

INTRAVENOUS AUGMENTATION THERAPY DOES NOT DECREASE AIRWAY
EPITHELIAL CELL INFLAMMATION IN INDIVIDUALS WITH ALPHA-ONE
ANTITRYPSIN DEFICIENCY

By

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To Sam: You are the reason I'm doing what I'm doing.

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Abstract of Thesis Presented to the Graduate School
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Alpha-1-Antitrypsin (AAT) deficiency is an autosomal recessive genetic disease associated with the development of early onset emphysema, bronchiectasis and chronic bronchitis. Homozygous individuals have deficient production of alpha-1 antitrypsin, an anti-protease protein with known additional anti-inflammatory properties. Individuals with two severe deficiency alleles maintain a markedly increased genetic risk for developing progressive emphysema. However, a significant variability in the development of lung disease is consistently observed. Exposure to environmental toxicities such as cigarette smoke clearly contributes to an accelerated decline in lung function in deficient patients. Other factors such as asthma, history of previous pneumonias or other childhood respiratory illnesses, chronic bronchitis and sex have all been associated with a more rapid development of severe lung disease. The effect of these factors suggests that both acute and chronic airway inflammation contributes to the development of airflow obstruction and progressive emphysema. Intravenous (IV) augmentation therapy with human blood-derived AAT has been demonstrated to restore blood and epithelial lining fluid concentrations of AAT to protective levels and to decrease inflammatory markers in both expectorated sputum and in bronchoalveolar lavage fluid,

presumably by limiting neutrophil chemoattraction. The effect of IV augmentation therapy on limiting airway epithelial cell inflammation remains undetermined. Therefore, this study was designed to investigate the potential reduction in bronchial airway epithelial cell inflammation following IV augmentation therapy. This study was a prospective, observational pilot study in 11 AAT deficient individuals to evaluate the effect of IV AAT augmentation therapy on epithelial cell inflammation as measured by epithelial cell gene expression of the inflammatory markers TNF- α , IL-8, IL-15, HSP-1 and OSM-receptor. BIP and IRE-1, genes associated with the unfolded protein response, were also evaluated, as was AAT gene expression. After 12 weeks of therapy, no significant changes in gene expression were noted for any of the selected genes. AAT gene expression was demonstrated in the airway epithelial cells and also remained constant after 12 weeks of therapy. The results of this pilot study suggest that AAT IV augmentation therapy does not significantly affect airway epithelial cell inflammation and that additional therapeutic strategies are warranted in order to reduce the progression of airways disease in AAT deficient individuals.

CHAPTER 1 INTRODUCTION

Alpha-1-Antitrypsin Deficiency

Alpha-1-Antitrypsin (AAT) deficiency is an inherited disorder characterized by low serum concentrations of the AAT protein and is associated with the early onset of severe lung disease. AAT deficiency, the most common inherited cause of COPD, can lead to the development of severe emphysema even with minimal exposure to tobacco smoke. Individuals with the most common AAT deficiency phenotype, Pi ZZ, have an 18-fold greater risk of developing severe emphysema compared to individuals with adequate AAT levels. AAT deficiency occurs with a prevalence rate of 1 in 1,600 to 1 in 4,000. Up to 10% of individuals with COPD have at least one copy of the Z gene mutation. Currently, no cure exists for AAT deficiency [1].

AAT deficiency is associated with the premature onset of progressive COPD, characterized by basilar emphysema, chronic bronchitis and bronchiectasis, especially in smokers [2]. AAT is a serum acute-phase glycoprotein that inhibits several types of proteolytic enzymes, notably neutrophil elastase (NE). The increased activity of NE in AAT deficient individuals has been long believed to be the primary cause of lung damage. However, a growing body of clinical evidence shows that the absence of sufficient AAT in the lower respiratory tract is associated with an increased burden of pro-inflammatory factors such as TNF-alpha, IL-6, IL-8 and C-reactive protein (CRP) and that AAT may have broad anti-inflammatory properties [3] [4] [5].

AAT and Airway Inflammation

Asthma-like symptoms and airway hyper-responsiveness are common and important findings in many patients with AAT deficiency. One large cohort study revealed 82% of AAT deficient patients reported wheezing at their well baseline and almost 90% reported wheezing with cold symptoms [6]. Almost 50% of the cohort had a significant response to bronchodilator therapy.

