Inflammatory pulmonary fibrosis is associated with pulmonary autotaxin expression

Vassilis Aidinis*

Division of Immunology, Alexander Fleming Biomedical Sciences Research Center, Athens, Greece

Abstract

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive, fibrotic form of diffuse lung disease occurring mainly in older adults and characterized by a progressive worsening of lung function and a poor prognosis. To date, no management approach has proven efficacious. The disease is largely unresponsive to corticosteroid and immunosuppressive therapy. To study the pathogenetic mechanisms that govern disease activation and perpetuation, a number of animal models have been developed. Among them, the bleomycin (BLM) model is the most widely used and best characterized. Current research suggests that the mechanisms driving IPF reflect abnormal wound healing in response to pulmonary epithelial damage, involving increased activity and possibly exaggerated responses by a spectrum of proinflammatory and profibrogenic factors. Lysophos Phatidic Acid (LPA) is a phospholipid mediator that evokes growth-factor–like responses in almost all cell types, including pulmonary fibroblasts, smooth muscle cells, and epithelial cells. Its diverse functions are attributed to at least six LPA receptors (LPARs) with overlapping specificities and widespread tissue distribution, including the lung. LPARs couple to more than three distinct G proteins, which in turn feed into multiple effector systems. LPA concentrations were found to be increased in the bronchoalveolar lavage fluids (BALFs) of IPF patients and a BLM murine model, and were shown to mediate fibroblast recruitment and vascular leakage. Moreover, the genetic or pharmacological inhibition of LPAR1 attenuated the development of BLM-induced pulmonary fibrosis, suggesting a primary role for LPA in disease pathogenesis.

Accepted on 18 November, 2021

Introduction

LPA is produced under various conditions both in cells and in biological fluids, where multiple synthetic reactions occur. In blood, LPA is mainly converted from lysophospholipids, predominantly through the lysophospholipase D (lysoPLD) activity of the enzyme autotaxin. ATX is a secreted glycoprotein, widely present in biological fluids, and originally isolated from the supernatants of melanoma cells as an autocrine motility stimulation factor. The complete genetic deletion of ATX results in embryonic lethality because of vascular and neuronal defects. Increased ATX expression has been detected in a large variety of cancers, chronic liver diseases, Alzheimer's disease, and multiple sclerosis. More importantly, we recently reported significant ATX expression from arthritic synovial fibroblasts, both murine and human. The conditional genetic deletion of ATX from synovial fibroblasts attenuated the development of the modelled disease, suggesting a major role for the ATX/LPA axis in chronic inflammation.

To examine a possible role for ATX in the development of IPF, we have analyzed its expression in fibrotic lungs of patients and in the BLM animal model, and found it to be up-regulated. Following these observational results, the conditional genetic deletion of ATX from murine pulmonary cells diminished disease severity, whereas the pharmacological inhibition of ATX in mice was shown to be efficient in the attenuation of the modeled disease. Some of the results of these studies were previously reported in the form of abstracts.

Discussion

In this study, we demonstrate that ATX, the enzyme largely responsible for extracellular LPA production, is significantly up-regulated in the fibrotic lungs of IPF (and fNSIP) patients and BLM-challenged mice. ATX expression was localized mainly in bronchial epithelial cells and alveolar macrophages. Conditional genetic deletion in both pulmonary cell compartments reduced the severity of the modeled disease, confirming the multiple sources of ATX in the lung and implicating ATX in the pathogenesis of the disease. Accordingly, the pharmacological inhibition of ATX attenuated disease development.

ATX shows a broad tissue distribution, with the highest mRNA concentrations detected by in situ hybridization and/or Northern blot analysis in the brain, ovary, placenta, kidney, intestine, and lung. In the lung, in situ hybridization localized ATX mRNA expression to normal bronchial epithelium. Moreover, ATX was identified, using genome-wide linkage analysis coupled with expression profiling, as a candidate gene controlling lung function, development, and remodeling. In pathophysiological disorders of the lung, ATX expression was found to be up-regulated in poorly differentiated carcinomas, in line with the suggested role of the ATX/LPA axis in carcinogenesis. In septic-like, intratracheal LPS-induced, acute lung injury, both ATX and LPA concentrations were found to be increased in the BALFs of animal models. Likewise, we detected increased ATX concentrations in the BALFs of BLM-

challenged mice, where increased LPA concentrations were reported previously. The immunostaining of fibrotic murine lungs indicated strong expression in bronchial epithelial cells and inflammatory alveolar macrophages. ATX expression from LPS-stimulated transformed macrophages (the human acute monocytic leukemia [THP-1] cell line) has been reported previously. However, most transformed cell lines have also been reported to express ATX. On the contrary, we did not detect any ATX expression in bone marrow–derived primary macrophages, even upon their stimulation (unpublished data). Therefore, the noted ATX expression from alveolar macrophages in the fibrotic lung could be localized in specialized subsets and is worthy of further investigation, especially because of the renewed interest in the role of macrophages in the pathogenesis of IPF.

