Oncostatin M and TNF- α Induce Alpha-1 Antitrypsin Production in Undifferentiated Adipose Stromal Cells

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Alpha-1 antitrypsin (A1AT), a circulating acute-phase reactant antiprotease, is produced and secreted by cells of endodermal epithelial origin, primarily hepatocytes, and by immune cells. Deficiency of A1AT is associated with increased risk of excessive lung inflammation and injury, especially following chronic cigarette smoke (CS) exposure. Exogenous administration of mesenchymal progenitor cells, including adipose tissue-derived stromal/stem cells (ASC), alleviates CS-induced lung injury through paracrine effectors such as growth factors. It is unknown, however, if mesodermal ASC can secrete functional A1AT and if CS exposure affects their A1AT production. Human ASC collected via liposuction from nonsmoking or smoking donors were stimulated by inflammatory cytokines tumor necrosis alpha (TNF α), oncostatin M (OSM), and/or dexamethasone (DEX) or were exposed to sublethal concentrations of ambient air control or CS extract (0.5%–2%). We detected minimal expression and secretion of A1AT by cultured ASC during unstimulated conditions, which significantly increased following stimulation with TNF α or OSM. Furthermore, TNF α and OSM synergistically enhanced A1AT expression and secretion, which were further increased by DEX. The A1AT transcript variant produced by stimulated ASC resembled that produced by bronchial epithelial cells rather than the variant produced by monocytes/macrophages. While the cigarette smoking status of the ASC donor had no measurable effect on the ability of ASC to induce A1AT expression, active exposure to CS extract markedly reduced A1AT expression and secretion by cultured ASC, as well as human tracheobronchial epithelial cells. ASC-secreted A1AT covalently complexed with neutrophil elastase in control ASC, but not in cells transfected with A1AT siRNA. Undifferentiated ASC may require priming to secrete functional A1AT, a potent antiprotease that may be relevant to stem cell therapeutic effects.

Keywords: human adipose progenitor cell, alpha-1-proteinase inhibitor, cigarette smoke

Introduction

ALPHA-1-ANTITRYPSIN (A1AT) is a major lung-protective antiprotease with activity primarily against neutrophil elastase (NE) that also extends against caspases to increase the survival of structural alveolar cells [1]. Patients with A1AT deficiency are at high risk of developing chronic obstructive pulmonary disease (COPD) characterized by inflammation- and protease-mediated destruction of the alveolar air sacs surrounding blood vessels, termed panacinar emphysema. Whereas circulating A1AT originates primarily from hepatocytes, smaller amounts of A1AT are produced by other epithelial cells, including those of the respiratory tract

and by nonepithelial immune cells, such as mononuclear cells [2].

Inflammatory signals markedly increase systemic and local A1AT output, as part of the acute-phase reaction. However, genetic mutations or cigarette smoke (CS) exposure render A1AT dysfunctional, the latter due to oxidation of critical amino acid residues in the reactive center loop of the protein required for binding and inhibition of target proteases [3,4]. We have shown that when administered systemically to animals exposed to CS, adipose tissue-derived stromal/stem cells (ASC) inhibit lung inflammation, apoptosis, and airspace enlargement [5]. Since ASC effects are mostly exerted in a paracrine manner, we investigated their potential to

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secrete A1AT. Since inflammatory signals regulate A1AT expression, we hypothesized that ASC require inflammatory stimulation to induce and secrete functional A1AT.

Inflammatory signals play a critical role in upregulating A1AT RNA expression and protein secretion in epithelial cells of endoderm-derived tissues, including the liver (hepatocytes), lung [6,7], and gut [8,9] and also by immune cells such as macrophages [10] and neutrophils [11]. The combination of the inflammatory cytokine oncostatin M (OSM) and the corticosteroid dexamethasone (DEX) is the most powerful stimulus for A1AT secretion in various lung epithelial cells and hepatoma-derived hepatocytes [7,12]. Neutrophils in the alveoli are an important source of OSM during lung inflammation [13] and are elevated in sputum of COPD patients [14]. Therefore, the ability of airway epithelial cells to respond to OSM by secreting A1AT may be an important antiprotease response during inflammation to limit neutrophilic tissue damage.

Another key inflammatory cytokine in the airways that is elevated in COPD is tumor necrosis factor α (TNF α) [15]. Although TNF α by itself did not affect A1AT secretion by hepatoma cells [16], since it is a potent inducer of paracrine factor secretion by ASC [17], its ability to induce A1AT in ASC was also the focus of our investigations.