Longitudinal study in patients with AAT deficiency demonstrates that the most rapid decline in airway function, measured by the forced expiratory volume in one second (FEV₁), occurs in the third and fourth decades of life, which is also when the onset of self-reported wheezing occurs [7]. Additionally, the diagnosis of asthma and significant airway hyper-responsiveness have both been associated with a more rapid decline in lung function in AAT deficiency [7, 8]. These results suggest that the absence of sufficient AAT in the respiratory tract is associated with increased airway inflammation. Increased airway inflammation likely results in airway remodeling, worsened airflow obstruction, and accelerated decline in FEV₁.

Airway disease, including bronchiectasis and changes in the airway walls, has been identified in subjects with AAT deficiency. Approximately 40% of patients with AAT deficiency have chronic cough and sputum expectoration [9]. Clinically significant bronchiectasis (radiologic bronchiectasis in 4 or more bronchopulmonary segments together with symptoms of regular sputum production) has been demonstrated in 27% of patients with AAT deficiency [10]. These findings demonstrate that while emphysema is the predominant component of AAT deficiency related lung injury, airway inflammation has an important impact on disease progression. To date, studies evaluating airway inflammation in AAT deficiency have utilized either sputum or bronchoalveolar lavage (BAL) fluid, neither of which specifically targets the inflammatory changes in bronchial airway epithelial cells. An improved understanding of the airway epithelial cell inflammatory response to AAT deficiency and augmentation therapy has important implications for patient outcomes and also for drug development, specifically aerosolized therapeutics, and is therefore a crucial area of investigation.

CHAPTER 2 MATERIALS AND METHODS

Study Design

This study was a prospective, observational pilot study approved by the Institutional Review Boards at the University of Florida and the University of Texas at Tyler.

The specific aim of this pilot study was to determine the effect of intravenous AAT augmentation therapy on the gene expression of inflammatory markers in airway epithelial cells. Markers of inflammation tested were TNF- α , IL-8, IL-15, HSP-1 and OSM-receptor. BIP and IRE-1 were tested to evaluate markers of the unfolded protein response. Additionally, AAT gene expression was determined in all samples. The primary hypothesis tested was that intravenous AAT augmentation therapy would decrease bronchial airway epithelial cell inflammation.

Study Overview

After providing voluntary informed consent, subjects receiving exogenous AAT therapy underwent a 5 week washout period. Prior to receiving study related AAT augmentation therapy, all subjects underwent baseline bronchoscopy with bronchial brushings to obtain bronchial epithelial cells. Subjects then received functional human AAT (either Kamada API or Prolastin) via IV drip at a dose of 60mg/kg body weight and weekly for 12 weeks. Subjects then were evaluated with a second bronchoscopy and bronchial brushing to investigate the effect of AAT augmentation therapy on bronchial airway epithelial cell inflammation.

Subjects

Inclusion criteria included age \geq 18 years, presence of “at-risk” alleles associated with serum AAT levels \leq 11 μ M, and evidence of AAT deficiency related lung disease identified by either FEV₁ < 80% predicted post bronchodilator, loss of lung function over a one year period of greater than 35mL in FEV₁ or high resolution CT (HRCT) evidence of pulmonary emphysema.

Subjects who were actively treated with augmentation therapy prior to enrollment had to agree to not receive any exogenous AAT product (i.e. washout period) for five weeks prior to the first study infusion. All subjects had to be on a stable dose of inhaled corticosteroids for two weeks prior to first bronchoscopy and through the remainder of the study.

Exclusion criteria involved safety and efficacy parameters for delivering intravenous AAT therapy and for undergoing bronchoscopy. Current or recent (within the past 3 months) tobacco use, known allergy to plasma proteins, uncontrolled hypertension and/or tachycardia excluded participation. Factors potentially affecting immune function including laboratory evidence of severe immunoglobulin A deficiency, pregnancy or lactation, current malignancy, previous organ transplantation, history of infection with HCV, HBV, and or HIV, and any acute respiratory tract infections within the prior 6 weeks requiring antibiotics or systemic corticosteroid treatment excluded participation. Finally, $FEV_1 < 45\%$, allergy to lidocaine, or any other inability to undergo bronchoscopy also excluded participation.

