

Alpha-one antitrypsin aerosolized augmentation abrogates neutrophil elastase induced expression of Cathepsin B and Matrix Metalloprotease 2 *in vivo* and *in vitro*

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ABSTRACT

Neutrophil elastase (NE) activity is increased in lung diseases such as alpha-1-antitrypsin (A1AT) deficiency and pneumonia. We have recently demonstrated that NE can induce expression of cathepsin B and MMP 2 *in vitro* and in a mouse model. We postulated that increased Cathepsin B and MMP-2 in acute and chronic lung diseases are due to the presence of high levels of extracellular NE and that expression of these proteases could be inhibited by A1AT augmentation therapy. Cathepsin and MMP activities were assessed in bronchoalveolar lavage fluid (BAL) from A1AT deficient, pneumonia and control patients. Macrophages were exposed to BAL rich in free NE from pneumonia patients, following pre-treatment with A1AT. MMP-2, Cathepsin B, SLPI and lactoferrin levels were determined in BAL from A1AT deficient patients pre and post aerosolization of A1AT. BAL from both pneumonia and A1AT deficient patients, containing free neutrophil elastase, had increased cathepsin B and MMP-2 activities compared to BAL from healthy volunteers. Addition of A1AT to pneumonia BAL greatly reduced NE-induced cathepsin B and MMP-2 expression in macrophages *in vitro*. A1AT augmentation therapy to A1AT deficient individuals also reduced cathepsin B and MMP-2 activity in BAL *in vivo*. Furthermore, A1AT deficient patients had higher levels of SLPI and lactoferrin post A1AT augmentation therapy. This study describes a novel role for A1AT - inhibition of neutrophil elastase-induced up-regulation of expression of MMPs and cathepsins both *in vitro* and *in vivo*.

INTRODUCTION

Elevated levels of proteases are typically observed in acute and chronic lung diseases at sites of inflammation and infection. Proteases have a multitude of effects including tissue destruction, tissue remodeling and cleavage of soluble innate factors. We previously demonstrated that cathepsin B can cleave and inactivate key innate immunity proteins including lactoferrin¹ and SLPI². We have also recently described a novel hierarchy in protease regulation demonstrating that NE could induce expression of cathepsin B and MMP 2 *in vitro* and in a mouse model³. MMPs and cathepsins have previously been demonstrated along with NE in conditions such as emphysema and cystic fibrosis (CF)^{4,5}. We postulated that increased extracellular NE activity *in vivo* may induce expression of both cathepsins and MMPs. These proteases are capable of significant tissue destruction and inflammation both directly and indirectly by cleaving key innate anti-inflammatory and antimicrobial proteins. We hypothesized that NE-mediated upregulation of cathepsins and MMPs could be inhibited by A1AT, a naturally occurring inhibitor of NE. Reduced expression of these destructive proteases could have significant implications in reducing both lung inflammation and cleavage of innate immunity proteins.

In this study we demonstrate that NE upregulates cathepsin B and MMP 2 *in vitro* and *in vivo* in pneumonia and A1AT deficiency and that this effect of NE can be prevented by A1AT. We demonstrate increased levels of the key innate immunity proteins, lactoferrin and SLPI following A1AT aerosolized augmentation. Protease inhibitors are attractive therapeutic targets in many lung diseases. Inhibition of NE may provide a therapeutic target in conditions with elevated cathepsin B and MMP2 activities such as A1AT deficiency, CF, COPD and pneumonia. By inhibiting NE, it may be possible to abrogate not just the deleterious effects of this key serine protease, but also the effects of its cysteinyl and metallo protease secretagogues, cathepsin B and MMP 2 respectively.

METHODS

Selection and description of A1AT deficiency and pneumonia patients

An augmentation study of aerosolized A1AT in individuals with A1AT deficiency was carried out in the Department of Medicine, University of Florida, Gainesville using plasma purified alpha 1-antitrypsin produced by Aventis Behring (now ZLB Behring, King of Prussia, PA, USA). A1AT was converted to dry powder by spray drying and packaged in unit dose blisters prior to delivery by a pulmonary delivery system (Nektar, formerly Inhaled Therapeutics, San Carlos, CA, USA). Briefly, a Dry Powder Pulmonary Device was used that disperses fine, dry, respirable powders in a reproducible fashion for to the lung. The mass median aerodynamic partical size was two microns. This was a dose escalation study evaluating different doses of A1AT from 6-96mg given once daily for 14 days. After adequate sedation and analgesia, bronchoscopy and BAL were performed with the subject in the supine position. The bronchoscope was wedged into the anterior segment of the right or left upper lobe. BAL was performed in each lobe with five sequential 20-mL aliquots of normal saline solution, which were infused quickly with no dwell time between infusion and aspiration. BAL fluid from both lobes was combined, and the percentage of the recovered volume was measured. Mucus was removed from BAL fluid using two layers of sterilized cotton gauze. Cells were separated from the BAL fluid at 2,000 revolutions per minute for 15 min. The BAL fluid then was separated and stored at -70°C. The dosage was given once a day for 14 days. Patients were lavaged prior to entering the study and at a time point after the last aerosol. The pre-BAL was day -13, -14 i.e. 13-14 days pre study and the post BAL was 24+/- 4 hours after last dose i.e. day 15. For the purpose of this study we evaluated only those individuals with free NE in their BAL prior to aerosolized augmentation therapy and those for whom we had access to post aerosolized augmentation BAL samples (6mg of A1AT) (n=11) (Table 1). There was no selection bias before the data was analysed. BAL was obtained according to standardized guidelines and as approved by the Beaumont Hospital and University of Florida Review Board committee. All subjects had a diagnosis of A1AT deficiency, PiZZ phenotype confirmed by nephelometry and isoelectric focusing.

