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The Biological Effects of Double-Dose Alpha-1 Antitrypsin Augmentation Therapy
A Pilot Clinical Trial

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Abstract

Rationale: Augmentation therapy with intravenous AAT (alpha-1 antitrypsin) is the only specific therapy for individuals with pulmonary disease from AAT deficiency (AATD). The recommended standard dose (SD; 60 mg/kg/wk) elevates AAT trough serum levels to around 50% of normal; however, outside of slowing emphysema progression, its effects in other clinical outcomes have not been rigorously proven.

Objectives: To evaluate the biological effects of normalizing AAT trough levels with double-dose (DD) therapy (120 mg/kg/wk) in subjects with AATD already receiving SD therapy.

Methods: Clinically stable subjects were evaluated after 4 weeks of SD therapy, followed by 4 weeks of DD therapy, and 4 weeks after return to SD therapy. At the end of each phase, BAL fluid (BALF) and plasma samples were obtained.

Measurements and Main Results: DD therapy increased trough AAT levels to normal and, compared with SD therapy, reduced serine protease activity in BALF (elastase and cathepsin G), plasma elastase footprint ($A\alpha$-Val^{360}), and markers of elastin degradation (desmosine/isodesmosine) in BALF. DD therapy also further downregulated BALF ILs and cytokines including Jak-STAT (Janus kinases–signal transducer and activator of transcription proteins), TNFα (tumor necrosis factor-α), and T-cell receptor signaling pathways, cytokines involved in macrophage migration, eosinophil recruitment, humoral and adaptive immunity, neutrophil activation, and cachexia. On restarting SD after DD treatment, a possible carryover effect was seen for several biological markers.

Conclusions: Subjects with AATD on SD augmentation therapy still exhibit inflammation, protease activity, and elastin degradation that can be further improved by normalizing AAT levels. Higher AAT dosing than currently recommended may lead to enhanced clinical benefits and should be explored further.

Clinical trial registered with www.clinicaltrials.gov (NCT 01669421).

Keywords: alpha-1 antitrypsin deficiency; antiinflammatory; dosing; immunomodulation

At a Glance Commentary

Scientific Knowledge on the Subject: AAT (alpha-1 antitrypsin) has antiinflammatory and immunomodulatory functions, in addition to antiprotease properties. Augmentation therapy with intravenous AAT is the only specific therapy for individuals with AAT deficiency. Although the recommended standard dose of 60 mg/kg/wk keeps trough AAT levels around 50% of normal (range, 20–53 μM) and slows radiologic progression of emphysema, its proven clinical benefits have been elusive. This, along with the variability of disease manifestations and progression in individuals on augmentation therapy, suggests that the standard dose may not be optimal for all patients.

What This Study Adds to the Field: This study describes the effects of double-dose AAT therapy (120 mg/kg/wk) on several biological parameters associated with the development and progression of chronic obstructive pulmonary disease. Overall, double-dose therapy was shown to restore serum AAT levels to greater than 25 μM and to further reduce circulating and airway levels of serine proteases, reduce elastin degradation in the lung, and diminish airway inflammation in subjects already receiving standard dose therapy. Therefore, increasing AAT levels into the normal range may provide additional clinical benefits and have a more robust impact on clinical outcomes in subjects with AAT deficiency requiring augmentation therapy.

AAT (alpha-1 antitrypsin) deficiency (AATD) is a genetic disorder characterized by low circulating levels of AAT (also known as alpha-1 protease inhibitor) (1). AATD predisposes the lung to the unopposed action of proteases, such as cathepsin G and proteinase 3 but primarily NE (neutrophil-derived elastase) (2). This results in progressive and irreversible destruction of lung parenchyma (emphysema) usually

associated with an accelerated decline in lung function, particularly in the context of exposure to tobacco smoke inhalation (3). Compared with smoke exposure–associated chronic obstructive pulmonary disease (COPD), individuals with AATD seem to have higher levels of inflammatory markers in both BAL fluid (BALF) (4) and sputum (5, 6), and a higher prevalence of airway hyperreactivity (7) and bronchiectasis (8).

Independent of its antiprotease properties, AAT has important antiinflammatory and immunomodulatory functions (3, 9, 10). Accumulating evidence suggests that AAT reduces the activity of proinflammatory cytokines and increases the release of antiinflammatory mediators (11, 12). For example, AAT upregulates the expression of the antiinflammatory cytokine IL-Ra (IL-1 receptor antagonist) and blocks the release of IL-1 and TNFα (tumor necrosis factor-α) in stimulated peripheral blood mononuclear cells (13, 14). In neutrophils, AAT also has broad effects, such as inhibiting superoxide production, induction of IL-1Ra, and decreasing chemotaxis and adhesion (15). AAT inhibits bacterial- and endotoxin-induced proinflammatory responses *in vitro* and *in vivo*, such as lowering levels of IL-8 and MCP-1 (monocyte chemotactic protein-1), two major chemokines involved in trafficking inflammatory cells (16, 17). AAT also acts on the lung endothelium, an active participant in the inflammatory response, by assisting in the resolution of chronic inflammation (18).