Undifferentiated cells of embryonic mesoderm origin such as mesenchymal stem or progenitor cell (MSC) and ASC are not known to produce A1AT. Prolonged 2–3-week differentiation into hepatocyte-like cells [18,19] induces the expression of A1AT mRNA in MSC and ASC [18–22], but it is not reported if this results in functional A1AT secretion. Investigators have relied primarily on viruses to induce significant A1AT expression in both hepatocyte-like cells differentiated from bone marrow-derived MSC [23] and in undifferentiated ASC [24]. Interestingly, OSM and DEX, which upregulate A1AT expression in epithelial cells, are also both developmentally implicated in the proper maturation of fetal hepatocytes [25,26] and can be used to differentiate ASC to hepatocyte-like cells [18,19].

Using cultured human ASC, we determined that undifferentiated ASC can be induced to express and secrete functional A1AT following short-term cytokine stimulation and that active exposure to CS dampens this response.

Materials and Methods

ASC isolation and culture

The work was conducted under protocol approved by the Indiana University Institutional Review Board. ASC isolation, expansion, and flow characterization were previously described [17,27,28]. Briefly, ASC were obtained from discarded adipose tissue obtained by liposuction from human donors that was digested using collagenase I (Worthington, Lakewood, NJ) under mechanical agitation for 2 h at 37°C and centrifuged at 300g for 8 min to obtain a pellet consisting of the stromal vascular fraction. This fraction was filtered using 250 μ m Nitex filters (Sefar America, Buffalo, NY) and treated with red blood cell lysis buffer. The treated cells were then cultured using EGM2-MV media (Lonza, Allendale, NJ) and passaged at 60%–80% confluence. ASC cultured in EGM2-MV were extensively characterized by us and others [27,29]. Cells were used for experiments between passages 4 and 6.

To stimulate ASC, cells were washed twice with phosphate-buffered saline (PBS) to remove residual fetal bovine serum (FBS) containing A1AT and then incubated in basal EBM2 media (Lonza) with 20 ng/mL TNF α (R&D, Minneapolis, MN), 100 ng/mL OSM (R&D), and/or 1 μ M DEX (Sigma, St. Louis, MO) for 24 h. Serum starvation had minimal effect on ASC viability assessed by Annexin V/propidium iodide staining (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/scd), which agrees with previous report [30]. Detailed demographic information of the ASC donors used for A1AT expression studies and age-matched nonsmoker versus smoker cohort is provided in Supplementary Tables S1 and S2.

Annexin V/propidium iodide staining

ASC-conditioned media containing detached cells were combined with adherent cells detached by 0.05% trypsin/0.53 mM EDTA (Corning, Corning, NY) and then centrifuged, washed with PBS, and resuspended in Annexin V binding buffer with Annexin V-FITC and PI (TACS Annexin V-FITC Kit; Trevigen, Gaithersburg, MD). Gates were set using unstained and Annexin V- and PI-only stains. Side scatter-A versus forward scatter-A plots were drawn to exclude debris.

BEAS-2B culture

The bronchial epithelial cells that transformed cell line BEAS-2B were obtained from ATCC, cultured using Dulbecco's modified Eagle's medium (DMEM) with 10% FBS and 1% penicillin/streptomycin, and passaged at 70%–90% confluence. To stimulate BEAS-2B, cells were washed twice with PBS to remove residual FBS and then incubated in basal DMEM with indicated stimuli for up to 8 h, to avoid cell death due to prolonged serum deprivation.

Human tracheobronchial epithelial cells were isolated from lung and tracheal tissue of deidentified healthy (without a history of lung diseases) organ donors, using an institutional review board (IRB)-exempt honest broker system. Tissues were incubated overnight in a 0.2% protease solution (Sigma) and then underwent filtration removal of mucus, followed by plating onto collagen-coated dishes. Human tracheobronchial epithelial cells were grown until 70% confluence, then trypsinized, and stored at –80°C, as passage 0 cells. Passage 0 cells were then expanded in 60-mm dishes until they reached 70% confluence, followed by trypsinization and seeding onto 12-well plates where they were grown at the air/liquid interface and, on reaching 80% confluence, treated as indicated.

