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Evidence for Unfolded Protein Response Activation in Monocytes from Individuals with α-1 Antitrypsin Deficiency

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The hereditary disorder α-1 antitrypsin (AAT) deficiency results from mutations in the SERPINA1 gene and presents with emphysema in young adults and liver disease in childhood. The most common form of AAT deficiency occurs because of the Z mutation, causing the protein to fold aberrantly and accumulate in the endoplasmic reticulum (ER). This leads to ER stress and contributes significantly to the liver disease associated with the condition. In addition to hepatocytes, AAT is also synthesized by monocytes, neutrophils, and epithelial cells. In this study we show for the first time that the unfolded protein response (UPR) is activated in quiescent monocytes from ZZ individuals. Activating transcription factor 4, X-box binding protein 1, and a subset of genes involved in the UPR are increased in monocytes from ZZ compared with MM individuals. This contributes to an inflammatory phenotype with ZZ monocytes exhibiting enhanced cytokine production and activation of the NF-κB pathway when compared with MM monocytes. In addition, we demonstrate intracellular accumulation of AAT within the ER of ZZ monocytes. These findings change the current paradigm regarding lung inflammation in AAT deficiency, which up until now was derived from the protease–anti-protease hypothesis, but which now must include the exaggerated inflammatory response generated by accumulated aberrantly folded AAT in circulating blood cells. The Journal of Immunology, 2010, 184: 4538–4546.

Newly synthesized proteins destined for secretion (such as α-1 antitrypsin [AAT]) are transported into the lumen of the endoplasmic reticulum (ER) where they are folded and assembled. ER homeostasis is essential for normal cell function and survival. Environmental perturbations, such as disturbances in calcium storage, compromise the ER protein folding capacity resulting in the accumulation of unfolded or misfolded protein within the ER. Any imbalance between the load of unfolded proteins entering the ER and the ability of the ER to process this load is termed ER stress. ER stress can also be induced by a range of pathophysiological conditions, including ischemia, diabetes, viral infection, and mutations that impair host protein folding (1). To maintain homeostasis, the ER has evolved to sense stress and transduce signals to the cytoplasm and the nucleus, striving to adapt for survival or induce apoptosis. The protective system includes the translational attenuation of global protein synthesis, transcriptional induction of ER-resident chaperones, and ER-associated degradation (ERAD) (2). Three integral ER-resident transmembrane sensors, protein kinase RNA-like ER kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring kinase 1 (IRE1), form a tripartite management system that coordinates the unfolded protein response (UPR) (3).

The first and immediate step in UPR engagement is translational attenuation. This reduces the load of host protein synthesis in the ER and further prevents accumulation of unfolded proteins. This is carried out by PERK, through phosphorylation of eukaryotic initiation factor 2α (eIF2α) (4). Although it reduces global protein synthesis, eIF2α phosphorylation paradoxically promotes the translation of ATF4 mRNA, a basic leucine zipper transcription factor (5). ATF4 is involved in the expression of genes essential for amino acid import, glutathione biosynthesis, and resistance to oxidative stress (5). The transcriptional arm of the UPR uses IRE1 and ATF6, which regulate chaperone induction, ERAD, and expansion of the ER in response to ER stress (2). IRE1 is an endoribonuclease, and ER stress causes it to oligomerize and activate its RNase domain (6), and a target of IRE1 is the basic leucine zipper transcription factor X-box binding protein 1 (XBP-1). The stress-induced spliced form of XBP-1 travels into the nucleus and can bind to both ER stress response elements and UPR elements, activating the transcription of ER chaperone genes, ER quality control genes, and folding enzymes (7, 8). In a later phase of the UPR components of the ERAD pathway including p97/valosin-containing protein (p97/VCP) and Derlin-1 are called into action.