Intravenous AAT is currently the only specific therapy available for individuals with AATD. The recommended standard dose (SD) of 60 mg/kg/wk aims to increase AAT plasma levels above the putative protective threshold level (>11 μM) (19). Findings from the RAPID clinical trial program confirmed the benefits of SD AAT therapy in slowing progression of emphysema radiologically, providing evidence of a disease-modifying therapy for AATD (20, 21). However, no randomized trial has shown an effect of SD therapy on conventional COPD outcomes, such as exacerbation frequency and severity, quality of life, lung function decline, or mortality, which largely reflects their poor sensitivity to detect change and the natural influence of aging (22). The therapeutic protective threshold goal of 11 μM is still much lower than levels found in nondeficient individuals

(20–53 μM) (23). The clinical variability of disease manifestations and progression in individuals on therapy (24), suggests that the SD may not be sufficient for all patients and suggests higher doses may prove more beneficial.

Our hypothesis states that patients with AATD receiving SD AAT therapy (60 mg/kg/wk) may have residual systemic and pulmonary inflammation that could be further improved with a higher dose aimed at increasing AAT to physiologic levels. In this prospective, open-label pilot study, we evaluated the biological effects of 1 month of double-dose (DD) AAT therapy (120 mg/kg/wk) in subjects with AATD previously receiving SD therapy. This was an exploratory study to evaluate the feasibility and justification for further trials of higher-dose AAT therapy. Some of the results of this study have been previously reported in the form of abstracts (25, 26).

Methods

Detailed and expanded methodology is included in the online supplement.

Patients and Medications
Patients aged 18–75 years with a clinically symptomatic diagnosis of COPD and AATD receiving SD AAT therapy (60 mg/kg/wk) for at least 1 month before study entry were included. For detailed inclusion and exclusion criteria please refer to Table E1 in the online supplement. To compare SD and DD augmentation therapy, all study treatments were performed using Zemaira (CSL Behring LLC). Importantly, all patients were on standard COPD medications, including at least one long-acting bronchodilator and an inhaled steroid, for at least a month before entering the study. Patients who experienced an exacerbation during phase 1 (SD therapy) where continued on SD therapy until at least 1 month after resolution of the episode before starting the study protocol. An acute exacerbation requiring antibiotics or systemic steroids after any study bronchoscopy was an indication for study termination.

Written informed consent was obtained from all participants and the study was approved by the institutional review board of the University of Miami, School of Medicine. The protocol was registered in clinicaltrials.gov (NCT 01669421).

Outcomes

The primary outcome was change in pulmonary inflammatory markers measured in BALF; secondary outcomes were improvement in serum/plasma inflammatory markers and elastin degradation markers in serum and BALF.

Safety

All subjects were carefully monitored for adverse events (AEs) and serious AEs (SAEs) characterized by their seriousness, severity, and relationship to the administration of AAT therapy at 120 mg/kg/wk.

Procedures

After signing the informed consent, participants already receiving SD therapy entered a three-phase protocol: 4 weeks of SD AAT therapy (60 mg/kg/wk), followed by 4 weeks of open-label DD AAT therapy (120 mg/kg/wk), and a final 4-week phase in which subjects returned to standard dosing (60 mg/kg/wk). At the end of each phase, subjects underwent bronchoscopy and plasma sampling. Serum trough AAT levels were measured at the end of each phase to provide the nadir AAT level with SD and DD therapy; the study was not designed for full pharmacokinetic characterization. All sample collections were obtained during the stable clinical state. The study protocol is outlined in Figure 1. Administration of DD augmentation therapy required an Investigational New Drug designation by the Food and Drug Administration (IND #14636).

Cytokine, Chemokine, and Growth Factor Measurements

Human cytokines, chemokines, and growth factors were determined in BALF and plasma using bead assays (Bio-Rad Magnetic cytokine, chemokine, and growth factor bead panels; Bio-Rad) performed with the Bio-Rad Bio-Plex 200 system. BALF data were standardized to total BALF protein and urea concentrations. Total protein was determined by bicinchoninic acid assays, according to the manufacturer's instructions (Thermo Fisher).