Human alveolar macrophage culture

Alveolar macrophages were collected by airway lavage of deidentified nondiseased human explanted lungs and enriched by 2 h attachment to tissue culture plastic in RPMI media with 1% penicillin/streptomycin. Nonadherent cells were then removed by PBS wash and incubated in RPMI with 2% FBS with vehicle (0.1% bovine serum albumin in PBS) or 50 ng/mL ultrapure LPS-EK (InvivoGen, San Diego, CA) for 24 h.

CS extract preparation

Aqueous CS extract was prepared using research grade cigarettes (3R4F; University of Kentucky Tobacco Reference

Product, Lexington, KY). One hundred percent extract was prepared by bubbling smoke from two cigarettes into 20 mL PBS that were burned at a rate of taking 1 min per cigarette to reach 0.5 cm above the filter mark [31].

A1AT gene expression and transcript analysis

Total RNA (1,000–2,000 ng) extracted from cell culture (RNeasy Mini Plus; Qiagen, Germantown, MD) was used to synthesize cDNA (High-Capacity cDNA Reverse Transcription; Thermo Fisher Scientific, Waltham, MA). Real-time quantitative polymerase chain reaction (qPCR) was performed on the StepOnePlus System (Thermo Fisher Scientific) using SYBR Select Master Mix (Thermo Fisher Scientific) and TaqMan Universal PCR Master Mix (Thermo Fisher Scientific) and primers specific for human A1AT (Supplementary Table S3) [19]. Relative fold expression was calculated using the double delta Ct method and endogenous control human *GAPDH* (TaqMan Hs99999905_m1; Thermo Fisher Scientific).

Real-time qPCR assays (Supplementary Table S3) targeting the noncoding exons in A1AT were described previously [32]. Briefly, both monocyte-specific (M1 assay) transcript and nonspecific (ME1 assay) transcript (covering both monocyte and epithelial) were detected using primers and a quenchable probe from the Universal Probe Library (Roche, Indianapolis, IN). Real-time qPCR was similarly performed using TaqMan Universal PCR Master Mix on the StepOnePlus platform.

Transfection of A1AT siRNA in ASC

siRNA (ON-TARGETplus; GE Dharmacon, Marlborough, MA) targeting A1AT (SERPINA1, L-008847-00-0010) and control (nontargeting Pool, L-012379-00-0010) were transfected into human ASC using Lipofectamine RNAiMax (Thermo Fisher Scientific) as previously described [17]. Briefly, ASC that reached 30% confluence in T150 flasks were switched to antibiotic-free EGM2-MV for 1 day and then incubated with siRNA/Lipofectamine complexes in OPTI-MEM media (Thermo Fisher Scientific) for 12 h. siRNA complexes were formed using 360 pmol siRNA (GE Dharmacon) and 45 μ L RNAiMAX reagent in 3 mL OPTI-MEM and diluted with 12 mL OPTI-MEM to cover the entire T150 flask. Transfected cells were then washed twice with PBS to clear any residual serum and incubated in EBM2 basal media with OSM, TNF α , and DEX for 24 h.

A1AT western blot

ASC- or BEAS-2B-conditioned media mixed with Laemmli buffer were separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE) using Criterion TGX 4%–20% gradient gels (BioRad, Hercules, CA), transferred onto PVDF membrane using TransBlot Semi-Dry (BioRad), and probed using goat-anti-hA1AT antibody (Bethyl, Montgomery, TX). ASC-conditioned media were concentrated 10-fold using microcon-10 kDa columns (EMD Millipore, Darmstadt, Germany). ChemiDoc MP (BioRad) was used to image Stain-free Criterion TGX 4%–20% gels (BioRad) for total protein staining.

The anti-human A1AT antibody is minimally cross-reactive with bovine A1AT that may be found in cell culture FBS, which was nevertheless removed before treatment by

rinsing with PBS to remove any residual bovine A1AT. Under these conditions we did not detect any bovine A1AT in vehicle-treated ASC controls, as assessed by both western blot and ELISA.

A1AT ELISA

A1AT ELISA (Bethyl) was performed on conditioned media according to the manufacturer's instructions. In experiments in which A1AT was below detection limit of 0.27 ng/mL, conditioned media were concentrated using microcon-10 kDa columns (EMD Millipore).