ERAD is the process whereby terminally misfolded ER proteins are prevented from progressing along the secretory pathway, retrotranslocated from the ER lumen into the cytoplasm, and degraded by the ubiquitin–proteasome system (9). AAT deficiency (AATD) is a genetic disorder characterized by decreased serum levels of a serine protease inhibitor and classically presents with emphysema in young to middle-aged adults and liver disease in childhood and occasionally in adulthood (10, 11).
However, AATD can be classified as an ER stress-related disease as it involves mutations that affect AAT structure, resulting in retention of abnormal AAT cargo in the ER (12). AAT is a 52 kDa gp produced primarily by hepatocytes and its main site of action is the lung, where it serves to protect the fragile alveolar, connective, and epithelial tissues from proteolytic attack (13). AAT is the archetype of the serine protease inhibitor or serpin superfamily, inhibiting the serine proteases neutrophil elastase, cathepsin G, and proteinase 3 (14). The commonest cause of AATD is the Z mutation, which differs from the normal M variant by a single glutamic acid to lysine substitution at position 342 (Glu342Lys) (15). This mutation changes the structure of the molecule leading to protein misfolding, polymerization, and accumulation of rogue Z protein within the ER of cells that produce AAT. The failure in AAT secretion leads to low circulating levels of AAT and inadequate antiprotease protection in the lower respiratory tract, eventually causing progressive lung destruction and emphysema (13). The retention and accumulation of aberrantly folded Z AAT in the liver, which is associated with juvenile hepatitis, cirrhosis, and hepatocellular carcinoma (11), implicates a role for ER stress in the pathogenesis of this condition.

In addition to its role in maintaining ER homeostasis, it has become apparent that ER stress-induced UPR activation can influence the expression of certain inflammatory cytokines. The spliced form of XBP-1, a key inducer of chaperones and ERAD, is spliced out of its precursor during ER stress. XBP-1 splicing is regulated by the transcription factor IRE1α and is activated by protein unfolded or misfolded in the ER (16). XBP-1 splicing can be rescued by treatment with agonists designed to prevent splicing of XBP-1, such as thapsigargin (17). Similarly, other cytokines, peptides, or ligands can activate XBP-1 splicing, and the extent of XBP-1 splicing can be used as a readout of ER stress and the effectiveness of potential therapies to combat ER stress.

Cytokine arrays
Monocytes were isolated as described and left untreated or stimulated with LPS (20 μg/ml) at 37°C. After 24 h, cell supernatants were recovered, incubated with the cytokine array membranes (RayBiotech, Norcross, GA), and developed according to the manufacturer’s instructions. Relative protein levels were quantified by densitometry and normalized for total protein using the SynGene GeneSnap and GeneTools software (Cambridge, U.K.).

ELISA
Monocytes were isolated as described and cultured for 24 h. Supernatants were recovered and IL-8 and AAT protein concentrations determined by ELISA with specific Abs to IL-8 (R&D Systems, Minneapolis, MN) and AAT (ICN Biomedicals, Solon, OH).

cDNA synthesis and RT-PCR
RNA was isolated using TRI reagent (Sigma-Aldrich) according to the manufacturer’s instructions. Equal quantities of RNA were reverse transcribed into cDNA using the Quantitect Reverse Transcription kit (Qiagen, Valencia, CA). The resulting cDNA was template for quantitative real-time PCR. Oligonucleotide primers were synthesized (MWG Biotech, Ebersberg, Germany) and the quantitative PCR reactions performed in 20 μl containing 2 μl template cDNA, 2× SYBR Green master mix (Roche, Basel, Switzerland), and 10 pmol of each primer. Amplification was performed using the LightCycler 480 PCR system (Roche) with the expression of target genes relative to β-actin determined using the 2^-ΔΔCt method (27).

Analysis of XBP-1 mRNA cleavage
XBP-1 mRNA splicing was analyzed using an assay described by Harding et al. (28). Briefly, RNA was isolated using TRI reagent (Sigma-Aldrich) and cDNA synthesized as described. The following primers were used to amplify XBP-1 cDNA: forward 5’-AACAGATGAGCATCGCTCTTCG-3’; reverse 5’-GGTGGTCAGACGTTGGA-3’. The cDNA fragments were resolved on a 2.5% agarose gel with unspliced XBP-1 yielding a product of 480 bp, whereas spliced XBP-1 was 454 bp.