Desmosine/Isodesmosine Quantification

HPLC and tandem mass spectrometry were used as previously described (27). Analyses of desmosine/isodesmosine (DES/IDES) levels were performed in triplicate in both BALF and plasma with eight samples per group on subsequent days. In BALF, levels were normalized to BALF protein concentrations. The coefficient of variation for the method is 8%.

Protease Activity Measurement

The NE footprint Aα-Val[360] was measured in plasma samples using a highly specific assay as described previously (28). BALF NE activity was determined using 50 μM fluorogenic substrate N-(methoxysuccinyl)-Ala-Ala-Pro-Val-7-amino-4-methylcoumarin (Enzo Life Sciences) in 0.1 M N-2-hydroxyethylpiperazine-N′-ethane sulfonic acid, 0.5 M NaCl, pH 7.5 by excitation at 360 nm, and emission at 460 nm. Experiments were performed with and without NE inhibitor (1 mM N-[methoxysuccinyl]-Ala-Ala-Pro-Val-chloromethyl ketone). NE was also confirmed by performing immunoblots on BALF using an anti-NE polyclonal antibody (ab68672; Abcam). Cathepsin G activity was determined in 50 μl BALF with a colorimetric cathepsin G activity assay kit (ab126780; Abcam), according to the manufacturer's instructions.

Pathway Analysis

Downregulated proteins were submitted to the Database for Annotation, Visualization and Integrated Discovery to identify biological processes/pathways relevant to lung pathophysiology (29).

Statistical Analysis

For this pilot study, we aimed to enroll a minimum of 10 subjects based on estimating a 30–50% decrease in inflammatory markers. The variability of these markers was calculated based on published data for patients with usual COPD because no BALF data were available for patients with AATD at the time of the study. For example, the levels of IL-8 in BALF from patients with usual COPD are in the range 0.104 ± 0.03 ng/ml (30); assuming that treatment reduced IL-8 by 50%, the calculated N was 8 ($\alpha = 0.05$; power, 90%) and for a 30% reduction, the calculated N was 18 ($\alpha = 0.05$; power, 90%).

Statistical analysis was performed using D'Agostino and Pearson normality tests and paired Student's t tests on normally distributed data and Wilcox matched-pair signed rank testing on data not normally distributed. Two-tailed analysis was performed on all data and P values for significance were set at 0.05. All analyses were performed using GraphPad Prism software version 6.0h for Mac OS X. All samples were collected and analyzed; an intention-to-treat analysis was not performed.

Results

A total of 10 subjects were enrolled in the study; two withdrew after bronchoscopy-related events (one hemoptysis after procedure; one after respiratory distress after procedure). Overall, eight subjects completed all procedures. Baseline demographic and clinical characteristics are provided in Table 1 (see Table E2 for patient pulmonary function test results after therapy).

Study week	1	2	3	4	5	6	7	8	9	10	11	12	13
Study day	1	8	15	22	29	36	43	50	57	64	71	78	85
	60 mg/kg/week (SD)				120 mg/kg/week (DD)				60 mg/kg/week (SD)				
AAT dose	X	X	X	X	X	X	X	X	X	X	X	X	
Blood draw			X		X		X		X		X		X

Bronchoscopy and serum sampling at trough

Figure 1. Summary of study procedures. AAT = alpha-1 antitrypsin; DD = double dose; SD = standard dose.

Table 1. Baseline Demographic and Clinical Characteristics for the Full Population of Patients Who Entered the Study

Characteristic	Data ($N = 10$)
Mean age, yr (StD)	60.5 (8.1)
Sex, n (%)	
M	7 (70)
F	3 (30)
Genotype, n (%)	
ZZ	9 (90)
SZ	1 (10)
Baseline mean (StD) AAT level, μM*	6.1 (2.5)
Mean (StD) post-bronchodilator FEV_1, %	55.0 (11.7)
Mean (StD) number of exacerbations in previous year	1.9 (1.2)
Chronic bronchitis, n (%)	8 (80)
Median number of months on SD AAT therapy, median (IQR)	81 (29–246)
St. George's respiratory questionnaire, median (IQR)	
Total score	48.9 (34–60.2)
Symptoms	63.7 (46.5–73.6)
Impacts	33.3 (24–46)
Activity	63.6 (40.3–72.5)
BODE score, median (IQR)	2 (1–3.5)
Smoking history	
Ever-smokers, n (%)	6 (60)
Mean (StD) pack-years	19.08 (14.7)
Active smokers, n	0

Definition of abbreviations: AAT = alpha-1 antitrypsin; BODE = body mass index, airflow obstruction, dyspnea, and exercise; IQR = interquartile range; SD = standard dose; StD = standard deviation.
*Historical baseline AAT levels were obtained from medical records and correspond to levels recorded before the initiation of AAT therapy.