A1AT/NE complex formation

Human NE (16-14-051200; Athens) was resuspended using 50 mM sodium acetate, pH 5.5. NE (0, 20, 50, and 100 ng) in 20 μ L volume was mixed with 30 μ L of 10-fold concentrated ASC-conditioned media and incubated with agitation for 1 h at room temperature. Reducing Laemmli buffer was added to stop the reaction and samples were separated by SDS-PAGE. A1AT and complexed A1AT bands were both detected by western blotting using goat-anti-hA1AT antibody (Bethyl).

Ethics approval and consent to participate

All collection of human adipose tissue was approved by Indiana University School of Medicine Institutional Review Board IRB No. 1011003615. No consent was required from donors; the obtained tissue was deidentified.

Results

A1AT production in OSM- and TNF α -stimulated undifferentiated ASC

Undifferentiated ASC during basal culture conditions expressed and secreted minimal A1AT, measured at 0.20 ng/mL/ 10^6 cells, with standard deviation (SD) of 0.17 (Fig. 1A–D). Previous work in airway epithelial and hepatocyte-derived cells identified that in combination, OSM and DEX were the most potent inducers of A1AT [7,12]. To test whether undifferentiated ASC can induce A1AT expression in response to similar stimuli, we treated human ASC isolated from three separate nonsmoking donors with OSM. OSM treatment for 24 h upregulated A1AT mRNA by 9.4-fold (SD=2.85; Fig. 1A). Since we have previously shown that TNF α induces ASC secretion of anti-inflammatory effectors [27], we next investigated whether TNF α also increases A1AT expression in ASC. TNF α treatment for 24 h upregulated A1AT mRNA by 12.5-fold (SD=1.9) in undifferentiated ASC (Fig. 1B).

We next used ASC from donors No. 290 to determine if combinations of OSM, TNF α , and/or DEX synergize to induce A1AT production in ASC. We found that the greatest induction of A1AT mRNA, of 125-fold (SD=75), was obtained when all three stimuli were utilized: OSM, TNF α , and DEX (OTD) (Fig. 1C). Using western blot, we noted that A1AT expression was paralleled by A1AT secretion into conditioned media (Fig. 1D), with the combination of all three stimuli also yielding the highest levels of A1AT secretion by ASC. A1AT is a glycoprotein exhibiting extensive glycosylation following synthesis in epithelial cells

FIG. 1. A1AT expression in ASC following $\text{TNF}\alpha$ and OSM stimulation. Expression of *hA1AT* RNA in ASC from three different donors in response to inflammatory cytokines OSM (A) and $\text{TNF}\alpha$ (B) after 24h of stimulation. Cytokine-induced *hA1AT* expression (C) and secretion (D) from donor 290 using indicated combinations of OSM, $\text{TNF}\alpha$, and DEX: OD (OSM and DEX), OT (OSM and $\text{TNF}\alpha$), and OTD (OSM, $\text{TNF}\alpha$, and DEX). $n=3$ independent experiments (C) and representative western blot (D). Equal volume of conditioned media was loaded for each treatment group. $*P<0.05$. $***P<0.001$. ANOVA with Bonferroni's multiple comparisons. ANOVA, analysis of variance; ASC, adipose tissue-derived stromal/stem cells; DEX, dexamethasone; OD, oncostatin M and dexamethasone; OSM, oncostatin M; OT, oncostatin M and tumor necrosis factor alpha; OTD, oncostatin M, tumor necrosis factor alpha, dexamethasone; $\text{TNF}\alpha$, tumor necrosis factor alpha.

such as hepatocytes [33]. Whereas unglycosylated A1AT migrates at ~ 45 kDa size [34–36], we noted that ASC-secreted A1AT migrates at 54 kDa (Fig. 1D), similar to A1AT secreted by bronchial epithelial cells (Fig. 2B), suggesting extensive glycosylation of ASC-secreted A1AT.

TNF α stimulation of A1AT production in bronchial epithelial cells

Since $\text{TNF}\alpha$ alone had been found to have minimal effect on A1AT secretion in hepatocyte-derived cells [16], we next tested whether $\text{TNF}\alpha$ induction of A1AT and synergy with OSM are unique to mesodermal ASC. We used an endodermal cell type that, similar to hepatocytes, is known to

secrete A1AT, such as BEAS-2B, a transformed cell line derived from normal bronchial cells. Similar to ASC, $\text{TNF}\alpha$ synergized with OSM to induce A1AT expression and secretion in bronchial epithelial cells (Fig. 2A). To compare the relative magnitude of A1AT secretion in ASC versus lung epithelial cells, we measured A1AT levels in their conditioned media by western blot (Fig. 2B) and ELISA. A1AT secretion by OTD-stimulated ASC was 6.1 ng/mL/ 10^6 cells ($\text{SD}=0.4$), compared to 15.1 ng/mL/ 10^6 cells ($\text{SD}=5.0$) by similarly stimulated BEAS-2B (Fig. 2C); both cell types required $\text{TNF}\alpha$ costimulation to increase A1AT above 5 ng/mL/ 10^6 cells. We next confirmed that the bronchial epithelial cell response was not restricted to a transformed cell line in submerged cell culture conditions. We