BAL fluid was also obtained from community acquired pneumonia (CAP) patients (n=9, 4 female and 5 male) of mean age 54 ± 1.63 years, of whom 3 were nonsmokers, 1 was a former smoker for 5-10 years (with 10-20 pack-years of history), and 5 were current smokers (10-35 cigarettes/day with 15-30 pack-years of history). CAP was diagnosed using established criteria. All BAL samples were found to be negative by quantitative culture. Sputum and blood Gram stain and culture were performed. *Streptococcus pneumoniae* was identified in just under half of the patients. Cytospin preparations of BAL cells were prepared for cytologic analysis. Filtered BAL fluid was aliquoted and stored at -80 °C. Controls were recruited from medical outpatient clinics at Beaumont Hospital and had no history of pneumonia, current infection, were PiMM phenotype confirmed by nephelometry and isoelectric focusing and were non-smokers (n=9). ELF levels were determined by measuring urea levels in BALF and serum (from blood taken at the time of bronchoscopy). Ethical approval was obtained and all subjects consented to participate in the study.

Culture and stimulation of monocyte cells

Myelomonocytic cells (U937) (European Collection of Cell Cultures Health Protection Agency, Salisbury, Wiltshire, UK) were cultured in RPMI 1640 medium (Gibco) and were differentiated

to macrophage-like cells for 48 hours with phorbol myristic acetate (PMA). The macrophage-like cells were incubated in fresh medium for a further two days before stimulation. Stimulation was performed with pneumonia BAL fluid (pooled from 9 patient samples with community acquired pneumonia possessing a mean NE activity of 146.85 ± 35.06 nM/epithelial lining fluid (ELF)) for 1 hour in fresh serum-free medium, washed and cultured in fresh serum-free medium for either 3 h or 24 hours before harvesting, if needed for RNA or protein isolation, respectively.

NE Measurements

NE activity in BAL fluid or cell supernatant was determined by use of the NE-specific substrate *N*-methoxy-succinyl-Ala-Ala-Pro-Val *p*-nitroanilide (Sigma). Release of *p*-nitroaniline was measured at 405 nm every 2 minutes over a 20-min time period and was compared with an NE standard of known activity (Low-endotoxin elastase derived from human sputum (approx. 50% active), Elastin Products, Owensville, MO, USA)⁶. All assays were performed in triplicate.

Semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR)

After treatment, cells were harvested in Tri reagent (Sigma-Ireland) and RNA was extracted as detailed in the manufacturer's protocol. RNA (2 µg) was reverse-transcribed at 37°C with 1 mM deoxynucleotide mix (Promega, Southampton, UK), 1.6 µg oligo-p[dT]15 primer (Roche, Lewes, UK) and 1 µl M-MLV reverse transcriptase (Promega, Southampton, UK) in a 20 µl volume as described in the manufacturer's protocol. 2 µl of each cDNA was amplified with 1.25 U Taq DNA polymerase, 1×PCR buffer and 10 mM dNTPs (Promega) in a 50 µl volume containing 100 pmol each of the following primers: 5'- ATG TGG CAG CTC TGG GCC T-3' and 5'-TAC TGA TCG GTG CGT GGA ATT-3' for cathepsin B; 5'-GCC CCC AAA ACG GAC AAA GA-3' and 5'-TCC CAA GGT CCA TAG CTC ATC G-3' for MMP-2; 5'-AAC TCT GGT AAA GTG GAT-3' and 5'-TAC TCA GCG CCA CCA GCA TCG-3' for GAPDH. PCR products were quantified densitometrically at cycle numbers between 10 and 40 to determine the appropriate cycle number at which exponential amplification of products was occurring, and to identify the cycle number at which sufficient discrimination was possible to accurately quantify increases or decreases in gene expression. After a hot start the amplification profile was 32 cycles of 1 min denaturation at 94°C, 1 min annealing at 58°C and 1 min extension at 72°C. RT-PCR amplification of cathepsin B, MMP-2 and GAPDH generated products of 1004 bp, 525 bp and 211 bp respectively. PCR products were commercially sequenced (MWG Biotech AG, Ebersberg, Germany) to verify gene identity. PCR products were resolved on a 1% agarose gel containing 0.5 µg/ml ethidium bromide (Sigma). The ratio of PCR fragment intensities of cathepsin B and MMP-2 relative to GAPDH was determined by densitometry.