Baseline Procedures

Patients were enrolled at the University of Florida and the University of Texas at Tyler. Patients meeting inclusion criteria and agreeing to provide informed consent underwent further screening with a routine medical assessment, a complete physical examination and blood tests for hematologic, biochemical, and virology screening. Evidence of lung disease was confirmed by an HRCT and complete pulmonary function testing (PFTs) including spirometry, lung volumes, and diffusing capacity.

Study Procedures

After baseline procedures were completed, all subjects underwent bronchoscopy with bronchial brushing within 10 days of, but no less than 2 days prior to, the first infusion of AAT. Blood samples for the measurements of functional and antigenic AAT levels were drawn

immediately prior to the infusion. Subjects received an infusion of functional human AAT via IV drip at a dose of 60 mg/kg body weight. Initial week one infusions were administered at the bronchoscopy center at either research site. Subsequent weekly doses (2-11) were administered IV via each individual's home health agency. Between weeks 10 and 12, all subjects undergoing repeat bronchoscopy received complete PFTs.

Bronchoscopy

Prior to undergoing bronchoscopy, subjects could not have a history of adverse reactions to the local anesthetic, sedatives or pre-medications employed. Intravenous morphine and midazolam were used for conscious sedation in all subjects. Subjects were monitored by electrocardiogram and pulse-oximetry throughout the procedures. All subjects received inhaled bronchodilator therapy prior to receiving local airway anesthesia.

Bronchial airway brushings were obtained from the fourth through sixth segmental airways utilizing a sheathed cytologic brush system (Olympus® Endo Therapy disposable cytology brush). Brushing samples were processed immediately after bronchoscopy. Brushing specimens were initially collected in sterile RPMI-1640 media and centrifuged at 800X g force for 15 minutes at 4° C. Cell pellets were resuspended in 1mL RTL buffer/ β -mercaptoethanol mixture and stored at -80° C until future processing.

Quantification of Gene Expression

Total RNA was isolated and cDNA synthesis performed according to commonly standardized protocols. Quantitative PCR was then performed to measure gene expression of human TNF- α , IL-8, IL-15, HSP-1A, OSM-receptor, BIP, IRE-1 and AAT. Quantitative PCR was performed as follows. Primers and FAM/VIC labeled probes were purchased as either pre-developed assays or custom designed assays using the Applied Biosystems's Assay-on-demand and Assay-by-design

service, respectively (Applied Biosystems, Foster City CA). Primer sequences for each gene probed are included:

1. AAT (primer ID - HS00165475_m1) gcaaatggga gagaccctt gaagcaagg acaccgagga agaggacttc cacgtggacc aggtgaccac cgtgaaggtg cctatgatga agcg
2. HSP-A1A (HS00359163_s1) tgcagagat gaatttatac tgccatctta cgactatttc ttcttttaaa tacacttaac tcaggccatt tttaagttg gttactcaa agtaaataaa cttaaaatt caaaaaaaaa aaaaaa
3. TNF alpha (HS00174128_m1) c aaacctcaa gctgaggggc agctccagtg gctgaaccgc cgggccaatg cctcctggc caatggcgtg gagctgaga
4. IRE-1 (HS00176385_m1) aact tcctttacc atcccagaat tgggtcagc atcccctgc cgaagttcag atggaatcct ctacatgggt aaaaagca
5. BiP (HS00607129_gh) c ggcgtgtca agaacggccg cgtggagatc atcgccaacg atcagggcaa ccgcatacgc ccgtcctatg tcgcctcac tctgaaggg gaacgtctga ttggcgatgc cgccaagaac cagctcacct ccaaccccga gaaca
6. IL-8 (HS00174103_m1) gtgca gttttgcaa ggagtgtctaa agaactaga tgcagtgca taaagacata ctccaaacct tccacccca aattatcaa agaactgaga gtgattg
7. IL-15 (HS99999039_m1) ccagttg caaagtaaca gcaatgaagt gctttctctt ggagttacaa gttattcac ttgagtccgg agatgcaagt attcatgata cagtagaaaa tctgatcctc ctagcaaca acagttgtc ttcta
8. OSM-receptor (HS01051640_m1) gg gtggggaatt acagcaccac tgtgaagtgg aaccaggttc tgcattggag ctgggaatct gagctcctt tggaatgtgc cacacacttt gtaagaataa agagtttggg ggacgatgcc aagttccctg agcca.