DD Therapy Restored AAT to Normal Levels

DD AAT therapy results in AAT serum levels that were closer to those for AAT-competent subjects (normal range, 20–53 μM) (23). Trough levels of AAT increased from 16.7 ± 2.3 μM with SD therapy to 27.2 ± 5.0 μM with DD therapy (Figure 2), and returned to baseline (16.0 ± 2.6 μM) after SD therapy reinstitution.
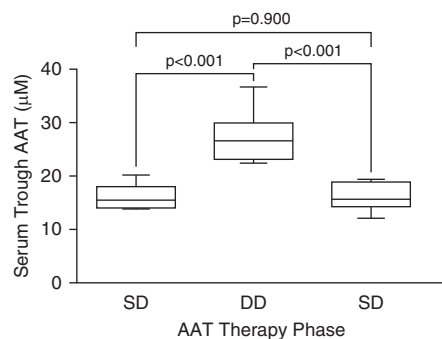

Figure 2. Trough serum AAT levels. For definition of abbreviations, *see* Figure 1.

DD Therapy Increased Antiprotease Activity and Decreased Elastin Degradation in Subjects with AATD Receiving SD Therapy

Compared with baseline values while receiving SD therapy, treatment with DD therapy (120 mg/kg/wk) produced significant reductions in serine protease activity in BALF (NE and cathepsin G) (Figure 3A). Levels of NE reduced from 3.32 ± 0.86 nM after SD therapy to 1.61 ± 0.29 nM on DD therapy ($P = 0.008$), and cathepsin G reduced from 247.00 ± 60.37 nM to 56.32 ± 15.28 nM ($P = 0.005$), respectively. The activity of these proteases in BALF began to rise (although not significantly) by 4 weeks after resuming SD therapy with levels of NE and cathepsin G of 1.75 ± 0.33 nM and 86.06 ± 26.43 nM, respectively, which suggests a possible carryover effect of the DD therapy (31, 32). Plasma levels of Aα-Val[360] were also significantly reduced with DD therapy, from 10.15 ± 1.19 nM after SD therapy to 7.89 ± 0.57 nM on DD therapy ($P = 0.016$) (Figure 3B). This marker increased again a month after SD therapy was resumed.

Increasing AAT levels with DD therapy also produced significant reductions in local markers of elastin degradation. In BALF, DES/IDES levels fell after DD therapy (0.65 ± 0.34 pg/μg protein compared with 3.74 ± 3.7 pg/μg protein on SD therapy; $P = 0.050$) (Figure 4A) and a possible carryover effect was noted, because levels a month after SD was resumed were as low as after a month on DD therapy. In plasma, however, we did not observe changes in DES/IDES with DD therapy compared with baseline (0.38 ± 0.03 ng/ml compared with 0.42 ± 0.03 ng/ml, respectively; $P = 0.493$) (Figure 4B).

Normalization of AAT Levels Significantly Reduced Airway Inflammation in Subjects with AATD Receiving SD Therapy

BALF concentrations of multiple cytokines, chemokines, and growth factors were significantly reduced after 120 mg/kg/wk dosing of AAT (Figure 5). For some measures, the levels returned back to those observed with SD therapy (60 mg/kg/wk) at study start after reinstitution; however, in other measures, including CCL3 (chemokine [C-C motif] ligand 3), CCL11, TNFα, IFNγ, and bFGF (basic fibroblast growth factor), a possible carryover effect was observed. In addition, DD therapy significantly downregulated several other inflammatory markers, including ILs and cytokines that affect the Jak-STAT (Janus kinases–signal transducer and activator of transcription proteins), TNFα, and T-cell receptor signaling pathways. DD therapy also affected cytokines involved in macrophage migration, eosinophil recruitment, humoral and adaptive immunity, neutrophil activation, and cachexia (*see* Table E3). However, no significant differences in levels of some cytokines, chemokines, and growth factors were observed after DD AAT therapy (*see* Tables E4 and E5).