Presence of cathepsin B

Cathepsin B activity was determined from either BAL or medium taken from macrophage-like cells 24 hours after stimulation with or without BAL. Cathepsin B activity was determined in 100 µl of each sample using the substrate Z-Arg-Arg-AMC (0.1 mM). A cathepsin B inhibitor CA-074 (10 µg/ml) was used as a control for the specificity of the cathepsin B substrate. The reaction buffer used for cathepsin B activity estimation was 0.2 M sodium acetate, 2mM EDTA, 1 mM DTT, 1 µM pepstatin, and 2 mM Pefabloc, pH 5.5. The samples were incubated with substrate for 60 min at 37°C, and fluorescence (substrate turnover) was determined by excitation at 355 nm and emission at 460 nm. Results were expressed as a change (delta) in fluorescence units over a 60-minute period (FU).

Zymography

Gelatin zymography was performed on either BAL samples or medium collected from unstimulated or BAL fluid stimulated cells. Samples were subjected to 7% SDS-polyacrylamide gel electrophoresis with a gel-containing gelatin (1mg/ml). After electrophoresis was performed gels were incubated in 50mM Tris (pH 7.5), 5mM CaCl₂, 1μM ZnCl and 2.5% (v/v) Triton X-100 for 30 minutes. The gels were washed in the same buffer without the Triton X-100 for 5 minutes and then incubated at 37°C overnight in the same buffer supplemented with 1% (v/v) Triton X-100. The gels were stained with 0.125% Coomassie blue and washed with 10% acetic acid and 40% methanol in water. The presence of MMPs appears as transparent bands. Latent MMP-2 and active MMP-2 were observed at 72 and 66kDa, respectively. Densitometry was carried out to compare the intensity of the MMP transparent bands.

Western blot

BAL from healthy controls and A1AT deficient patients were investigated for the presence of cathepsin B. BAL samples were separated by electrophoresis on 10% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane (Sigma-Aldrich) and this was probed using rabbit anti-cathepsin B antibody (Calbiochem). Binding was detected using the appropriate horseradish peroxidase-conjugated secondary antibody and visualised by chemiluminescence (Pierce).

Densitometric analysis

Gels were analysed by densitometry and compared in a semiquantitative manner using the GeneGenius Gel Documentation and analysis system (Cambridge, UK) and GeneSnap and GeneTools software. All expression values were verified by at least two independent experiments.

Statistical analysis

Data were analyzed with the PRISM 3.0 software package (GraphPad, San Diego, CA). Results are expressed as the mean ± SE and were compared by *t* test. When more than 2 groups were being compared an ANOVA test was used, followed by a Tukeys post hoc test. Differences were considered significant at $p \leq 0.05$.

RESULTS

BAL characteristics

BAL samples were collected from A1AT deficient, pneumonia and control patients. Cytospin preparations were analyzed to determine cell numbers per milliliter of BAL fluid. The sample data for the A1AT deficient patients is summarized on Table 1. NE activity levels were reduced from 297.0 ± 325.4 to 0.00 nM/ELF following A1AT aerosolized augmentation therapy but without reducing the percentage of neutrophils within the BAL fluid. Samples from the pneumonia-infected lung contained elevated numbers of cells compared to controls ($1.59 \times 10^6 \pm 8.3 \times 10^5$ cells/ml vs. $3.89 \times 10^5 \pm 7.6 \times 10^4$ cells/mL; $P < 0.05$). The macrophage: neutrophil ratio in the infected ELF was 5:3 compared to 4:1 in the uninfected. CAP was characterized by neutrophil influx, with 5.5-fold more neutrophils at the infection site for control and infected ($8.4 \times 10^4 \pm 6.2 \times 10^3$ cells/mL and $4.7 \times 10^5 \pm 2.4 \times 10^4$ cells/mL; $P < 0.05$).

Protease profile from normal, A1AT Deficiency and pneumonia BAL

Cathepsin B and MMP-2 activities were measured in BAL from individuals with A1AT deficiency and community-acquired pneumonia. Elevated cathepsin B activity was observed in A1AT deficient BAL compared to BAL from non-smoking, healthy controls using the cathepsin substrate Z-Arg-Arg-AMC (Figure 1a). Incubation of A1AT deficient BAL with the cathepsin B inhibitor, CA-074, significantly reduced turnover of the substrate Z-Arg-Arg-AMC indicating that cathepsin B is the primary cathepsin present in A1AT deficient BAL (Figure 1a, $P < 0.0001$, when all experimental groups were compared). This was further confirmed by Western blot, which demonstrated the presence of cathepsin B in A1AT deficient BAL, but no cathepsin B was detected in BAL from healthy control subjects (Figure 1b). Cathepsin B and MMP-2 activities were measured in BAL from individuals with pneumonia. High cathepsin B activity was observed in BAL from pneumonia patients compared to BAL from controls (Figure 1a, $P < 0.0001$). Active forms of MMP-2 were present in A1AT deficiency and pneumonia BAL unlike control BAL (Figure 1c).