Immunofluorescence and confocal microscopy
Monocytes were isolated and fixed in 4% paraformaldehyde in PBS. The cell membranes were disrupted in 0.2% Triton X-100 in TBS. Non-specific binding of Abs and fluorescent conjugates were blocked by preincubation in 4% BSA/1% gelatin in PBS. Colocalization of AAT and ER-specific chaperones was detected by immunofluorescence using goat polyclonal anti-AAT-FITC (Abcam, Cambridge, U.K.) and mouse monoclonal anti-KDEL (Stressgen, Ann Arbor, MI) Abs, with an anti-mouse tetramethylrhodamine isothiocyanate secondary conjugate (Abcam) for visualization of the anti-KDEL Abs. Cells were mounted in Vectashield (Vector Laboratories,
Burlingame, CA) containing DAPI and examined using a LSM510 Meta laser scanning confocal microscope (Zeiss, Oberkochen, Germany). Images were captured at ×63 magnification and ×4 zoom with excitation wavelengths for FITC, tetramethylrhodamine isothiocyanate, and DAPI of 488 nm, 543 nm, and 364 nm, respectively. The images presented are single focal plane scans of 1-μm depth at the midsection of the fixed cells. The images are representative of three independent experiments and were collected using identical scanning parameters.

Immunoblotting
Monocytes were pelleted by gentle centrifugation and cells resuspended in 1 ml hypotonic buffer (10 mM HEPES [pH 7.9], 1.5 mM MgCl2, 10 mM KCl, 0.5 mM PMSF, and 0.5 mM DTT) (Sigma-Aldrich). Cells were lysed for 30 min on ice before centrifugation at 14,000 rpm for 10 min at 4°C. The supernatant was recovered for immunoblotting and stored at −80°C until required. Whole cell lysates were separated by electrophoresis on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Sigma-Aldrich). Nonspecific binding was blocked with 5% BSA (Sigma-Aldrich) in PBS containing 0.1% Tween-20 (Sigma-Aldrich). Immunoreactive proteins were detected by incubating the membrane with specific Abs to total Akt, phospho-Akt (Ser473) (Cell Signaling Technology, Beverly, MA), IkBa or β-actin (Santa Cruz Biotechnology, Santa Cruz, CA).

Statistical analysis
Data were analyzed with GraphPad Prism 4.0 software package (GraphPad Software, San Diego, CA). Results are expressed as mean ± SE and compared by Student t test and ANOVA. Differences were considered significant when the p value was <0.05.

Results
ZZ monocytes produce increased levels of cytokines compared with MM monocytes
We investigated cytokine production from monocytes isolated from MM and ZZ individuals. MM (n = 3) and ZZ (n = 3) monocytes were maintained for 24 h in the presence and absence of Pseudomonas LPS. LPS is a pathogen-associated molecular pattern, which signals through the TLR 4 pathway, and regulates a plethora of host defense genes in immune cells, including monocytes (29). The monocyte supernatants were applied to cytokine arrays to investigate whether there was an abnormal immune response in ZZ individuals. LPS was found to induce higher levels of secreted IL-6, IL-8, and IL-10 in monocytes from ZZ individuals compared with MM individuals (Fig. 1A). IL-6 and IL-8 protein levels were 1.5-fold higher (p = 0.0015 and p = 0.0003, respectively), whereas IL-10 production was almost 3-fold higher (p = 0.0015) in LPS-treated ZZ monocytes compared with LPS-treated MM monocytes. Significant differences in LPS-induced CXCL1 and MIP-1α protein production between the two groups were also observed (data not shown).