Safety

During the study, three subjects experienced an SAE that required urgent medical evaluation or hospitalization (*see* Table E6). The SAEs were all acute respiratory exacerbations or possible bronchoscopy complications and unrelated to the administration of DD augmentation therapy. There were seven minor AEs reported in six patients, none of which were judged to be related to the administration
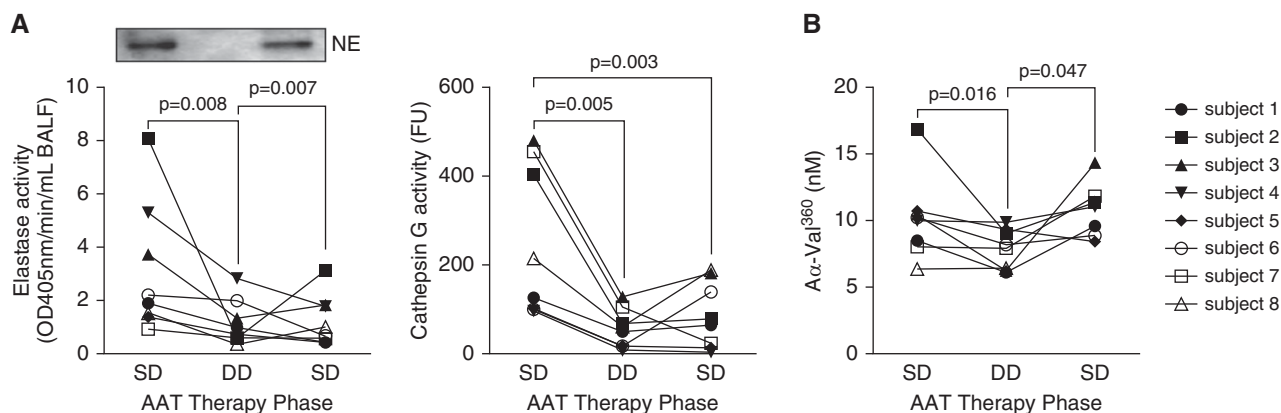
Figure 3. Activity of serine proteases in BAL fluid (BALF) (elastase activity, cathepsin G) and plasma (Aα-Val[360]). Eight subjects with AAT (alpha-1 antitrypsin) deficiency and chronic obstructive pulmonary disease on standard dose (SD) therapy (60 mg/kg/wk; Zemaira, CSL Behring) underwent BALF and plasma sampling after 4 weeks of SD, 4 weeks of double-dose (DD), and finally 4 weeks of SD therapy. (*A*) Elastase and cathepsin G activity were measured in BALF. (*B*) Aα-Val[360] was measured in plasma. Representative immunoblot for neutrophil elastase in BALF sample from one subject. Graphs are represented as individual subject response to AAT therapy; each measurement performed three times (*n* = 8 per group). NE = neutrophil elastase.

of DD AAT therapy. Of these, three (mild stridor after bronchoscopy, arm bruise, and nausea and anxiety 1 d after bronchoscopy) were judged to be probably or likely related to study procedures.

Discussion

This study describes the impact of "normalizing" AAT levels on several biological parameters implicated in development and progression of COPD in subjects with AATD. Although these subjects were already receiving the currently approved augmentation therapy dose of 60 mg/kg/wk (SD therapy), we show that they still exhibit residual inflammation and elastin degradation that can be further improved by increased dosing. SD therapy aims to increase AAT levels above the "protective" threshold estimated at 11 μM (19). This estimation, which is based on the observation that never-smokers with AATD genotype SZ usually exhibit AAT levels above this level

and rarely develop lung disease (33), is approximately 50% of normal. As previously shown, DD therapy (120 mg/kg/wk) leads to trough serum AAT levels similar to those observed in non–AAT-deficient individuals and can be administered without any major AEs (34). We show here that with only 1 month of weekly DD intravenous infusions, significant reductions in serine protease activity in BALF and plasma can be achieved, which coincided with reductions in BALF concentrations of DES/IDES, indicating reduced local elastin degradation in the lung. In combination with diminished airway inflammation, these results suggest that DD therapy may have a more pronounced impact on slowing disease progression in subjects with AATD compared with SD therapy.

Most augmentation therapy scheduling and dosing studies have focused on achieving equivalent AAT levels to SD therapy, the only dose approved for use in AATD more than 25 years ago. For example, dosing at 250 mg/kg every 28 days confers "protective" serum AAT levels and antielastase activity in epithelial lining fluid for "at least" 25 days after the infusion (35), whereas a regimen of 120 mg/kg every 2 weeks could not maintain nadir serum levels above the 11 μM threshold for the entire 14-day dosing interval (36). Our observations highlight that this putative protective value of 11 μM as a goal of therapy should be revisited and that subjects with AATD in need of therapy
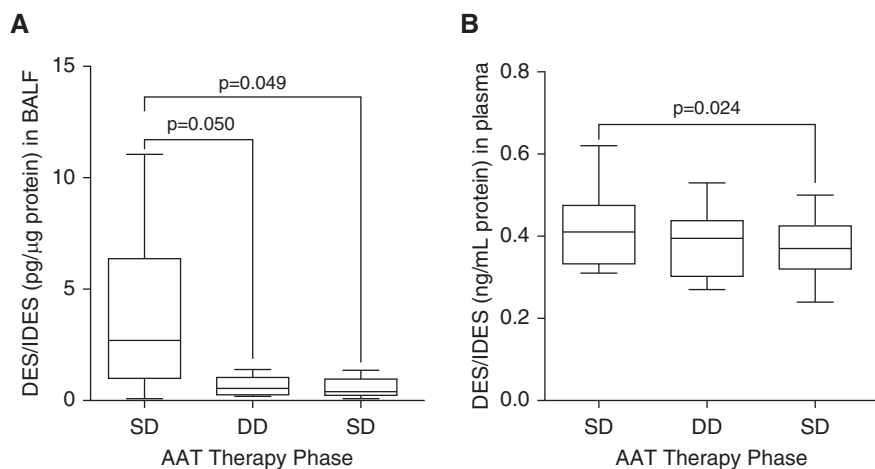