Cathepsin B and MMP-2 gene expression and activity from macrophages exposed to pneumonia BAL containing free NE

Previous investigators have used U937 cells to investigate MMP and cathepsin activity extracellularly secreted from monocytes and macrophages.^{7,8} To examine whether exposure of differentiated U937 cells to BAL samples obtained from pneumonia patients could mimic the effect of NE, differentiated U937 cells were exposed to pneumonia BAL containing (146.85 ± 35.06 nM) active NE. Cathepsin B and MMP-2 mRNA expression levels were investigated by RT-PCR (Figure 2a). Cathepsin B ($P = 0.0057$) and MMP-2 ($P = 0.002$) genes expression were observed to increase when cells were stimulated with PB but this increased expression was suppressed when cells were pre-treated with A1AT. No NE activity was recorded in pneumonia BAL fluid or cell supernatant following addition of A1AT (data not shown). Cathepsin B and MMP-2 activities were measured in the supernatants and pneumonia BAL-stimulation was found to elevate cathepsin B activity compared to non-stimulated control cells (Figure 2b). Pneumonia BAL stimulation resulting in cathepsin B activity was inhibited when cells were pre-treated with A1AT ($P = 0.0005$, when all experimental groups were compared). Increased active MMP-2 was observed when cells were treated with Pneumonia BAL (Figure 2c), which was abrogated when Pneumonia BAL was incubated with A1AT.

Protease and antiprotease activity in A1AT deficiency patients following aerosolized augmentation therapy

Cathepsin B and MMP-2 activity was measured in BAL from eleven A1AT deficient patients before and after A1AT treatment. Patients were treated with 6mg A1AT and BAL was recovered 12 hours later. Cathepsin B activity was statistically demonstrated to diminish when patients were treated with A1AT (Figure 3a, $P=0.0019$). A1AT treatment was also observed to result in a statistically significant reduction in latent and active MMP-2 (Figure 3b, $P=0.0392$ and 0.0396 , respectively). The band observed at 78 kDa is active MMP-9.

SLPI and lactoferrin levels in BAL from A1AT deficient patients before and after A1AT treatment, were examined by ELISA (Figure 3c). Lactoferrin levels were observed as significantly increased following A1AT treatment ($P=0.0349$). SLPI levels had increased following A1AT treatment but not significantly ($P=0.2857$).

DISCUSSION

The pulmonary extracellular matrix is a constantly changing infrastructure comprised of a complex mixture of proteoglycans, collagen and elastin. It is estimated that as much as one tenth of the extracellular matrix is degraded and replaced each day in the normal lung⁹. Members of the serine, matrix metalloproteases and cysteinyl protease families have been associated traditionally with lung inflammation and airway extra cellular matrix destruction. Cathepsin B is a cysteinyl protease that is expressed ubiquitously in lung tissue, particularly in bronchial epithelial cells and macrophages¹⁰. Cathepsin B degrades types IV and X collagen and also fibronectin *in vitro*^{11,12} and intratracheal instillation of cathepsin B has been shown to induce emphysema in hamsters¹³. Cathepsin B activity is also elevated in infective and inflammatory conditions such as pneumonia and Cystic Fibrosis. Schmidt *et al*¹⁴ demonstrated increased cathepsin B activity in BAL from patients with both chronic bronchitis and pneumonia. Recently, we have shown that Cathepsin B activity is elevated in CF bronchoalveolar lavage (BAL) and that activity was several hundred fold increased compared to BAL from healthy subjects⁵.

A number of secretagogues of cathepsin B have been described. For example, IFN- γ causes emphysema and alterations in pulmonary protease/antiprotease balance when expressed in pulmonary tissues in mice by inducing cathepsins B, H, D, S, and L as well as several MMPs. Furthermore this inflammation can be attenuated by cathepsin inhibition¹⁵. Cigarette smoke has been shown to stimulate cathepsin B activity in alveolar macrophages of rats¹⁶. LPS has been shown to accelerate caspase-independent but cathepsin B-dependent cell death of human lung epithelial cells and inhibition of cathepsin B was shown to prevent lung cell apoptosis. Recently, cathepsin B was one of several cathepsins implicated in the pathogenesis of bronchopulmonary dysplasia¹⁷. Clearly, cathepsin B is a key protease involved in a variety of lung diseases. Work by Burnett *et al*¹⁸ demonstrated that the predominant form of cathepsin B (Mr 42,000) is converted to an active form (Mr 38,000) upon treatment with NE and we showed that extracellular NE is not only required to activate cathepsin B³. Data in this manuscript demonstrates similar findings in BAL from human subjects and describes a novel way to potentially prevent cathepsin B and MMP 2 mediated lung inflammation and destruction by inhibiting their upregulation by NE.