Similar conclusions on ATX expression in fibrotic lungs were extracted from the expression patterns of ATX in different types of IIPs, with different extents of disease progress and treatment response. In particular, ATX showed strong staining intensity within the alveolar epithelium immediately adjacent to fibroblastic foci, in interstitial macrophages, and in fibroblast-like cells, as well as in areas of bronchiolar metaplasia in IPF lung samples. A similar expression profile was demonstrated in fibrotic NSIP samples, a histopathological pattern sharing common pathologic features with UIP. On the contrary, ATX presented with minimal expression within both the inflammatory components of cellular NSIP lung samples and in areas of loose connective tissue, called Masson bodies, representing the pathogenetic hallmark of COP. These two pathologies represent two forms of IIPs with favorable prognoses and excellent treatment response to corticosteroids, indicating that ATX up-regulation is closely associated with more progressive and irreversible forms of pulmonary fibrosis, such as IPF/UIP and fNSIP.

The conditional genetic deletion of ATX from the majority of bronchial epithelial cells or macrophages, the major sources of ATX in the fibrotic lung, resulted in reduced disease severity, confirming both the expression of ATX in these cell types and a role for ATX in disease development. No major effects in fibrosis development were evident in mice with increased or decreased serum concentrations of ATX, suggesting a minimal contribution of circulating ATX, and highlighting the importance of local pulmonary ATX. However, the increased ATX/LPA concentrations in the transgenic mice, given the reported effects of the ATX/LPA axis in the vasculature and the bleeding diathesis of ATX transgenic mice, could exert indirect effects on inflammation, as seen with the increased infiltration of inflammatory cells in their lungs.

The conditional genetic deletion of ATX indicates a role for ATX in the development of pulmonary inflammation and fibrosis. The similar effects observed with both the epithelial and macrophage ATX deletion suggest that simultaneous deletion from both cell types could exert more pronounced effects in disease development. Accordingly, ATX inhibition with a pharmacological ATX inhibitor abrogated disease development, suggesting that ATX is a major contributor to disease development and a novel therapeutic target. Minor effects in disease attenuation were observed with phosphonate, a dual-function pan-antagonist of LPA receptors and a weak inhibitor of ATX. However, the attenuation of fibrosis with GWJ-A-3 was potent and comparable with the reported disease attenuation upon the genetic or pharmacological inhibition of LPAR1. Moreover, the administration of GWJ lowered LPA concentrations in BALFs despite its rapid turnover, suggesting that the effects of ATX inhibition are attributable to diminished LPA signaling.

Because ATX is a constitutively active enzyme, the biological outcomes of ATX action (essentially LPA production and signaling in the context of IPF pathogenesis) will largely depend on its expression levels, the local availability of its substrates (lysophosphatidylcholine [LPC]), the abundance and activity of the different LPA receptors in the microenvironment, and the relative activity of other LPA metabolizing enzymes.

Conclusion

Very little is known about the regulation of ATX expression. We recently reported that TNF, the major proinflammatory factor, stimulates ATX expression from synovial fibroblasts during the pathogenesis of rheumatoid arthritis, suggesting that ATX induction in arthritic synovial fibroblasts is a downstream event of exacerbated TNF signaling in the synovium. Moreover, anti-TNF treatment of an inflammatory arthritic model attenuated ATX expression. TNF has also been shown to induce ATX expression from hepatocytes in an dependent manner. Because TNF signaling is a prerequisite for BLM-induced pulmonary fibrosis, we could envisage a similar situation in modeled pulmonary fibrosis. Epithelial damage that induces epithelial TNF secretion, inflammation, and additional TNF secretion from macrophages could stimulate ATX expression from epithelial cells and macrophages in a paracrine or autocrine manner.