To confirm the findings from the cytokine arrays, we investigated the expression of IL-6, IL-8, and IL-10 genes in the same monocytes from Fig. 1A. We found significant differences in basal IL-6, IL-8, and IL-10 levels between unstimulated MM and ZZ monocytes as measured by RT-PCR. Furthermore, there were significant differences in LPS-induced IL-6, IL-8, and IL-10 mRNA levels
between the MM and ZZ groups (Fig. 1B). IL-6 gene expression was 8-fold higher \( (p = 0.0143) \), IL-8 gene expression 11-fold higher \( (p = 0.0143) \), and IL-10 gene expression 5-fold higher \( (p = 0.0286) \) in LPS-treated ZZ monocytes compared with LPS-treated MM monocytes. Significant differences were again observed in LPS-induced CXCL1 and MIP-1α expression between the two groups (data not shown). Collectively, these data demonstrate that monocytes isolated from ZZ individuals exhibit an exaggerated immune response compared with monocytes from MM individuals.

Normal monocytes undergoing ER stress produce increased levels of cytokines

It is well-established that treatment with TG, by depleting Ca\(^{2+}\) levels of cytokines Normal monocytes undergoing ER stress produce increased individual. LPS-induced CXCL1 and MIP-1α expression between the two groups (data not shown). Collectively, these data demonstrate that monocytes isolated from ZZ individuals exhibit an exaggerated immune response compared with monocytes from MM individuals.

Monocytes from ZZ individuals secrete less AAT protein

To determine whether the production of AAT was impaired in monocytes from ZZ individuals, we cultured monocytes from MM and ZZ subjects for 24 h and quantified AAT gene expression and AAT protein secretion in these cells. We found that while there was no difference in AAT mRNA between both cell types, ZZ monocytes secreted 4-fold less AAT protein than the MM monocytes \( (p = 0.0091) \) (Fig. 3A). To clarify this finding was specific to AAT and not due to impairment in global protein production we quantified IL-8 mRNA and IL-8 protein produced from the same monocytes. We found that quiescent ZZ monocytes express significantly more IL-8 mRNA \( (p = 0.0028) \) and secrete twice as much IL-8 protein \( (p < 0.0001) \) compared with monocytes from MM individuals (Fig. 3B). These findings replicate our earlier data (Fig. 1), showing increased cytokine production from the ZZ monocyte but more importantly support our contention that misfolded Z AAT protein is retained within these cells.

TG induces the UPR in normal monocytes

To investigate whether the UPR could be induced in normal monocytes, we treated monocytes from MM individuals with increasing concentrations of TG. Treatment with TG caused an increase in ATF4 mRNA and caused the activation of XBP-1, transcription factors essential for UPR activation (Fig. 4A, 4B). The effect of TG on the ER chaperones glucose-regulated protein (grp) 58, grp78, and grp94 and on the ERAD proteins Derlin-1 and p97/VCP was also investigated. TG dose dependently induced the expression of the chaperones grp58, grp78, grp94, and ERAD components Derlin-1 and p97/VCP (Fig. 4C).

AAT accumulates within the ER of ZZ monocytes

As ZZ monocytes exhibited impaired AAT secretion we investigated whether intracellular accumulation of AAT was occurring in these cells. The subcellular location of AAT was examined by immunofluorescence. As shown in Fig. 5 the expression of AAT was increased in ZZ monocytes. Using an ER-specific marker that recognizes the KDEL motif present in ER-resident chaperones grp78 and grp94, we colocalized AAT to the ER of ZZ monocytes. In addition, the expression of KDEL was increased in ZZ monocytes compared with MM. Taken together, this shows that AAT protein accumulates within monocytes isolated from ZZ individuals, and is localized specifically to the ER.

ATF4 expression is increased and XBP-1 is spliced in ZZ monocytes

Next, we explored UPR activation in vivo in monocytes isolated from MM and ZZ individuals by investigating the expression of ATF4 and splicing of XBP-1 mRNA. ATF4 expression was significantly increased in ZZ monocytes \( (n = 7) \) compared with MM monocytes \( (n = 7) \) as measured by quantitative RT-PCR \( (p = 0.006) \) (Fig. 6A). Spliced XBP-1 mRNA was also increased in ZZ monocytes compared with MM monocytes (Fig. 6B). TG- and TM-treated normal monocytes were included as positive controls for
ER-stress mediated splicing of XBP-1. TM induces ER stress by inhibiting N-linked glycosylation that causes an accumulation of unfolded protein (31). This data indicates that the UPR is activated in monocytes from ZZ individuals.