Figure 4. Changes in desmosine (DES) and isodesmosine (IDES) levels in (*A*) BAL fluid (BALF) and (*B*) plasma. Eight subjects with AAT (alpha-1 antitrypsin) deficiency and chronic obstructive pulmonary disease on standard dose (SD) therapy (60 mg/kg/wk; Zemaira, CSL Behring) underwent BALF and plasma sampling after 4 weeks of SD, 4 weeks of double-dose (DD), and finally 4 weeks of SD therapy. DES/IDES levels were measured in (*A*) BALF and (*B*) plasma. Graphs are represented as individual subject response to AAT therapy; each measurement performed three times (*n* = 8 per group). Month 1: SD, 60 mg/kg/wk. Month 2: DD, 120 mg/kg/wk. Month 3: SD, 60 mg/kg/wk.
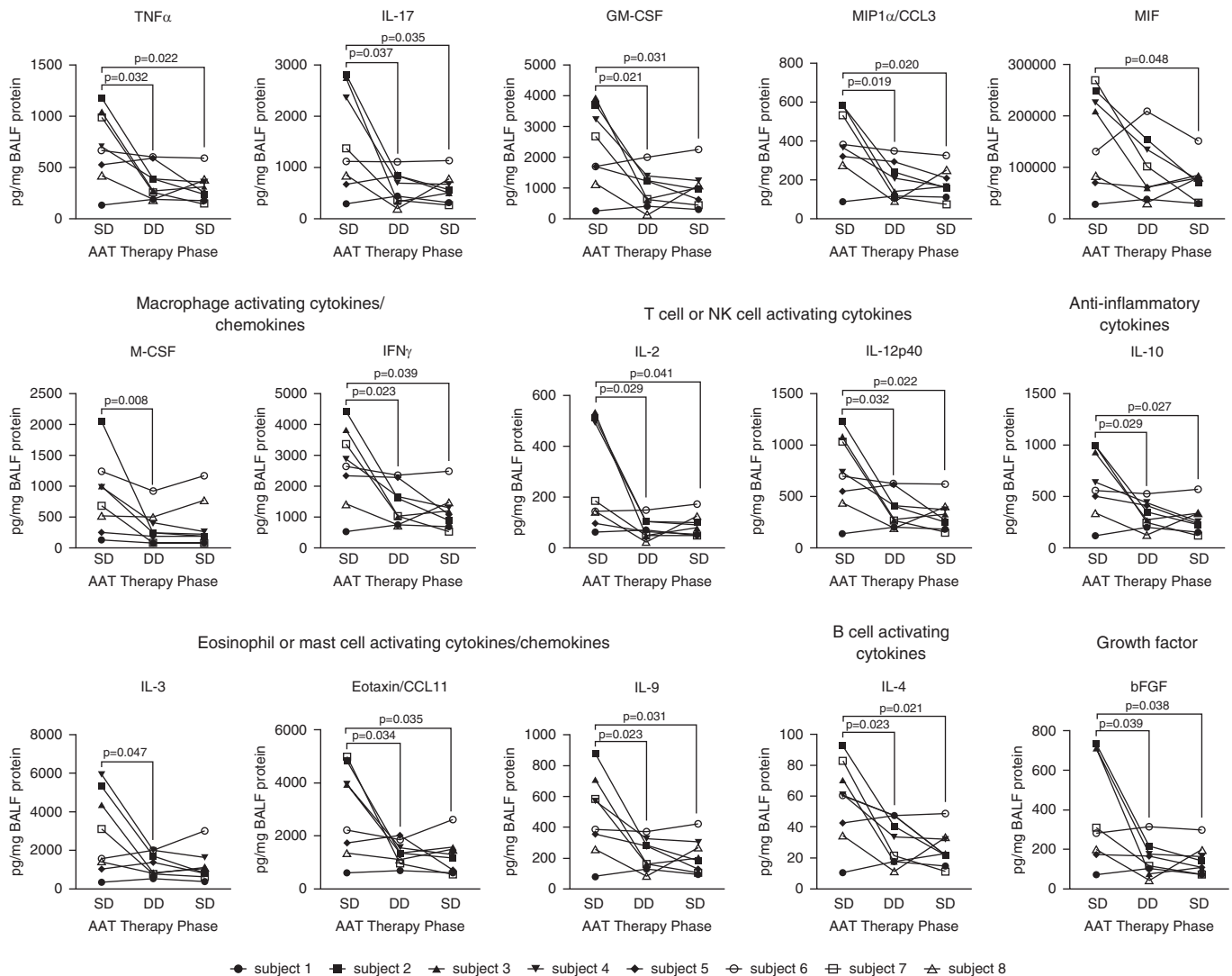
Figure 5. BAL fluid (BALF) concentrations of several ILs and related cytokines were significantly reduced after 120 mg/kg/wk dosing of AAT (alpha-1 antitrypsin). Eight subjects with AAT deficiency and chronic obstructive pulmonary disease on standard dose (SD) therapy (60 mg/kg/wk; Zemaira, CSL Behring) underwent BALF and plasma sampling after 4 weeks of SD, 4 weeks of double-dose (DD), and finally 4 weeks of SD therapy. BALF levels were determined by Luminex assays. Graphs are represented as individual subject BALF concentrations; each measurement performed three times ($n = 8$ per group). bFGF = basic fibroblast growth factor; CCL = chemokine (C-C motif) ligand; GM-CSF = granulocyte–macrophage colony–stimulating factor; M-CSF = macrophage colony–stimulating factor; MIF = macrophage migration inhibitory factor; MIP = macrophage inflammatory protein; NK = natural killer; TNF = tumor necrosis factor.

