproteinase balance may also play a role in the liver disease of AAT deficiency, most believe that retention of misfolded and polymerised AAT within the endoplasmic reticulum of hepatocytes of affected individuals and the response of hepatocytes to this retained protein are more likely culprits in this form of AAT deficiency associated disease.<sup>7 11 50-52</sup> The AAT deficiency associated vasculitides, necrotising panniculitis, and Wegner's granulomatosis have even more abstruse pathophysiology.

## PAST AND CURRENT SPECIFIC TREATMENTS

With this background, scientists both within and without the biopharmaceutical industry have attempted develop specific treatments for AAT deficiency. AAT is an acute phase reactant and, as such, AAT synthesis is stepped up during episodes of systemic inflammation or stress. Early attempts at devising specific treatment for AAT deficiency relied on this attribute.<sup>53</sup> Danazol, a synthetic androgen, is capable of stimulating the acute phase response and was advocated to stimulate hepatic production of AAT.<sup>54</sup> While statistically significant increases in circulating AAT levels were documented with this treatment, a clinical response to these small changes was impossible to detect.

In the 1980s it was appreciated that AAT could be purified in quantity from the plasma of healthy individuals and delivered intravenously to individuals with AAT deficiency.<sup>55</sup> This intravenous augmentation therapy was shown to raise the circulating levels of AAT as well as the levels in bronchoalveolar lavage (BAL) fluid. Based on an evaluation of the circulating AAT levels in a relatively small number of Pi SZ individuals with and without pulmonary emphysema, it was found that individuals with circulating AAT levels of  $>15 \mu\text{M}$  (80 mg/dl) seemed to be protected from lung destruction and this became the target trough level for intravenous augmentation therapy. A dose of 60 mg/kg body weight of this purified AAT administered weekly appeared to maintain levels above this target threshold and to raise BAL fluid levels of AAT significantly. This became the recommended dose for Prolastin when it was approved for marketing in the US by Cutter Laboratories at the end of 1987.<sup>56-57</sup> Currently marketed by Bayer Biologicals (West Haven, Connecticut, USA) in the US, Canada, Germany, Spain, Italy, and Sweden, it remains the most widely prescribed specific treatment for the pulmonary disease associated with AAT deficiency. In the USA, two additional plasma derived intravenous augmentation products have now received marketing approval (Zemaira, ZLB Behring, PA, and Aralast, Baxter Healthcare, IL). Based on clinical trials currently underway, it is expected that at least two more companies will enter the intravenous augmentation therapy market in the coming years.

A major concern regarding these products is that conclusive documentation of effectiveness at preventing AAT deficiency associated lung disease has been lacking. Where marketed, approval for all these products has been based on biochemical efficacy and safety criteria alone. None has been evaluated using a placebo controlled, randomised, blinded/masked clinical trial design to document effectiveness in treating or preventing emphysema. Even the two newer US products were approved based on small trials documenting their "non-inferiority" to Prolastin in trough AAT blood levels, BAL fluid AAT levels, and safety.<sup>58</sup>

The efficacy of Prolastin has been evaluated in a number of uncontrolled trials as well as in one randomised trial with an

unconventional dosing regimen and a small patient population. In the early 1990s the German and US AAT deficiency registries each looked at the mortality and rate of decline in lung function for enrolled individuals who received Prolastin compared with those who did not.<sup>59-60</sup> Both groups reported that those receiving Prolastin had a decreased rate of decline in lung function and a decreased mortality compared with those who never received Prolastin. The decreased rate of decline in lung function only reached statistical significance in the group identified as having moderate lung function impairment. The German group went on to confirm that, when patients were used as their own controls, comparing the decline in lung function before and after starting Prolastin in the same individuals, a decrease in the rate of decline in lung function could be documented in the post-Prolastin period, especially in individuals who had a rapid decline in lung function during their baseline measurements.<sup>61</sup> An additional analysis compared German patients receiving Prolastin with Danish patients who did not.<sup>62</sup> Again, the group with moderately impaired lung function receiving Prolastin demonstrated a significant decrease in the rate of decline of their lung function. A randomised study of 56 patients in Denmark and the Netherlands showed a trend to less loss of lung tissue in Prolastin treated individuals, as judged by lung densitometry using computed tomography of the lungs.<sup>63</sup> Finally, a study employing an internet based questionnaire suggested that Prolastin treatment reduced the frequency of exacerbations in lung affected individuals with AAT deficiency.<sup>64</sup> None of the evidence accumulated so far has been sufficiently compelling to expand approval of intravenous augmentation therapy throughout Europe.