ER chaperone and ERAD gene expression is increased in monocytes from ZZ individuals

To further elucidate activation of the UPR in ZZ monocytes, we investigated additional genes induced by ER stress. Real-time PCR analysis showed that expression of chaperones grp58, grp78, and grp94 is significantly increased in ZZ monocytes (n = 7) compared with MM monocytes (n = 7) (p, 0.05), suggesting that misfolded Z AAT protein is inducing chaperone expression in an attempt to refold the rogue protein (Fig. 7, top). Moreover, Derlin-1 and p97/VCP mRNA expression was also increased in ZZ monocytes compared with MM monocytes indicating that components of ERAD are upregulated in monocytes producing Z AAT protein (Fig. 7, bottom).

FIGURE 4. TG causes ER stress and upregulates components of the UPR in normal monocytes. Monocytes isolated from healthy MM individuals (n = 4) were treated with increasing concentrations of TG for 24 h and the activation of several UPR genes was investigated. A, ATF4 gene expression was analyzed by real-time RT-PCR and normalized for β-actin. B, Representative gel of PCR showing spliced XBP-1 (XBP-1s) and unspliced XBP-1 (XBP-1u) bands. C, Real-time RT-PCR analysis of five UPR genes normalized for β-actin. *p < 0.05, Student t test compared with unstimulated cells.
Akt is phosphorylated and IκBα degraded in monocytes from ZZ individuals

In the search for a mechanism linking exaggerated cytokine production to UPR activation, we investigated the possible involvement of Akt and NF-κB. ER stress pathways can involve Akt-dependent activation of NF-κB (32) and it has been shown that spliced XBP-1 can activate Akt phosphorylation in a zebrafish embryonic cell line (33). We demonstrated that Akt phosphorylation and IκBα degradation, a hallmark of NF-κB pathway activation, were increased in ZZ monocytes compared with MM monocytes (Fig. 8). When ER stress was induced in MM monocytes by treatment with TG, Akt phosphorylation was also increased.

Discussion

In this study, we demonstrate for the first time that the UPR is activated in monocytes from AATD individuals and that this affects monocyte function, specifically inflammatory gene expression. We have shown that monocytes from ZZ individuals exhibit an exaggerated immune response, particularly in the production of IL-6, IL-8, IL-10, MIP-1α, and CXCL1, when responding to bacterial challenge. IL-6 is a potent proinflammatory cytokine, IL-8, MIP-1α, and CXCL1 are neutrophil and monocyte chemoattractants, whereas IL-10 is an anti-inflammatory cytokine (34). We found that normal monocytes subjected to ER stress display a similar enhanced immune response. In support of our intracellular AAT accumulation and ER stress hypothesis, we have shown ZZ monocytes display impaired AAT secretion and we provide evidence of AAT accumulation within the ER of ZZ monocytes. We have demonstrated that key components of the UPR are activated in unstimulated monocytes from ZZ individuals. Finally, we propose a mechanism linking exaggerated cytokine production in ZZ individuals to UPR activation involving the prosurvival serine/threonine kinase Akt and activation of the NF-κB pathway.