Similarly to cathepsins, over-expression of MMPs has been associated with destruction associated in the lung¹⁹. MMP-2 has been demonstrated in pneumocytes, fibroblasts, and alveolar macrophages in both emphysematous and normal lung tissue samples with significantly more immunoreactivity in the emphysematous samples²⁰. Interleukin-13 (IL-13) up-regulates MMP 2 and several other MMPS as well as cathepsins to causes emphysema in a mouse model^{15,21}.

We have recently shown that NE can induce cathepsin B and MMP 2 expression in macrophages and a mouse model of *Pseudomonas* infection³. We demonstrated that NE can activate NF- κ B in macrophages, and inhibition of NF- κ B or TLR-4 or transfection of macrophages with dominant-negative IL-1R-associated kinase-1 resulted in a reduction of NE-induced cathepsin B and MMP-2. Given that these proteases are responsible for much of the lung inflammation and destruction in acute and chronic lung diseases, we postulated that inhibition of NE might abrogate their upregulation and production. Inhibition of NE's ability to upregulate such key destructive proteases could provide potential new strategies for therapeutic interventions.

A1AT is the principal inhibitor of neutrophil elastase. Deficiency of A1AT in the lung results in insufficient anti-elastase protection in the lower respiratory tract, thus allowing neutrophil elastase to destroy alveolar structures leading to premature and aggressive emphysema. Inhibition of NE in the A1AT deficient lung has been investigated as a therapeutic target for the past 20 years. The goal of A1AT aerosolized augmentation therapy in A1AT deficiency is to raise lung A1AT levels and anti-neutrophil elastase capacity thereby providing adequate protection against neutrophil elastase and preventing elastase-mediated degradation²². Previous augmentation studies have employed intravenous and aerosol delivery systems. Both modes of delivery have shown increased A1AT levels and anti-NE capacity in the lung ELF. Similar to the inflammatory manifestations of A1AT deficiency, neutrophil-dominated inflammation on the respiratory epithelial surface also occurs in cystic fibrosis (CF) resulting in a chronic epithelial burden of NE. A1AT has been given by aerosol form to CF patients and has successfully suppressed NE in ELF and restored the ELF anti-NE capacity²³. This treatment also reversed the inhibitory effect of CF ELF on *Pseudomonas* killing by neutrophils, which suggested that it may augment host defence in CF.

Replacement therapy for A1AT deficiency-associated emphysema may be associated with a reduction in the frequency and severity of lung infections as well a reduction in inflammation^{24,25}. Lactoferrin and SLPI have key anti-inflammatory and antimicrobial roles. Lactoferrin has been shown to inhibit the LPS-induced expression and proteoglycan binding ability of Interleukin-8 in human endothelial cells²⁶. Lactoferrin can bind unmethylated CpG motifs, which are bacterial DNA products capable of stimulating various innate and acquired immune responses in human and murine models²⁷. It is bactericidal against many of the common pathogens seen in the lung, is fungicidal and can prevent viral replication²⁸⁻³⁰. SLPI possesses many similar attributes having important anti-bacterial, anti-viral and anti-inflammatory properties. SLPI can inhibit LPS-induced NF- κ B activation by inhibiting degradation of IRAK, I κ B α and I κ B β and can also impair lipoteichoic acid (LTA) and LPS induced proinflammatory gene expression in monocytes and macrophages in vitro^{31,32}. Lactoferrin and SLPI have been shown to act synergistically in bacterial killing³³.

In this study, augmentation with A1AT resulted in increased levels of lactoferrin in BAL from patients with A1AT deficiency. We speculate that aerosolized augmentation therapy resulted in decreased cathepsin cleavage of these key anti-inflammatory and anti-microbial proteins. Restoration of host innate immunity by abrogating cathepsin cleavage demonstrates a further potential therapeutic effect of aerosolized augmentation therapy.

The activities of multiple proteases in chronic lung disease are clearly deleterious to the host. We have elucidated a hierarchy in protease control and regulation whereby NE orchestrates tissue destruction and inflammation by activation of other proteases (Cathepsin B and MMP-2.) Neutralization of NE's effects with a specific antiprotease (A1AT) may be sufficient to lessen the overall protease burden in chronic infective lung disease without the need for inhibition of all proteases. This may not only prevent protease-induced lung destruction but may play an important role in restoring lung host defense.

COMPETING INTERESTS

The authors have no competing interests.