FIG. 2. A1AT expression in BEAS-2B following $\text{TNF}\alpha$ and OSM stimulation. Induction of *hA1AT* expression (A) and A1AT secretion (B, C) in the human bronchial epithelial cell line BEAS-2B following cytokine stimulation (8h; $n=3$ independent experiments). Equal volume of conditioned media was loaded for each treatment group (B). (C) A1AT secretion in supernatants of BEAS-2B was compared to ASC, using ELISA, normalized by cell number. (D) Induction of *hA1AT* expression in primary human tracheobronchial epithelial cells after OTD stimulation (24h; numbers indicate each of $n=3$ human tracheobronchial epithelial cell donors). $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$. ANOVA with Bonferroni's multiple comparisons.

tested the response of primary human tracheobronchial cells grown at the air/liquid interface exposed to similar concentrations and duration of OTD, and noted they also induce robust *A1AT* expression (Fig. 2D).

Comparison of *A1AT* transcript variants induced in stimulated ASC compared to bronchial epithelial cells

The *A1AT* transcription start site differs in epithelial cells compared to monocytes [2], such that epithelial cells primarily express a shorter transcript that starts in exon 1C. In contrast, monocyte-like cells primarily express a longer transcript that spans all three exons 1A, 1B, and 1C (Fig. 3A). The transcriptional variant of *A1AT* produced by ASC is unknown. We used primers specific to 1B and 1C junction (M1) for qPCR to detect monocyte-specific *A1AT* transcripts. We also used the primers spanning exon 1C and exon 2 junction (ME1) that cover a region present in both the longer and shorter forms of *A1AT* transcript, which renders the ME1 assay nonspecific.

We found that monocyte-specific *A1AT* transcripts are minimally expressed in stimulated ASC and BEAS-2B in contrast to cultured alveolar macrophages (Fig. 3B). In contrast, there was a marked induction of nonspecific *A1AT* transcripts (ME1 assay) in stimulated ASC (6.6-fold, SD=1.6), similar to those found in stimulated BEAS-2B (6.4-fold, SD=3.0) (Fig. 3C). These results suggest that ASC transcribe *A1AT* variants that are similar to epithelial cells rather than monocytes.

ASC-secreted *A1AT* covalently binds NE

The serpin function of *A1AT* is most potent against NE, which is inhibited through the formation of an irreversible covalent link between *A1AT* and NE that inactivates the enzyme. To investigate whether ASC-derived *A1AT* is functional, we coincubated conditioned media from stimulated ASC with increasing concentrations of human NE and detected the appearance of a 70 kDa band corresponding to *A1AT*-NE complex. As additional control, we used ASC in which *A1AT* expression was knocked down using siRNA (Supplementary Fig. S2). Compared to ASC treated with nontargeting (NT)

siRNA control, conditioned media from cells in which *A1AT* was knocked down had markedly decreased *A1AT* secretion and complex formation with NE (Fig. 4).

Effect of *in vivo* and *in vitro* CS exposure on *A1AT* production by ASC

Given that ASC may be used to alleviate CS-induced lung disease, and many affected individuals are active smokers, we next investigated whether CS use affects induction of *A1AT* expression in ASC. We first tested whether the smoking status of the ASC donors affected *A1AT* production following cytokine stimulation. We obtained information about the smoking status, age, and gender of deidentified ASC donors. We used ASC collected by liposuction from three smokers and four nonsmoker donors who were nondiabetic Caucasian females with comparable ages (mean $\pm$ SD: 39.3 $\pm$ 6.7 vs. 46.5 $\pm$ 7.8) and body mass index (BMI) (mean $\pm$ SD: 21 $\pm$ 1.7 vs. 24.3 $\pm$ 2) (Supplementary Table S2). The differences in the relative fold induction of *A1AT* by OTD stimulation of ASC obtained from smokers versus nonsmoker donors in this small sample size were not statistically significant (Fig. 5A).