## EMERGING NEW TREATMENTS

### Alternative routes of administration of current treatment

Even where available, current use of intravenous augmentation therapy is limited by supply of drug, lack of efficacy documentation, and extreme inconvenience of administration. All three limitations are currently being addressed by the evaluation of alternative routes of administration. Most studies are focusing on drug delivery by inhalation. There was initial reluctance to pursue development of inhaled AAT since it was difficult to document that the inhaled protein was able to be delivered to the pulmonary interstitium where proteolytic activity was thought to be doing its damage in pulmonary emphysema.<sup>65</sup> In recent years, however, it has been shown that the airways of individuals with AAT deficiency are under a constant inflammatory barrage<sup>31</sup> and that administration of exogenous inhaled AAT can reconstitute the lower respiratory tract antiproteinase screen and potentially reduce inflammation.<sup>65-66</sup> Interest in this route of administration has therefore been renewed.

Several companies that have developed products for intravenous administration have produced agents formulated for inhaled administration and have tested them in humans. The ease of administration compared with the intravenous route is obvious. Since these agents are being administered directly to the lungs, smaller doses are required than for intravenous administration, potentially allowing the limited plasma supply to treat a larger number of patients. Finally, it is expected that these products will be evaluated in randomised, blinded efficacy trials. Unfortunately, at the time of this review, no one has undertaken the step of beginning such an efficacy trial, presumably because of the cost and time involved in such a major clinical programme.

### Alternative sources of augmentation therapy

Another troubling aspect of intravenous augmentation therapy is the source of the drug—namely, human plasma.

The primary concerns relate to the limited supply of this raw material and the potential for transmission of infectious agents. Because of these concerns, alternative sources of augmentation therapy have been sought. This has prompted the development of transgenic/recombinant sources of the human AAT protein and the evaluation of synthetic inhibitors of neutrophil elastase. Transgenic production of human AAT protein has been accomplished in both sheep (PPL Therapeutics, Scotland, UK and Bayer Biologicals, West Haven, Conn, USA)<sup>67</sup> and goats (Genzyme, Boston, Mass, USA).<sup>68</sup> Human AAT has also been produced in yeast using recombinant technology (Baxter Healthcare, IL, USA and Arriva Pharmaceuticals, Alameda, CA, USA).<sup>69</sup> Because of anomalies in the glycosylation of the AAT protein in these various species, these proteins are cleared rapidly from the human circulation making intravenous administration impractical. The PPL product and the yeast derived AAT have been evaluated in human safety studies using an inhaled route of administration. Whether these will prove safe and effective at preventing lung destruction in AAT deficiency is yet to be shown.

Potent synthetic inhibitors of human neutrophil elastase have been in use for decades. Several have been evaluated in humans including agents administered intravenously and orally.<sup>70-74</sup> In an attempt to capture early hints of efficacy, these agents have been used to treat ARDS, cystic fibrosis, chronic bronchitis, and exacerbations of COPD. None of these trials has so far yielded results promising enough to justify embarking on expensive long term trials aimed at modifying the progression of pulmonary emphysema in AAT deficiency.

#### Treatments aimed at pulmonary emphysema

A number of agents for the treatment of pulmonary emphysema due to smoking are under development. While these are aimed at the broad COPD population, clinical evaluation of these agents in patients with AAT deficiency has proved attractive as individuals with AAT deficiency related emphysema tend to be younger, have fewer complicating medical conditions, and to have "pure" emphysema. Several stand out within this group.

The first are the retinoids. Studies using elastase induced emphysema in rats suggested that administration of all-trans retinoic acid (ATRA) was associated with reversal of the emphysematous changes.<sup>75</sup> Based on this finding, it was suggested that ATRA might stimulate the growth of new alveoli in humans with emphysema. Clinical trials in humans with emphysema have failed to demonstrate measurable improvements in indices of lung destruction so far but these studies are ongoing.<sup>76</sup>

Another line of investigation has been based on the observation that the lungs of individuals with emphysema tend to have dramatic reductions in the hyaluronic acid content.<sup>77</sup> When animals are administered hyaluronic acid they are protected from exogenous elastase induced emphysema.<sup>78-79</sup> These findings have led to trials of inhaled hyaluronic acid in individuals with AAT deficiency in the hope of preventing the progression of lung disease.

Finally, our understanding that oxidative inactivation of AAT may lead to loss of antiproteinase activity has led to consideration of drugs with antioxidant potential in the treatment of individuals with AAT deficiency related emphysema. Physicians have been suggesting the use of supplements containing vitamins A, C, and/or E based on this rationale, and more potent antioxidants are also being considered for evaluation.<sup>80-82</sup> So far there is little evidence of benefit to individuals with AAT deficiency from these approaches.