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FIGURE LEGENDS

Figure 1 Cathepsin and MMP-2 activity in A1AT deficient and pneumonia BAL versus control BAL. (a) Cathepsin activity in control BAL, A1AT deficient BAL alone or in the presence of the specific cathepsin B inhibitor CA-074 and pneumonia BAL. * $P < 0.0001$ when all experimental groups were compared. (b) Western blot of cathepsin B in control (lanes 1,3) and A1AT deficient BAL (lanes 2,4). (c) MMP-2 activity was determined in control (cBAL), A1AT deficiency (a1BAL) BAL and pneumonia (pBAL) BAL using gelatin zymography. Bands at 72 and 66kDa are representative of latent and active MMP-2, respectively and are highlighted. Protein loading was corrected to albumin levels. Experiments or analyses of results were performed at least 3 times and representative data and SE are shown (upper limits).

Figure 2 Cathepsin B and MMP-2 gene expression and activity from U937 macrophages exposed to pooled pneumonia BAL (pBAL) containing free NE. (a) RT-PCR of cathepsin B and MMP-2 were assessed compared to GAPDH from cells stimulated with pBAL or pBAL and A1AT. *Cathepsin B ($P = 0.0057$) and #MMP-2 ($P = 0.002$) when all experimental groups were compared. (b) Cathepsin activity 24 hours after stimulation with pBAL or pBAL and A1AT. * $P = 0.0005$ when all experimental groups were compared. (c) MMP-2 activity following stimulation with pBAL or pBAL and A1AT. Bands at 72 and 66kDa are representative of latent MMP-2 and active MMP-2, respectively. Protein loading was corrected to albumin levels. Experiments were performed at least 3 times and representative data and SE are shown (upper limits).

Figure 3 A1AT aerosolized augmentation therapy effects on protease and antiprotease levels in the A1AT deficient lung. (a) Cathepsin B activity in BAL from A1AT deficient patients pre and post 6mg A1AT treatment. Cathepsin B activity was corrected to albumin levels. * $P = 0.0019$ between the pre and post treatment BAL samples. (b) MMP-2 activity was determined using gelatin zymography and by densitometry. Bands at 72 and 66kDa are representative of latent MMP-2 and active MMP-2, respectively. The band observed at 78 kDa is active MMP-9. * and # $P = 0.0392$ and $P = 0.0396$ for MMP-2 activation (latent and active forms), respectively, between the pre and post treatments. Densitometry values are representative of all the patient population examined on three zymogram gels, ran on independent days. (c) SLPI and Lactoferrin levels in BAL from A1AT deficient patients pre and post 6mg A1AT treatment by ELISA. SLPI and lactoferrin levels were corrected to albumin levels. * $P = 0.0349$ and # $P = 0.2857$ between the pre and post treatment BAL samples. Analyses of results were performed at least 3 times and representative data and SE are shown (upper limits).

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Table 1: Clinical characteristics of the ZZ Alpha-1-antitrypsin population

Criteria	Pre	Post
Age	52 ± 8	
M/F breakdown	60/40	
FEV1 (predicted)	60.9 ± 12.4	60.8 ± 10.5
FEV1/FVC %	53.7 ± 15.3	52.0 ± 15.6
Neutrophil counts (% of total cells)	26.99 ± 20.51	31.33 ± 22.73
NE activity (nM/ELF)	297.0±325.4	0.00 *

Data are presented as median plus/minus standard deviation

*p<0.05 pre-treatment vs post-treatment.