We next investigated the direct effect of soluble CS extract on *A1AT* induction by ASC. The concentrations of CS extract used (1% and 2%) did not significantly affect ASC viability, as measured by Annexin V/propidium iodide staining (Supplementary Fig. S1), or on the total protein secreted in conditioned media, as measured by stain-free detection of loaded proteins on the electrophoresis gel used for western blotting (Fig. 5C). However, CS extract exposure markedly reduced the ASC *A1AT* induction following OTD stimulation, measured by qPCR (Fig. 5B) and secretion, measured by western blotting (Fig. 5C). CS extract similarly potentially reduced *A1AT* expression in primary human tracheobronchial cells stimulated with OTD, as measured by qPCR (Fig. 5D).

Discussion

Our results show for the first time that undifferentiated mesenchymal cells such as ASC can be stimulated to express and secrete functional *A1AT* in response to a combination

FIG. 3. *A1AT* transcript profile in ASC, BEAS-2B, and macrophages. (A) Schematic of *hA1AT* transcript variants and primers for TaqMan qPCR assays to detect monocyte-specific (M1) and nonspecific (ME1) *A1AT* transcripts that bind different areas of non-coding exon 1. (B) Expression of monocyte-specific *A1AT* transcript by ASC and BEAS-2B stimulated with inflammatory stimuli compared to cultured human AM. (C) Expression of monocyte-specific (M1) and nonspecific (ME1) transcript variants in ASC and BEAS-2B stimulated with inflammatory stimuli ($n=3$ donors for AM and $n=3$ independent experiments for each cell type). AM, alveolar macrophages; qPCR, quantitative polymerase chain reaction.

FIG. 4. Covalent interaction of ASC-secreted A1AT with NE. A1AT detected by western blot following incubation of increasing concentrations of human NE with conditioned media from ASC treated with NT- or A1AT siRNA and then exposed to the indicated inflammatory stimuli. NE, neutrophil elastase; NT, nontargeting.

of OSM, $\text{TNF}\alpha$, and DEX. Such ability may be due to their remarkable plasticity as multipotent cells to not only differentiate into mesoderm-derived tissue cells such as bone, adipose, and muscle but also into nonmesoderm-derived cells such as ectodermal neuron-like cells [37] and endodermal hepatocyte-like cells, the most prolific A1AT producing cells in the body. As plastic multipotent cells, ASC may be less epigenetically restricted and more competent for endodermal signaling than terminally differentiated mesenchymal counterparts. Nonetheless, efficient A1AT expression and secretion are largely limited to epithelial cells of endodermal origin containing the endodermal transcription factor HNF3 β (FOXA2) [38–40], which has been used to differentiate MSC into hepatocyte-like cells [41].

This finding may be relevant to pulmonary as well as vascular and other pathologies for which the activity of NE may be of significance. Further studies are needed to determine whether undifferentiated MSC from the bone marrow can be similarly stimulated to secrete A1AT and if any differences may be attributed to the hepatic differentiation potential than ASC, shown previously to be driven by OSM and DEX [19].

We also found for the first time that $\text{TNF}\alpha$ can synergize with OSM to induce *A1AT* RNA expression and protein secretion in both ASC and in a human bronchial epithelial cell line, BEAS-2B. Similar to a previous report using hepatocyte-derived cells [16], we found that $\text{TNF}\alpha$ alone had minimal

effect on A1AT secretion in either ASC or BEAS-2B. These data, along with our observation that stimulated ASC, similar to BEAS-2B, express minimal monocyte-specific *A1AT* transcript variants but induce nonspecific transcripts of similar magnitude suggest that ASC transcribe A1AT in response to similar signals and by similar transcriptional mechanisms as epithelial cells.

Since OSM and $\text{TNF}\alpha$ production is associated with neutrophilic inflammation, their role in stimulating A1AT transcription in ASC may be a physiologically important response to protect stromal tissue from proteases released by neutrophils. OSM and $\text{TNF}\alpha$ are increased in the lungs of COPD patients, especially during acute exacerbations of COPD, which are characterized by neutrophil recruitment to the lung. The relevance of adding DEX to further enhance A1AT production in ASC administered to COPD patients may be linked to the frequent use of synthetic steroids in both stable and acute exacerbations of COPD.