should preferably be treated with a regimen that normalizes AAT levels. Except for slowing emphysema progression, the clinical effects of SD augmentation therapy on other COPD outcomes has been deemed controversial by many. For example, slowing of lung function decline has been noted in observational studies but not within randomized controlled trials (37, 38). In addition, subjects on SD augmentation therapy exhibit high rates of exacerbations (24), and although

augmentation therapy may slow exacerbation rates in observational studies (39, 40) this has not been shown in randomized controlled trials (20, 41). Therefore, to prove the effects of augmentation therapy on lung function decline or exacerbation rates may require trials with larger numbers of patients and of longer duration than is currently feasible, unless patients are specifically recruited to enrich for the outcome in mind (22). However, our results also suggest that in

part the lack of clinical effect may be due to suboptimal dosing using SD therapy.

In our group of patients with moderate COPD severity on SD therapy, residual serine protease activity could be significantly reduced in both BALF (elastase activity and cathepsin G) and the footprint of this activity in plasma (Aα-Val[360]) after DD therapy. This control of serine protease activity is important because mice deficient in all three neutrophil serine proteases (proteinase 3, cathepsin G, and NE) are

substantially protected against lung tissue destruction after long-term exposure to cigarette smoke (2). The effect we observed on Aα-Val^{360} is also important because it represents a potential biomarker of disease activity given its significant correlation to physiologic, radiologic, and symptomatic markers of disease severity in untreated subjects with AATD (32). Overall, because of the protean effects of AAT, DD therapy produces a profound antiproteolytic effect, in addition to significant reductions in airway levels of other important proteases, such as collagenase (MMP1 [matrix metalloproteinase-1]) and gelatinase (MMP9), as we have previously described (42).

The overall reduction in proteolytic activity achieved with DD therapy translated into decreased elastin degradation even when this therapy was of relatively short duration (1 mo). DES and IDES, unique amino acid cross-links in mature elastin fibers, can serve as biomarkers of elastin degradation when measured in body fluids (43). The concentrations of DES/IDES present in body fluids are extremely low and recent methodologic advances have aided their detection. The specificity and sensitivity of DES/IDES measurement has improved with the development of an analytical method using HPLC followed by electrospray ionization (44). This method, in addition to tandem mass spectrometry, has resulted in enhanced detection of DES/IDES levels in plasma and sputum from subjects with usual COPD or AATD (27, 45). Furthermore, data support the use of DES/IDES as biomarkers to monitor emphysema progression and treatment response (46). Despite this enhanced detection method, 1 month of DD therapy did not lead to measurable changes in plasma levels of DES/IDES; however, significant changes were observed in BALF, highlighting the importance of directly studying lung samples to assess the impact of treatments aimed at halting elastin degradation. Overall, our findings reflect the importance of normalizing AAT levels to further decrease elastin degradation.