#### Treatments aimed at the liver

Since most deficient genotypes lead to production of an AAT protein that has fairly potent antineutrophil elastase capacity, one therapeutic concept has been aimed at trying to cause the liver to release its trapped AAT, thus relieving the congestion of the hepatocyte and reconstituting the circulating anti-elastase screen. The most promising candidates for this approach are the synthetic chaperones and molecular interventions that try to prevent intracellular polymerisation of the abnormal AAT.

Synthetic chaperones have been used in intracellular protein transport diseases such as cystic fibrosis.<sup>83</sup> The most studied candidate is 4-phenyl-butyric acid (4-PBA) and this has been studied in AAT deficiency at several centres.<sup>84</sup> Initial results suggest that improvements in liver retention of AAT and increases in serum levels of this protein are modest at best and that the gastrointestinal side effects of this treatment can be dose limiting. Still, this remains an area of active investigation.

With the elucidation of the molecular mechanisms of polymerisation of the Z protein in the liver, work has turned towards molecular interventions that could prevent these intermolecular interactions and allow the release of monomeric AAT molecules into the circulation. Two approaches have been described so far. The first uses small peptides designed to fit specifically into the open beta-sheet site on the abnormal AAT molecule where the intermolecular interaction takes place, thus blocking the insertion of the inhibitory site loop of one AAT molecule into the "sheet" of the next.<sup>85</sup>

The second approach attempts to target specific amino acids located within appropriate surface cavities on the AAT molecule and to replace them with more bulky or charged amino acids. This approach aims to close the insertion point of the "loop" by directly modifying the conformation of the AAT molecule.<sup>86</sup>

**Table 1** Specific treatments for  $\alpha_1$ -antitrypsin (AAT) deficiency

Therapeutic class	Status of use in humans with AAT deficiency
Plasma derived intravenous augmentation	Three manufacturers with approvals in at least one country Two manufacturers awaiting approval
Plasma derived inhaled augmentation	Two manufacturers have tested drugs by this route Two additional manufacturers considering starting
Recombinant/transgenic augmentation	IV therapy not practical Two manufacturers considering inhaled route
Synthetic elastase inhibitors	Orally bioavailable inhibitors have been developed by at least six manufacturers At least four have been tested in humans None currently being tested in AAT deficiency
Chaperones and polymerisation blockers	4-PBA currently under study Other synthetic chaperones being developed Polymerisation blockers not yet in human trials
Antioxidants	Empirical use of vitamins with antioxidant potential Candidate therapeutics near starting human trials
Gene therapies	Gene therapy safety studies about to begin

## Genetic approaches

As a condition caused by well characterised single gene mutations, the possibility of genetic approaches to mitigate or cure AAT deficiency have been entertained. Studies have been reported in which the normal human AAT gene has been inserted into muscle or liver cells. Novel gene repair technologies have also been studied. Additionally, consideration has been given to development of agents that can turn off or disrupt production of the abnormal gene product.

Animal studies at the University of Florida have succeeded in introducing the normal human AAT gene into striated muscle cells using an adeno-associated virus vector.<sup>87–88</sup> Such animals maintain potentially therapeutic human AAT levels in their blood for many months.<sup>89–90</sup> Human trials are expected to begin quite soon. Other approaches using different vectors and different target organs are being considered at other centres.<sup>91–92</sup>

Still, the classic gene therapy approach of introducing a normal gene into the cells of an individual with a genetic mutation has some drawbacks in AAT deficiency. The introduction of a normal gene is not likely to turn off production of the endogenous abnormal gene product. Thus, even if prolonged normal gene expression with production of copious normal AAT can be achieved, this approach would seem unlikely to be therapeutic to those at risk of liver injury due to AAT deficiency. A number of methods aimed at turning off production of the abnormal AAT protein are being considered. These include the use of antisense oligonucleotides and ribozyme technology to thwart translation of the mRNA message for the mutant protein.<sup>92–95</sup> While cell culture and animal studies have been promising, the effectiveness of these approaches in humans is still theoretical.

More speculative still is the concept of gene repair. This technology was initially designed around chimeraplasty,<sup>96</sup> which is the use of chimeric RNA/DNA oligonucleotides to “patch” a single gene mutation. RNA complementary to the area surrounding the point mutation was synthesised with a contiguous DNA oligonucleotide made of the corrected sequence. In model systems, chimeraplast constructs were capable of correcting targeted single site gene mutations. While touted in the lay press as a potential cure for fatal genetic diseases, this technology has not lived up to its early potential. Currently, this gene repair has advanced beyond the use of chimeric oligonucleotides. Single stranded bare DNA oligonucleotides appear to provide more consistent results *in vitro* and *in vivo* than the previously described methods.<sup>97</sup> The use of this technology in the treatment of AAT deficiency is currently being contemplated.