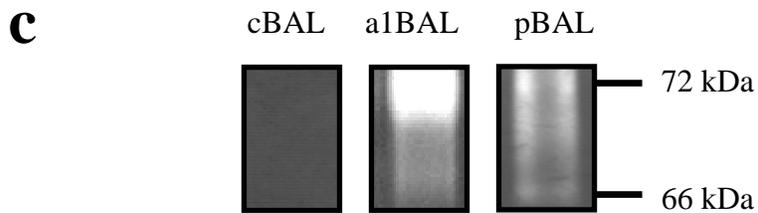
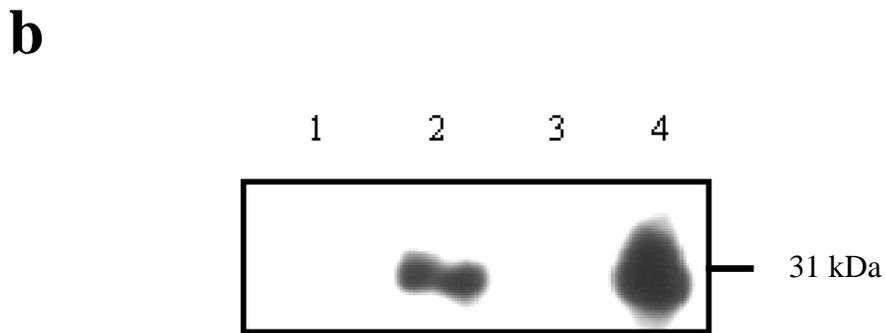
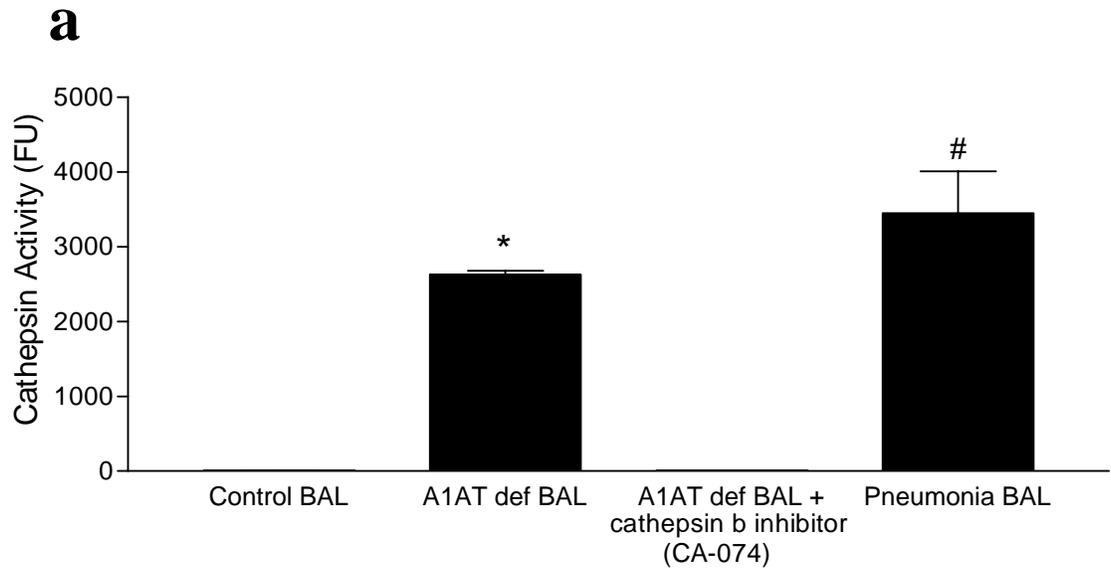