We assessed changes that occur in 58 different cytokines, chemokines, growth factors, and markers of tissue damage in the BALF and plasma of subjects when transitioning from SD to DD therapy. DD therapy significantly reduced a wide range of these inflammatory markers in BALF (CCL3, CCL11, IL2, IL3, IL4, IL9, IL10, IL12p40, IL17, GM-CSF [granulocyte–macrophage colony–stimulating factor], M-CSF [macrophage colony–stimulating factor], MIF [macrophage migration inhibitory factor], TNFα, IFNγ, and bFGF) compared with SD administration. These biomarkers are primarily linked with immune cell recruitment and activation (47), and these changes further confirm the additional role of AAT as a major immunomodulatory protein. MIF, GM-CSF, IL10, MCP7, CCL3, TNFα, IFNγ, IL2, and IL4 are major activators of macrophages and neutrophils. Equally, several T-cell activators are regulated by AAT, such as IFNγ, IL2, IL4, IL9, IL10, IL12p40, and IL15, and several of these targets are associated with emphysema formation and progression, such as IL-17 (48), IFNγ (49), and CCL11 (50). These changes are not surprising because AAT has been shown to have protean antiinflammatory effects, such as regulation of neutrophil chemotaxis (51), degranulation and autoimmunity (51), neutrophil apoptosis (52), antiinflammatory phosphatase responses (42), caspase activity (53), nitric oxide production (54), HIV type 1 infectivity and reproduction (55), TNFα converting enzyme activity (18), and endoplasmic reticulum stress (56, 57). We also observed a significant decrease in IL-10, an antiinflammatory cytokine that plays a central role in limiting local host immune responses. This decrease is expected because IL-10 is triggered by enhanced inflammatory states (58), now ameliorated with DD therapy.

Overall, further studies are required to determine the functional role of each of the targets affected by AAT level normalization identified in this study. The observation of a possible carryover effect in most of these markers, where the effect of DD therapy lasted at least a month after resuming SD therapy, should be further explored in studies of longer duration to determine when, and if, the potential carryover effect disappears. Clinical studies of DD therapy over a longer time period or the exploration of higher doses, which may yield greater improvements in biological parameters and potentially clinical parameters, should be implemented.

Some important limitations of this study should be mentioned. This was a small pilot study, which reported complete findings in eight patients with AATD. The primary outcome was change in pulmonary inflammatory markers measured in BALF and there are limited previous data on the natural variability of cytokine levels in BALF over such a short time period in healthy individuals, individuals with COPD, and in particular those with AATD. However, the significant changes observed despite this small sample size is a strong hint of the potent effects of DD therapy, at least in the individuals studied here. Unfortunately, we did not include non–AAT-deficient subjects with COPD, healthy control subjects, or a placebo arm to document the variability of inflammatory markers in BALF and better assess the magnitude of changes observed with DD therapy. For this reason, we opted for a three-phase design in which baseline conditions were restored, a design that followed a prior study evaluating the impact of AAT on sputum inflammation (12). We acknowledge that biological changes do not necessarily translate into changes in clinically relevant outcomes and further studies are required to validate the clinical impact of higher AAT dosing. It is important, however, to highlight that our results cannot be extrapolated to the entire AATD population, because the clinical manifestations in this condition are protean, from no lung symptoms to varying levels of COPD severity. We used defined inclusion criteria to enroll symptomatic subjects likely to have increased lung inflammation, such as presence of chronic bronchitis, exacerbations, or poor quality of life. Therefore, the magnitude of the effects observed might not be reflective of a broader population of patients with AATD.

It is important to note that although we standardized inhaler treatment that may affect lung inflammation (all subjects received a long-acting bronchodilator and an inhaled corticosteroid), different patterns of lung inflammation were observed among participants. For example, Subjects 2, 3, and 4 (Figures 2–4) always had high levels of cytokines, proteases, and lung degradation and a marked response to DD therapy, whereas Subject 1 had low levels that were not affected with increasing AAT dosing. This raises the question of applying precision medicine to tailor each subject's AAT requirement, similar to the personalized approach proposed to decide the initiation of augmentation therapy (59). Further studies are required to assess this,

ideally with reliable and accessible biomarkers to adjust therapy (not in BALF). The only plasma marker we observed that changed with DD therapy and followed the changes observed in BALF was Aα-Val^{360} and should be further explored.

Conclusions

Overall, we have confirmed that DD therapy is well tolerated by subjects with AATD, restores serum AAT levels to more than 25 μM, significantly reduces circulating and airway levels of serine proteases, reduces elastin degradation in the lung, and diminishes airway inflammation when compared with SD therapy. Further work in a larger cohort of patients is required to explore the long-term benefit or adverse effects of DD therapy because it could further slow the loss of lung function in subjects with AATD. Equally, the "protective" threshold concentration of AAT may require reevaluation. ∎

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