One hundred ng of cDNA (i.e. cDNA equivalent of 100 ng total RNA) of epithelial cell and human universal reference (18s) RNA-derived cDNAs were used as templates in hot-start PCR with gene-specific primers and Taqman probe using a universal PCR master mix (Applied Biosystems, Foster City CA) in a 25 μ l total reaction volume, using the protocol supplied by the manufacturer. Replicates were used so that statistical analysis could be performed on the data generated. Data analysis was performed using Ct values determined and normalized to the 18s housekeeping gene and analyzed using the delta-delta Ct method (Analysis of Relative Gene Expression data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta Ct}$ Method) to obtain fold change

in gene expression between experimental samples and human universal reference RNA control. Expression differences were expressed as fold-change ratio from baseline to 12 weeks.

Sample Size

This pilot study had no preliminary data to provide power calculations. A previously published study evaluating the effect of intravenous AAT augmentation therapy on sputum neutrophilic inflammatory markers found significant changes in sputum leukotriene B₄ concentrations with 12 subjects[4]. This previously published study suggests that a sample size of 12 or more subjects, although small, would be an adequate target enrollment for our pilot study.

Statistical Analysis

Changes in gene expression of human TNF- α , IL-8, IL-15, HSP-1A, OSM-receptor, BIP, IRE-1 and AAT were all independent primary analysis variables. Statistical analysis was performed for each probe using the Wilcoxon matched pairs t-test for non-parametric paired samples. Statistical significance was accepted at a level of $p < 0.05$.

CHAPTER 3 RESULTS

Fifteen individuals were initially enrolled, 11 at the University of Florida and 4 at the University of Texas at Tyler. One subject elected to withdraw from the study and two subjects were withheld from repeat bronchoscopy at the investigator's discretion due to the development of adverse events (pneumothorax, pulmonary embolism) subsequent to the first bronchoscopy. Inadequate cellular return and resulting inadequate RNA concentration prevented the final study of a fourth individual's samples. Therefore, paired baseline and after therapy bronchial epithelial cell brushings were available for analysis from 11 subjects. However, due to limited concentrations of RNA recovered from the bronchoscopies, gene expression analyses was performed on only eight samples for OSM-receptor and IL-15.

Demographics

The 11 subjects completing the study had an average age of 54.8 years of age. There were 7 males and 4 females in the sample group. Seven of the 11 were previous smokers with a pack year history ranging from 5 to 70 pack years. The average FEV₁% predicted for all subjects was 61% predicted. All but one of the study subjects had an obstructive ventilatory defect on pulmonary function testing determined by FEV₁ % predicted. That individual met study inclusion criteria by having evidence of pulmonary emphysema on high resolution CT (HRCT) of the chest (Table 3-1).

Treatment

All 11 subjects received AAT augmentation therapy throughout the course of the study. Serum AAT levels were drawn prior to initiation of therapy to demonstrate AAT deficiency and again prior to follow-up bronchoscopy to ensure appropriate response to therapy ($4.3 \pm 1.4 \mu\text{M}$ at

baseline, $18.9 \pm 5.2 \mu\text{M}$ after treatment) (Table 3-1). At the end of the study, all subjects had serum AAT levels equal to or greater than $11 \mu\text{M}$, the clinically accepted protective target [11].

Gene Expression Analysis

Quantitative PCR relative gene expression units for AAT, HSP-A1A, TNF- α , IRE-1, BiP, IL-8, IL-15 and OSM-receptor are summarized in Table 3-2. No significant changes from baseline and following AAT augmentation therapy were observed in any of the selected genes.

Adverse Outcomes

As noted, two individuals initially enrolled in the study did not undergo follow-up bronchoscopy due to investigator's discretion after development of a pneumothorax in one individual and a catheter-related pulmonary embolism in another. Neither of these events was study drug related. No study drug adverse events were observed.