Figure 1

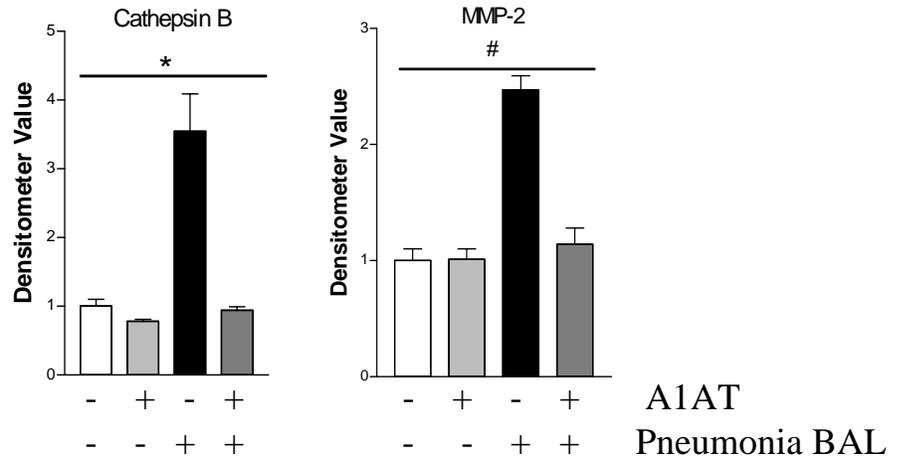
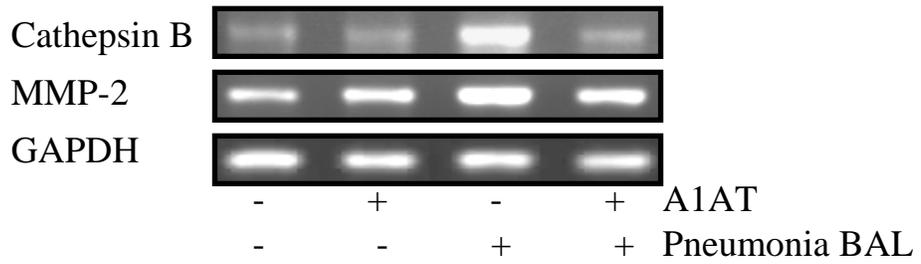
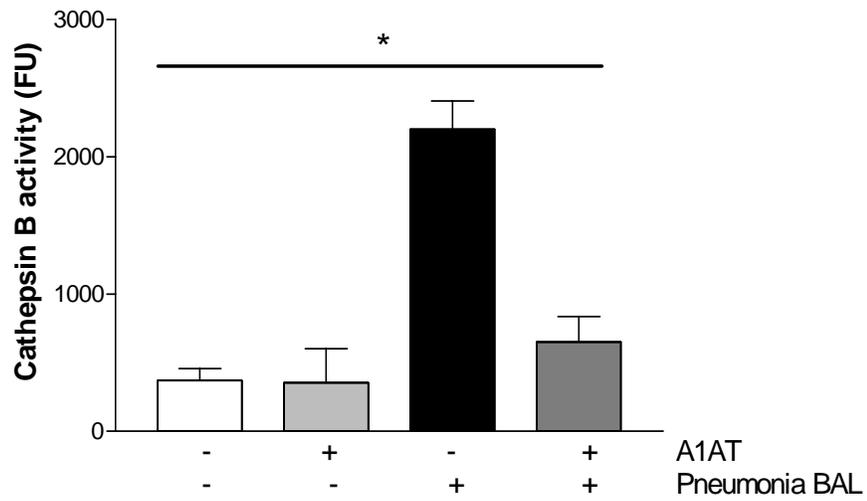
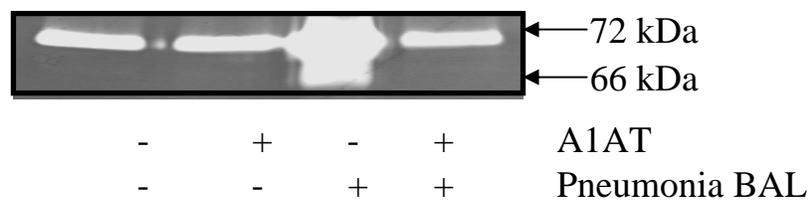
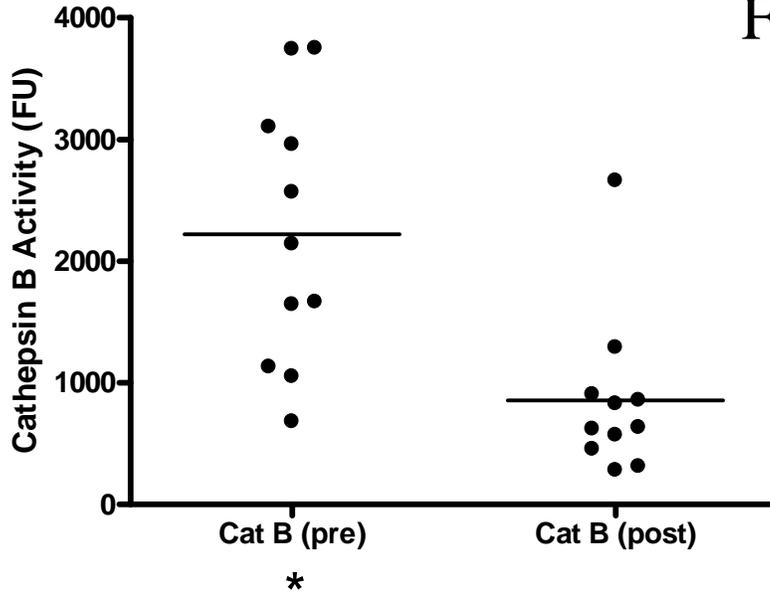
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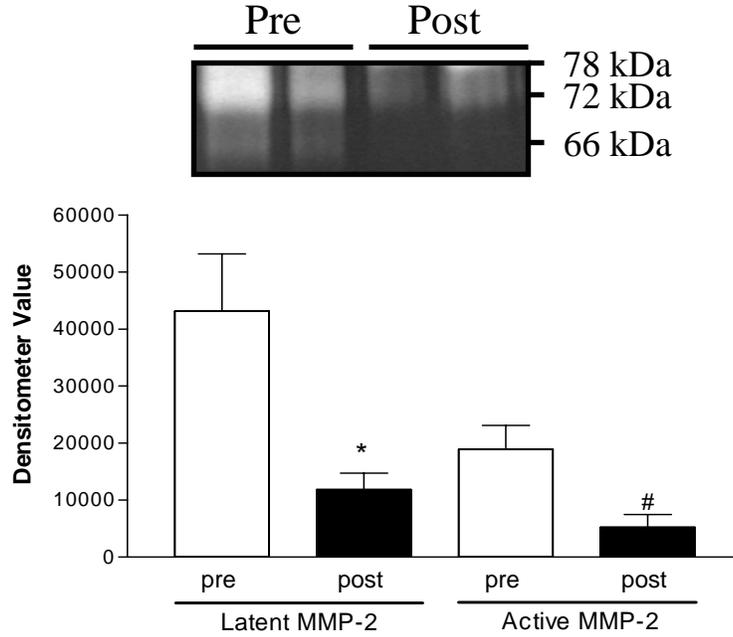
Figure 2

Figure 3

a



b



c