Stem cell based approaches to AAT deficiency are in their infancy. Possibilities include modification of an individual’s stem cells *ex vivo* to contain the normal AAT gene, then maturation towards hepatocytes and introduction into the liver of an affected individual.<sup>98</sup>

The current status of specific treatments for AAT deficiency is summarised in table 1.

## CONCLUSIONS

Great hope for the treatment of AAT deficiency was born with the introduction of intravenous augmentation therapy in 1987. While quite popular among those treating lung disease related to AAT deficiency in countries where this treatment is approved, its effectiveness is yet to be rigorously proven. In addition, it has been expensive and in short supply. Newer augmentation therapies with sources other than plasma and different routes of administration will be required to provide evidence of efficacy before regulatory approval. While this information will be welcome, these trials will be of long duration and therefore these products may not be available for years.

The status of more innovative approaches, including gene therapy and therapeutics aimed at preventing the liver disease associated with AAT deficiency, has been reviewed. Since there are many individuals with AAT deficiency who never develop disease of the lungs or liver, avoidance of known risk factors such as exposure to tobacco smoke, frequent lung infections, and exposure to occupational dust and fumes may be the most effective treatment currently available.<sup>99</sup> It is likely that there are additional genes—yet to be identified—that alter the likelihood that an individual with AAT deficiency will develop disease.<sup>100–101</sup>

Among the approaches described, several may apply to the general population of individuals with destructive lung or liver disease. A large percentage of those followed as “routine” COPD have undetected AAT deficiency.<sup>8–102</sup> Much of what we currently know about the pathogenesis of COPD in general has evolved from our understanding of the lung disease associated with AAT deficiency. Similarly, our understanding of conditions caused by protein conformational abnormalities has been aided by studies of the synthesis and intracellular trafficking of the AAT molecule. It is reasonable to assume, therefore, that this hereditary condition will continue to lead our knowledge towards new therapeutics for a variety of illnesses.

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## LUNG ALERT .....

### Mechanisms of tolerance and Th2 cell activation in asthma

▲ De Heer HJ, Hammad H, Soullié T, *et al*. Essential role of lung plasmacytoid dendritic cells in preventing allergic reactions to harmless inhaled antigen. *J Exp Med* 2004;**200**:89–98

▲ Ostroukhova M, Seguin-Devaux C, Oriss TB, *et al*. Tolerance induced by inhaled antigen involves CD4+ T cells expressing membrane-bound TGF-beta and FOXP3. *J Clin Invest* 2004;**114**:28–38

**M**ultiple factors are involved in the correct regulation of an immune response to common antigens. Two studies using asthma models in mice provide an insight into the immunological mechanisms of T helper 2 (Th2) cell activation in atopic asthma.

There are two subsets of human and mouse dendritic cells (DC): the myeloid (mDC) and plasmacytoid (pDC) types. de Heer *et al* show that inhaled antigen is taken up and presented to draining nodal T cells in the lung by both DCs. On selective depletion of pDCs, inhalation of a normally inert antigen induces Th2 cell activation and effector cytokine release (IL-5, IL-10, IL-13, interferon  $\gamma$ ), goblet cell hyperplasia, eosinophilic airway inflammation, and specific serum IgE production. However, the adoptive transfer of antigen exposed pDC in mice before subjecting them to an immunogenic asthma protocol led to tolerance through inhibition of mDC induced generation of Th2 cells. Atopic asthma may be caused by pDC dysfunction, but this hypothesis remains to be proven before treatments aimed at potentiating pDC activity can be considered.

Ostroukhova *et al* show that FOXP3, a transcription factor, is only present on the surface of CD4+ T cells in which tolerance has been induced by repeated exposure to a low dose inhaled antigen. The administration of CD4+ T cells bearing cell surface TGF- $\beta$  to naïve mice is shown to induce tolerance to subsequent antigen exposure. In addition, the depletion of TGF- $\beta$ <sup>+</sup> cells before antigen exposure resulted in proliferation of TGF- $\beta$ <sup>-</sup> cells and Th2 mediated allergic phenotype. The authors suggest that early antigen exposure could induce tolerance to commonly inhaled antigens in those at risk of allergic disease. However, important questions remain about the inhibitory pathways through which TGF- $\beta$ <sup>+</sup> cells induce tolerance, the role of FOXP3, antigen dosing and specificity, and defining the population predisposed to atopy.

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