Table 3-1. Patient characteristics

Subject	Age	Sex	AAT Genotype	Smoking history	Pack years	FEV1 actual	FEV1 % predicted	AAT μM baseline	AAT μM 12 weeks
1.	55	M	ZZ	Former	70	2.74L	68%	3.83	17.80
2.	71	M	ZZ	Former	10	3.00L	101%	3.85	17.90
3.	58	M	ZZ	Former	25	2.16L	57%	6.11	17.80
4.	59	F	ZZ	Never	0	1.73L	58%	3.73	18.70
5.	44	F	ZZ	Former	12	1.60L	41%	3.22	14.80
6.	55	M	MaltonMZ	Former	40	2.55L	63%	6.51	19.60
7.	52	F	ZZ	Former	30	1.73L	48%	2.15	26.60
8.	56	F	ZZ	Never	0	1.94L	76%	3.28	29.40
9.	53	M	ZZ	Never	0	2.19L	55%	5.81	18.70
10.	54	M	ZZ	Never	0	1.61L	48%	5.7	16.20
11.	46	M	ZZ	Former	5	2.18L	51%	3.14	10.50

Table 3-2. Quantitative PCR relative gene expression units at baseline and after 12 weeks of AAT augmentation therapy

Gene	Baseline	12 weeks	P value (comparing baseline to 12 weeks)
AAT	0.22	0.16	0.22
HSP-A1A	2.18	1.47	0.17
TNF- α	6.16	6.66	0.86
IRE-1	0.32	0.24	0.31
BiP	0.59	0.38	0.26
IL-8	3.84	5.26	0.55
IL-15	0.75	0.66	0.65
OSM-receptor	0.13	0.11	0.22