Two proximal indicators of UPR activation, ATF4 gene induction and splicing of XBP-1 mRNA, were upregulated in ZZ monocytes. This UPR engagement was independent of exogenous stimulation, and hints at a persistent state of UPR activation within quiescent monocytes from ZZ subjects. ATF4 is selectively activated by PERK-induced phosphorylation of eIF2α, whereas XBP-1 mRNA is processed by IRE1 to yield a transcription factor that regulates ER stress-responsive genes. Whereas IRE1 activation is difficult to reproducibly measure, processing of unspliced XBP-1 mRNA into mature XBP-1 mRNA represents a convenient, widely used indicator of IRE1 activation (35). The expression of several other genes involved in the UPR was shown to be increased in ZZ monocytes. Grp58, grp78, and grp94 mRNA expression was significantly increased in ZZ monocytes. These three ER-resident proteins were first identified as grps in hamster fibroblasts (36), and are ER-resident chaperones upregulated by ER stress (37). Grp58 (also called ERP57) is a thiol oxidoreductase, involved in protein folding and recognition of mutant proteins for ERAD (38). Grp78 is a member of the heat shock protein (Hsp) 70 family, binding to misfolded proteins and also playing a crucial role in the activation of PERK, ATF6, and IRE1 (3), whereas grp 94 is a member of the Hsp90 family (39). Immunofluorescence experiments also demonstrated increased grp78 and grp94 expression in resting ZZ monocytes. In addition to the chaperones, the expression of ERAD proteins Derlin-1 and p97/VCP was shown to be increased in ZZ monocytes. Derlin-1 and p97/VCP cooperate to ensure efficient

![FIGURE 5. Intracellular accumulation of AAT in ZZ monocytes. Monocytes were isolated from MM and ZZ individuals. The subcellular distribution of AAT was determined by immunofluorescence using Abs for AAT (green) and the ER marker KDEL (red). Colocalization is indicated by white arrows (yellow). Nuclei were stained with DAPI (blue). Imaging was acquired using a Zeiss LSM 510 Meta confocal microscope (original magnification ×63).](http://www.jimmunol.org/)

![FIGURE 6. ATF4 gene expression and XBP-1 mRNA splicing are increased in ZZ monocytes compared with normal monocytes. A. Monocytes were isolated from MM (n = 7) and ZZ (n = 7) individuals and ATF4 mRNA expression was analyzed by real-time RT-PCR and normalized for β-actin. B. Monocytes were isolated from MM (n = 3) and ZZ (n = 3) individuals and XBP-1 mRNA splicing investigated by RT-PCR. Representative gel showing spliced XBP-1 (XBP-1s) and unspliced XBP-1 (XBP-1u) bands. Monocytes from MM individuals treated with DMSO (vehicle control), TM (1 mg/ml), and TG (100 nM) for 24 h are included as controls. Data was normalized for β-actin for each sample. *p < 0.05, Student t test compared with MM monocytes.](http://www.jimmunol.org/)
retrotranslocation of misfolded proteins from the ER to the cytoplasm for proteasome-mediated degradation (40).

Lastly, our finding that phosphorylation of the serine/threonine kinase Akt is increased and that IκBα is degraded in monocytes from ZZ individuals suggests a mechanism linking exaggerated cytokine production to UPR activation and also provides a possible explanation for the continued survival of the ZZ monocyte. Prolonged ER stress can activate apoptosis by both mitochondria-dependent and -independent pathways (41, 42). The activation of Akt is a critical prosurvival response to ER stress as it promotes the expression of inhibitor of apoptosis protein family members (43) and inhibits several proapoptotic proteins by phosphorylation (44). Interestingly, XBP-1-mediated PI3K/Akt pathway activation has been shown to be responsible for the marked resistance of melanoma cells to chemotherapeutic drugs (45). The activation of NF-κB leads to a well-characterized prosurvival transcription program of cellular proliferation and the expression of genes encoding cytokines, chemokines, and growth factors (46), and NF-κB can be activated downstream of IRE1 in response to ER stress in vitro (47). These antiapoptotic but proinflammatory signals may facilitate the survival of the Z AAT-producing monocyte, preventing ER stress-induced apoptosis, whereas rendering the monocyte intrinsically proinflammatory. Further work will be required to tease out the exact mechanism of ER stress-induced cytokine production, and determine the involvement of the MAPK cascades that are known to be activated in cells undergoing ER stress (48–50).