In patients without A1AT deficiency, circulating levels of A1AT secreted by hepatocytes clearly exceed the A1AT levels secreted by stimulated ASC, as measured in the culture media. In these individuals, it remains to be determined whether impairments in the availability and functionality of A1AT in the CS-injured lung can be alleviated by exogenous administration of conditioned media from or cell infusions with primed ASC. It remains to be determined if it is feasible to use ASC-derived therapies to adequately increase A1AT in

FIG. 5. Effect of CS exposure on A1AT expression. (A, B) *hA1AT* mRNA (A, measured by qPCR) expression in ASC isolated from smoking donors ($n=4$) or from non-smoking donors ($n=3$), then cultured and exposed to indicated inflammatory stimuli. (C) *hA1AT* mRNA (A, measured by qPCR) and protein (B, shown in a representative western blot) secretion in ASC exposed to inflammatory stimuli and increasing concentrations of CSE. Equal volume of media was used for each group and loading was controlled using stain-free image of total protein per lane; $n=3$ independent experiments using ASC 290 loaded. (D) Effect of CSE on *hA1AT* mRNA expression in primary human tracheobronchial cells exposed to indicated stimuli (24 h, $n=3$ donors). * $P<0.05$, *** $P<0.001$, **** $P<0.0001$. ANOVA with Bonferroni's multiple comparisons. CS, cigarette smoke; CSE, cigarette smoke extract.

individuals affected with either the null-mutation, lacking circulating A1AT, or with the more common ZZ-mutation, where the accumulation of polymerized A1AT in hepatocytes decreases circulating A1AT. In these individuals, protective lung levels of A1AT (measured at 1.7 μ M in the alveolar lining fluid) are typically associated with A1AT circulating levels of at least 11 μ M [42].

The potential autologous use of ASC has raised much interest in understanding how the decreased “fitness” of the autologous ASC donor may impair the ASC multipotent progenitor to proliferate, differentiate, and secrete reparative and immunomodulatory factors. While obesity and age may impair ASC functionality [43–46], the effect of other health issues remains unclear. CS exposure has systemic adverse effects outside of the lung, including on the hematopoietic stem and progenitor cell (HSPC) niche in the bone marrow. We have previously shown in mice that HSPC expansion was impaired after as little as 3 days of CS exposure [17] and others have shown that allogeneic HSPC transplant survival outcome is lower in patients who smoke cigarettes [47].

While the effects of CS use on adipose stem cell number and function in general remain unclear, in our small number of donors tested, who were gender, age, and BMI matched, the CS exposure history of the donor did not appear to affect the ability of ex vivo stimulated ASC to produce A1AT. Our results are congruent to a recent study of MSCs from COPD patients (all former smokers), which were found to be phenotypically and functionally similar to those from non-COPD controls [48]. However, we did note measurable differences in the ability of stimulated ASC to produce A1AT when exposed directly to nonlethal concentrations of CS extract. CS extract is extensively used in the field to model exposure of nonairway cells to components of CS that are absorbed in the circulation across the alveolus capillary membrane.

The mechanisms by which CS exposure or other comorbidities would suppress A1AT transcription in ASC are not known. Such mechanisms may be related to the ability of CS extract to suppress transcription factors activated by TNF α such as nuclear factor-kappa B [49] and activator protein-1 [50]. Furthermore, these results may prompt future investigations into whether CS affects the ability of progenitor cells in the lung to secrete protective paracrine factors. The ability of CS extract to inhibit stimulated A1AT expression in primary tracheobronchial epithelial cells suggests that cigarette smokers may have impaired lung A1AT production, in addition to impaired A1AT functionality. Such impairment of A1AT production may explain why individuals with the milder PI*SZ genotype who have serum A1AT levels slightly below the protective threshold are at increased risk for emphysema only if they smoke [51].

In this study, we demonstrated that undifferentiated mesenchymal ASC transcribe and secrete functional A1AT on stimulation with inflammatory cytokines, that this response is heightened by steroids and inhibited by active exposure to CS. The mechanisms of A1AT production by ASC may be similar to those of epithelial cells. While the relative importance of A1AT secretion to the lung-protective paracrine effects of exogenously administered ASC or ASC-based therapy remains to be determined, our work highlights the potential requirement for preconditioning to induce optimized ASC secretion of protective paracrine factors.

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Author Disclosure Statement

I.P. and K.L.M. have patent applications related to therapeutic use of ASC. No competing financial interests exist for other authors.

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