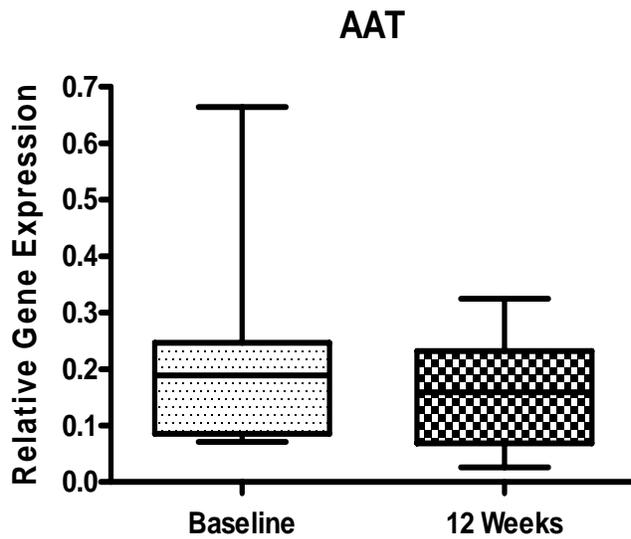


Figure 3-1. AAT quantitative PCR relative gene expression. $p=0.22$

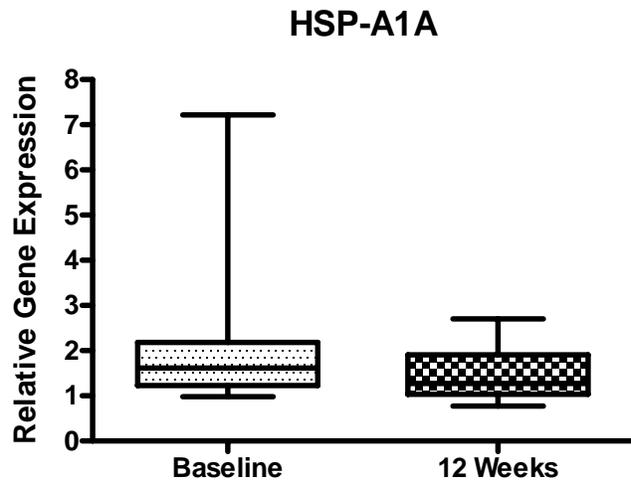


Figure 3-2. HSP-A1A quantitative PCR relative gene expression. $p=0.17$

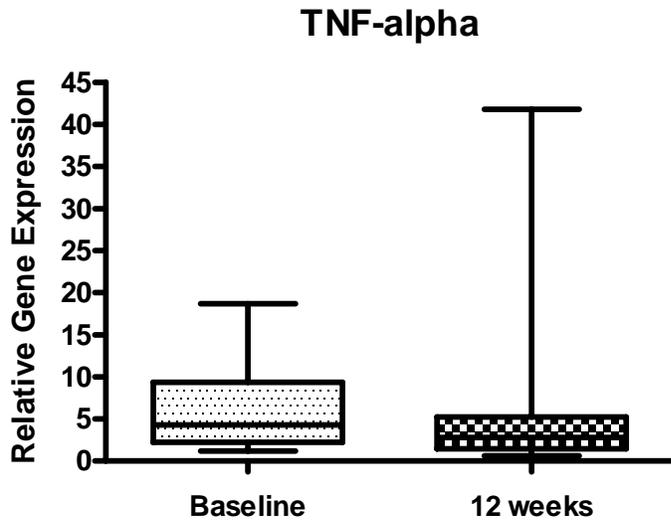


Figure 3-3. TNF- α quantitative PCR relative gene expression. $p=0.86$

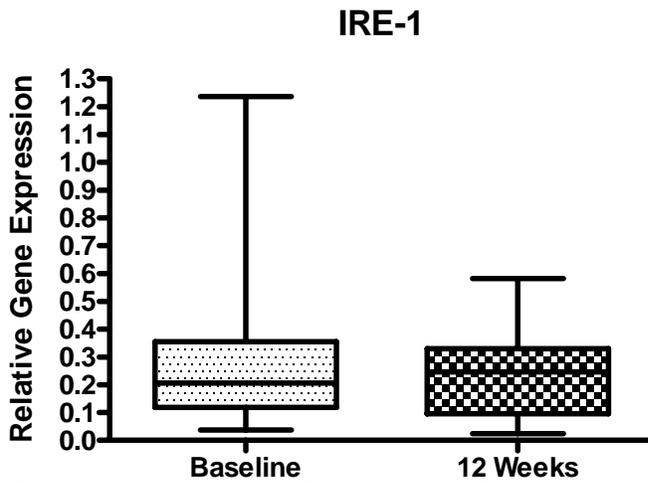


Figure 3-4. IRE-1 quantitative PCR relative gene expression. $p=0.31$

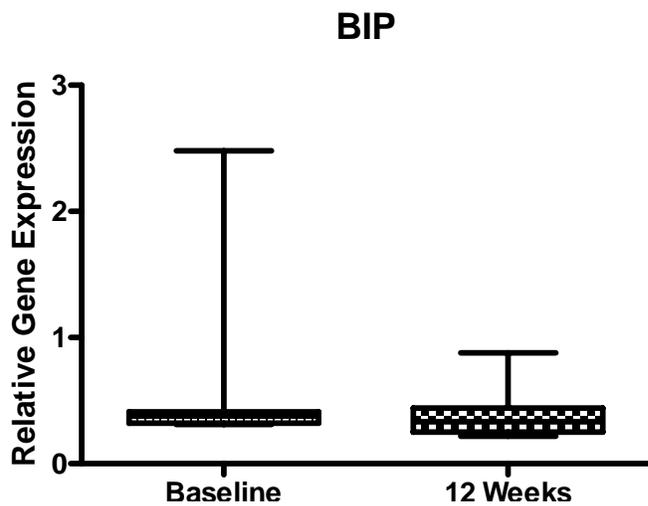


Figure 3-5. BiP quantitative PCR relative gene expression. $p=0.26$

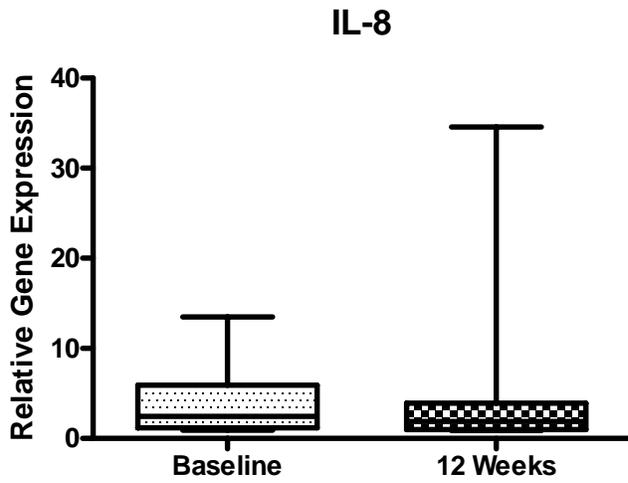


Figure 3-6. IL-8 quantitative PCR relative gene expression. $p=0.55$

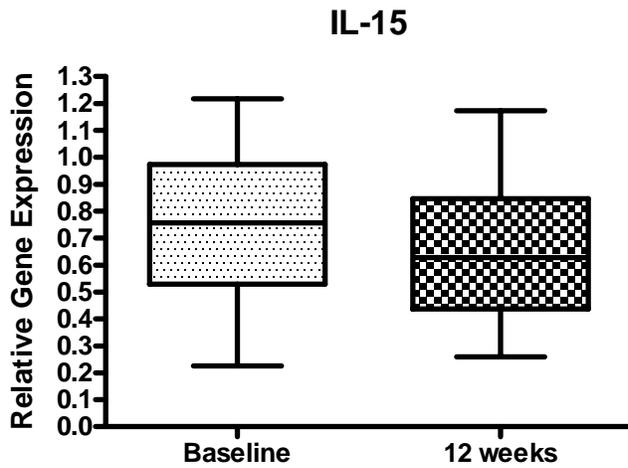


Figure 3-7. IL-15 quantitative PCR relative gene expression. $p=0.65$

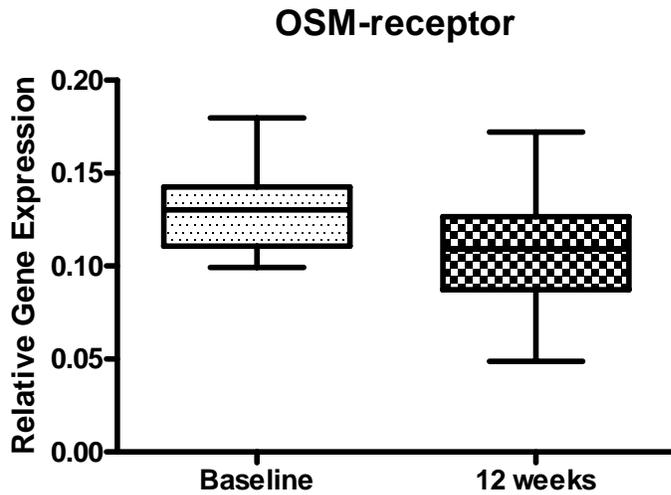


Figure 3-8. OSM-receptor quantitative PCR relative gene expression. $p=0.22$

CHAPTER 4 DISCUSSION AND CONCLUSION

Discussion

In this prospective, observational pilot study we tested the hypothesis that treatment of AAT deficient individuals with intravenous AAT augmentation therapy to clinically accepted serum concentrations would result in a decrease in airway inflammation measured by epithelial cell inflammatory gene expression. Our findings in 11 patients did not demonstrate significant changes in the inflammatory genes TNF- α , IL-8, IL-15, HSP-1 and OSM-receptor. BIP and IRE-1, genes associated with the unfolded protein response, were also not affected. We did demonstrate via cells obtained in vivo from bronchoscopy that bronchial epithelial cells express AAT, which to our knowledge has previously only been reported from cell cultures. No changes in AAT gene expression were observed following augmentation therapy in our study subjects.

These data have important implications. The link between AAT deficiency and increased airway inflammation resulting in clinically evident airways disease, such as bronchospasm and bronchiectasis, is well documented [2] [8]. This airway remodeling contributes to diminished airflow and further progression of lung disease [8]. Further supporting that a peripheral inflammatory process contributes to airflow obstruction in patients with AAT deficiency, the addition of extra-fine inhaled corticosteroids to long acting beta-agonists has been shown to decrease airway narrowing, mostly in the small airways, further reducing dynamic hyperinflation and resulting in a marked improvement in exercise tolerance and dyspnea [12]. While IV AAT augmentation therapy has been shown to decrease neutrophilic inflammation in the airways [4], a direct effect of therapy on diminishing airway epithelial cell inflammation has not been demonstrated.

