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Commentary

Liver gene therapy: The magic bullet for the sick lung

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Alpha1-antitrypsin (AAT) deficiency is one of the most common genetic diseases and is due to pathogenic variants in the *SERPINA1* gene that encodes for AAT, an acute-phase protein with anti-inflammatory and immunomodulatory properties. Although more than 150 pathogenic variants have been described, 95% of individuals with severe AAT deficiency are homozygotes for the Z allele, a substitution of glutamic acid for lysine at residue 342 (p.Glu342Lys) in *SERPINA1*. This single amino acid change makes Z-AAT prone to aggregation mostly in hepatocytes, the cells that abundantly synthetize and secrete AAT into the circulation. Retention of Z-AAT within hepatocytes results in liver disease by a toxic "gain-of-function" mechanism, whereas the lack of circulating AAT, which inhibits neutrophil elastase in the lung, makes affected individuals susceptible to early-onset emphysema by a "loss-of-function" mechanism. The lung is constantly under attack by pro-inflammatory insults, and it is incessantly damaged and repaired. In individuals with AAT deficiency, protease activity is not sufficiently balanced by anti-proteases, resulting in an excessive cleavage of structural proteins and innate immune proteins in the lung parenchyma, that ultimately lead to emphysema. Cigarette smoke and enhanced susceptibility to infections further accelerates and aggravates the lung disease.

Besides avoiding smoking, excessive consumption of alcohol, and excessive weight gain, the only disease-specific treatment available for AAT deficiency is augmentation therapy based on intravenous infusions of human plasma-derived, purified AAT. Although RNAi-based therapeutic silencing of hepatic Z-AAT is under clinical investigation,[1] currently there is no specific treatment for the liver disease, and life-threatening, progressive liver failure or uncompensated cirrhosis due to AAT deficiency can be treated only by liver transplantation.

Until recently, the only available model of AAT deficiency was the PiZ mouse, which is transgenic for the Z-AAT and recapitulates the pathology hallmarks of the human liver disease. However, PiZ mice do not develop any pulmonary disease, owing to the expression of their murine *Serpina1* genes.[2] In contrast to humans, who have only one gene, mice have up to six genes encoding AAT. For decades, this made it impossible to investigate the pathogenesis and therapies for lung emphysema in AAT deficiency. This obstacle was overcome by Mueller's group, who generated a mouse knockout for all murine *Serpina1* genes by using CRISPR-Cas9.[3] In a study published in *Molecular Therapy - Methods & Clinical Development*, the authors now report that these mice develop lung disease either spontaneously with aging or after exposure to cigarette smoke.[4] Moreover, they found that liver-directed gene transfer of *SERPINA1* can prevent development of the lung disease in this mouse model. This has been a long-awaited experiment that now conclusively supports the concept of alveolar destruction driven by uncontrolled elastase activity that is preventable through the expression and secretion into the blood of AAT by AAV-corrected hepatocytes.

Besides hepatocytes, Z-AAT has been detected in the respiratory epithelium of PiZ mice, where it was found to accumulate and to cause proteotoxicity, contributing to the lung disease. These results have challenged the paradigm of lung disease being solely due to the loss of antiprotease function.[5] *Serpina1a-e* knockout mice are not suitable to address the contribution of Z-AAT expression in respiratory cells, because these cells do not express Z-AAT. Nevertheless, this question might be investigated in the Z-AAT knockin mouse currently under development[3] or in the newly generated ferret model that is knockout for *SERPINA1* and knockin for Z-AAT.[6] Interestingly, Zieger and colleagues detected an increased neutrophil count in bronchoalveolar lavage of *Serpina1a-e* knockout mice that was significantly reduced in AAV-treated mice, supporting the role of neutrophil recruitment in disease progression in both humans and mice.[4] Whether neutrophils that express Z-AAT are more detrimental for the lung disease compared with those expressing the wild-type AAT or those completely devoid of *Serpina1* genes is another question that will require different animal models.

Polymers of Z-AAT are detected in the blood, bronchoalveolar lavage fluid, and lung tissue of patients with AAT deficiency. Extracellular polymers of Z-AAT have pro-inflammatory effects, and they are chemotactic and stimulatory for neutrophils, probably contributing to pulmonary inflammation. Although it is unclear whether they are formed by polymerization of secreted Z-AAT in the extracellular space, most extracellular Z-AAT polymers must come from the liver, since they become undetectable shortly after liver transplantation.[7] As recently shown, Z-AAT can also form heteropolymers made of wild-type AAT and Z-AAT *in vivo*,[8] and thus, gene therapy vectors driving expression of AAT in hepatocytes might aggravate the liver disease through increased formation of heteropolymers. Heteropolymers are expected to increase after liver-directed gene transfer of *SERPINA1*, and they might be secreted in blood, aggravating lung inflammation. Furthermore, Z-AAT polymers appear to sensitize cells to second insults that result in ER stress,[9] and there is increasing concern

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that ER stress and the unfolded protein response might be induced by viral-vector-mediated transgene overexpression, possibly compromising the duration of efficacy and safety of patients treated by gene therapy.[10] Unfortunately, these additional concerns cannot be investigated in the *Serpina1a-e* knockout mice, and studies in the Z-AAT knockin mouse or in the ferret will be required to address these issues.

Protein augmentation therapy by intravenous AAT therapy aims to achieve the "protective threshold" of 11 μM in serum AAT concentration in patients with AAT deficiency. However, this threshold is arbitrary and based on some controversial assumptions.[11] The study by Zieger and colleagues does not allow this question to be solved, because the serum AAT concentrations achieved by AAV-mediated liver-directed gene transfer were well above the 11 μM threshold. Moreover, the therapeutic threshold is likely to be species-specific and thus cannot be precisely established in mice. However, studies in mice remain valuable to compare augmentation therapy with gene therapy. Protein replacement therapy results in peak-and-drop pharmacokinetics in blood AAT concentrations, whereas liver-directed gene therapy results in sustained blood concentrations of AAT. It is likely that the therapeutic threshold with sustained expression of AAT by gene therapy may be different from the therapeutic threshold needed by protein augmentation products. The best timing to rescue or arrest the decline in lung pathology by gene therapy also remains to be investigated. The study by the Mueller group has set the stage for addressing these clinically relevant questions.

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