The concept of UPR involvement in the pathogenesis of AATD is not new. The synthesis of stress proteins Hsp70 and Hsp90 was shown to be increased in individuals with homozygous ZZ AATD and liver disease (51). In addition, it has been established that the secretion of AAT is decreased in monocytes from ZZ individuals due to the intracellular accumulation of Z AAT (52). More recently, Z AAT has been shown to associate in vitro with an array of chaperones, including grp78 and grp94 in several different cell lines (53), but conversely, work from the same group has shown that Z AAT does not cause UPR activation in mouse liver cells (54). However, work from our group indicates that the UPR is activated by Z AAT in human lung epithelial and liver cell lines, suggesting species- and cell-specific differences may exist. We have demonstrated that Z AAT induces proinflammatory IL-6 and IL-8 gene expression and activates specific ER stress responses (55). Using a novel in vitro model of Z AAT ER accumulation, we established that both the ER overload response and the UPR are activated by Z AAT, and not wild-type M AAT. The scenario of UPR activation in Z AAT-producing monocytes shown in this study adds another layer of complexity to the pathogenesis of AATD, and suggests a role for the monocyte in AATD-associated emphysema.

The model of UPR-induced inflammatory gene expression in monocytes producing Z AAT described here raises several intriguing scenarios with profound clinical and biological implications. First, previous studies have demonstrated that LPS, IL-6, IL-1β, neutrophil elastase, and TNF-α upregulate AAT production in monocytes and macrophages (56–58). The central role of the macrophage as a mediator of neutrophil accumulation is long established (59). If inflammatory stimuli, such as LPS, upregulate local AAT production in monocytes from AATD individuals, the subsequent increase in Z AAT production could cause activation of the UPR. Therefore, in monocytes producing Z AAT, UPR-induced cytokine production could represent a self-perpetuating cycle of amplified inflammatory gene expression and immune cell recruitment. As cytokine and chemokine levels become elevated in the local environment, increased numbers of neutrophils and monocytes would be attracted and contribute to the inflammatory milieu. In support of this theory, a 1991 study showed that alveolar macrophages from ZZ individuals spontaneously released 3-fold more chemotactic activity than normal macrophages (60).

Second, there is the possibility that moderate UPR activation in MZ individuals could confer an unexpected survival advantage. A recent study investigating monocytes from CF homozygotes and heterozygotes showed that a single allelic CFTR mutation was sufficient to augment IL-8 secretion in response to LPS. The authors postulated that the enhanced response to LPS in monocytes from CF
heterozygotes could be advantageous in priming the innate immune system, leading to the rapid eradication of bacterial invaders (25). In a similar manner this advantage could apply to individuals heterozygous for the Z mutation, and provide an explanation as to why the Z mutation has persisted for centuries. In support of this theory, a study of nonsmoking asymptomatic MZ subjects without airflow obstruction demonstrated IL-8–related neutrophilic inflammation in the airways (61). However, it is clear that many questions remain to be answered. What other UPR components are activated in monocytes from ZZ individuals? Is the UPR activated in airway epithelial cells that produce Z AAT? What precisely is the mechanism of UPR-regulated cytokine production?

Our findings change the current paradigm regarding inflammation in the lung disease associated with AATD from a protease/antiprotease-driven hypothesis to one where a gain of function from accumulated mutant AAT protein leads to ER stress and an exaggerated inflammatory phenotype. Previously, this group and others have demonstrated that polymerized Z AAT, in addition to being ineffective as an antiprotease, is a potent neutrophil chemotactant on the respiratory epithelial surface (62, 63). In this study, we present compelling data supporting a novel mechanism where Z AAT accumulation in the ER of monocytes activates the UPR and induces an exaggerated immune response. This model of UPR activation in ZZ monocytes may lead to renewed interest in pharmacological agents, such as 4-phenylbutyric acid, as interventions that enhance the secretion of Z AAT or accelerate the ERAD-mediated degradation of misfolded Z AAT may prove effective in reducing the inflammatory consequences of the accumulated Z AAT protein. Therapies that target elements of the UPR could represent novel strategies in the treatment of the lung disease associated with AATD.

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Disclosures
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