There are several possibilities as to why we did not observe decreased bronchial epithelial cell inflammation in this pilot therapeutic study. First, AAT concentrations obtained in the upper airway epithelial cells via IV augmentation therapy may be insufficient to down-regulate inflammation. While serum and lower airway/alveolar epithelial lining fluid (ELF) concentrations correlate well due to the close proximity of the alveolar-capillary membranes [11], the upper airways do not share this same type of vascular perfusion. Thus, it is unlikely that increased serum concentrations of AAT affect the epithelial cells in the upper airways the same as in the lower airways. Second, the increased sputum found in the upper airways also likely contributes to a lessened effect of IV augmentation. Not only does airway mucous create an additional barrier to serum AAT diffusion, but this mucous also contains many PMN's and other inflammatory cells, thus increasing AAT consumption. While we demonstrated that upper airway bronchial epithelial cells express AAT intrinsically and thus have some low level intracellular production, this production is unlikely to be adequate to ameliorate the observed airway inflammation, especially in AAT deficient individuals. Aerosolized AAT, an alternative therapeutic delivery mechanism currently being studied, may have a greater impact on airway inflammation by achieving much higher concentrations of AAT to the airway epithelial cells.

Third, the duration of the study intervals may have been inadequate. A washout period of 5 weeks for the therapeutic AAT augmentation therapy our subjects were receiving prior to beginning the study may not have been long enough for a complete return to a non-treatment baseline inflammation in their airway epithelial cells. Also, due to the factors affecting upper airway inflammation discussed previously, a therapeutic course longer than 12 weeks of therapy may be necessary to observe any significant down-regulation in airway inflammation. However, decreased neutrophilic inflammation in the sputum of AAT deficient patients and cystic fibrosis

patients receiving IV and aerosolized treatment have been observed in as little as 4 weeks of therapy [13] [4].

Finally, the absence of any significant down-regulation of bronchial epithelial cell inflammation following IV augmentation therapy observed in our study could also be due to Type II error because of our small sample size. No preliminary data existed for this pilot study, so adequate power calculations were not available. Previous studies investigating sputum neutrophilic inflammation in AAT deficient individuals suggested that a sample size of approximately 10 would be sufficient to observe a significant therapeutic effect [5] [4] [14]. However, the effect of increased serum AAT concentrations on sputum neutrophil inflammation may be much more profound than it is for upper airway epithelial cells, necessitating a larger sample size to detect a significant difference. Most studies investigating airway inflammation in AAT deficiency utilize expectorated sputum due to the high cost, difficulty, and invasiveness of bronchoscopy. Repeating this study on a larger scale with a larger sample size would be difficult due to the cost and invasiveness of the necessary sampling. Although no data exist for power calculations, another possible study would entail the use of aerosolized AAT rather than IV AAT augmentation therapy, thus increasing the therapeutic delivery to the target cells, and providing another means to test whether such treatment could modulate the expression of inflammatory markers.

Conclusion

Upper airway bronchial epithelial cells obtained from individuals with AAT deficiency express AAT in vivo. To our knowledge, this is the first report demonstrating that human bronchial epithelial cells express AAT outside of a cell culture model. Although increased airway inflammation is clearly associated with AAT deficiency, we were unable to demonstrate that intravenous AAT augmentation therapy decreases upper airway bronchial epithelial cell

inflammation. Further study regarding the role of AAT deficiency on bronchial epithelial cell inflammation is warranted, specifically utilizing aerosolized AAT as a therapeutic modality.

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BIOGRAPHICAL SKETCH

Eric Lee Olson, M.D., was born on November 26, 1970 in Fort Collins, Colorado. He graduated from Otis High School in Otis, Colorado and then pursued his undergraduate degree at the University of Colorado at Boulder. After completing his B.A. in molecular, cellular and developmental (MCD) biology at CU in 1993, Eric worked as a laboratory research technician in the Pulmonary and Critical Care Division at the University of Colorado Health Sciences Center and at the National Jewish Center for Immunology and Respiratory Medicine in Denver for two years. In the fall of 1995, Eric began his medical school training at the University of Vermont (UVM) College of Medicine in Burlington, VT. Upon graduation from UVM in 1999, Eric moved to the University of North Carolina at Chapel Hill, where he completed his internal medicine residency and then fellowship training in Pulmonary and Critical Care Medicine, concluding in 2005. He then accepted a position as an assistant professor of medicine at the University of Florida and has held that position since that time. In December, 2008, Eric received his M.S. in medical sciences with a concentration in clinical investigation from UF. Eric's research focuses on the patho-physiology of the airways and how genetic diseases such as Alpha-1 Antitrypsin deficiency and cystic fibrosis and environmental stimuli alter airway function.

Eric is married to Jennifer A. C. Olson and has one child, Samuel Olson.