Pulmonary and Critical Care Medicine

Mini Review

ISSN: 2398-3108

# Neuro-immune interaction during development of pulmonary fibrosis

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## Abstract

Pulmonary neuroendocrine cells are widely distributed throughout the airway mucosa of mammals. They may exist as solitary cells or aggregate to form Neuro-Epithelial Bodies (NEBs), which are important in early lung development. Pulmonary Fibrosis (PF) is a devastating disease caused by various pulmonary insults. The lung repair after injury is a complex process which involves a network of interactions among mediators, cytokines, and growth factors of inflammatory, endothelial, and epithelial cell origin. These interactions lead to lung remodeling, deposition of collagen, limitation of gas exchange, and dyspnea. The vagus nerve is related to each component of this network. Increasing evidence indicates that the NEB-Vagal System (NVS) plays an important role in neuro-immune interactions. NEBs are richly innervated and connect with the central nervous system through vagal afferent and efferent nerves. Stimulation of the vagus nerve in patients significantly increases the levels of TGF-β in plasma, a substance that promotes fibrosis. Based on current information, lung injury may activate the NVS, which then leads to PF via fibrogenic cells and molecules. Therefore, over-activation of the NVS could contribute to disease progression.

## Introduction

Pulmonary Fibrosis (PF) can result from various lung injuries (autoimmune diseases, drugs, occupational exposures). PF of unknown etiology manifests as a group of diseases classified as Idiopathic Pulmonary Fibrosis (IPF) [1]. Although the prevalence of IPF ranges from 6-14.6 per 100,000 people [2], it is uniformly fatal. The median survival is 3-5 years after diagnosis [3]. Despite extensive research efforts, the understanding of the underlying disease mechanisms is limited, and there exists no effective treatment. Growing evidence indicates that the neuro-endocrine system controls immune and cellular responses, so activation of the NVS may promote PF. This mini-review presents available information to support this hypothesis.

PF may result from chronic inflammation in rheumatoid arthritis, and in IPF, through profibrotic mechanisms with no inflammation. In IPF, repetitive injury to alveolar epithelium causes fibroblast recruitment and transformation to a myofibroblast phenotype. Myofibroblasts are fibrogenic cells that release abundant Extracellular Matrix (ECM) containing collagen and fibronectin into the lung interstitium, which leads to PF. This process is enhanced by fibrogenic cytokines and cells, and is regulated by the NVS (NEB-Vagal-System).

## The vagus nerve

The lung is innervated mainly by the vagus nerve, which is involved in the pathogenesis of asthma, COPD, ARDS, lung cancer, and potentially the development of PF [4]. The nervous and immune systems interact via shared ligands and receptors. For example, neurons express pro-inflammatory cytokines [5] and immune cells express neural mediators and their receptors [6]. Stimulation of vagal afferents exerts anti-inflammatory effects via Acetylcholine (ACh) released by vagal efferents [7]. Electrical stimulation of vagal efferents during endotoxemia inhibits TNF-α synthesis and prevents endotoxemic shock [8]. During lung injury and inflammation, tachykinin levels in nociceptive afferents increase [9] and a variety of neural, epithelial, endothelial, and phagocytic cells produce inflammatory cytokines, mediators, neuropeptides and other substances, like reactive oxygen species [10,11]. These agents activate vagal sensory afferents [12] to cause local (e.g. release of neuropeptides) or central reflexes to stimulate ECM production via T-cells, macrophages and fibroblasts. Stimulation of the vagus nerve in patients with depression significantly increased levels of transforming growth factor beta (TGF-β) , a substance which promotes fibrosis [13], while vagotomy eliminated the drug-induced activation of TGF-β in cerebrospinal fluid [14]. ACh can also activate M receptors to promote fibroblast proliferation in the lung [15]. Additionally, maternal nicotine exposure increased levels of collagen mRNA in pulmonary fibroblasts via α-7nAChR [16]. In inflammatory bowel diseases, vagotomy promotes type-1 helper T ($Th_1$) cytokines but inhibits type-2 helper T ($Th_2$) cytokines. In addition, vagal denervation decreases collagen deposition in the lung (Trichrome stain and ELISA), pulmonary consolidation, and the Ashcroft score [17].

## The NEBs

Pulmonary Neuroendocrine Cells (PNECs) are widely distributed throughout the airway mucosa of mammals. They may aggregate to form NEBs, which are innervated by vagal, spinal, and parabronchial nerves [18]. NEBs are connected to the central nervous system via the sympathetic nerve and nociceptors (C fiber receptors and high threshold A delta fiber receptors) in the vagus nerve [19]. In addition, NEBs are in direct contact with sensory afferents that immune-reactive for substance P (SP) [18] and may cause neurogenic airway inflammation [19]. PNECs/NEBs can release many bioactive substances with growth factors and mitogenic properties, such as bombesin/

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**Received:** July 26, 2017; **Accepted:** August 24, 2017; **Published:** August 28, 2017

Gastrin-Releasing Peptide (GRP), Calcitonin Gene-Related Peptide (CGRP), and 5-hydroxytryptamine (5-HT) [20]. These substances play an important role in lung morphogenesis, which is why NEBs prevail in fetal and neonatal lungs [21]. Morphogenesis is a process much like tissue remodeling, which is an important step in PF. NEB secretory products, such as bombesin/GRP, may exert airway remodeling effects, including proliferation of fibroblasts, and epithelial and smooth muscle cells [22]. Bombesin additionally induces alveolar wall thickening and myofibroblast proliferation, as demonstrated by intraperitoneal bombesin injection in mice [23]. 5-HT stimulates collagen synthesis by fibroblasts, which can be attenuated by 5-HT antagonists [24]. Furthermore, NEBs are thought to be stem cell niches [25], protective microenvironments for stem cells. For example, after naphthalene-induced injury, airway epithelial cells regenerate from cells within NEBs. Abnormalities of PNECs/NEBs are found in many human diseases, including congenital pneumonia, bronchopulmonary dysplasia, Wilson-Mikity syndrome (WMS, pulmonary dysmaturity), congenital central hypoventilation syndrome, sudden infant death syndrome, neuro-endocrine hyperplasia of infancy, cystic fibrosis, asthma, and pulmonary hypertension [20]. For example, in bronchopulmonary dysplasia, a disease in which airway epithelium is repeatedly injured, significant hyperplasia of PNECs/NEBs occurs. Altogether, it is clear that NEBs are involved in neuroendocrine interaction and respond to lung injury. In bleomycin-induced PF mice, the number and size of NEBs increased significantly within two weeks, supporting the hypothesis that they play an important role in the pathogenesis of PF [26].

## Cytokines

Cytokines, chemokines, and their receptors are significantly up-regulated in patients with IPF [27]. For example, **TGF-β** activation plays a major role in myofibroblast transformation, and its overexpression leads to PF [28]. This process can be prevented by inactivating the TGF-β transgene [29]. Transient overexpression of Interleukin-1β (IL-1β) in lung epithelial cells causes acute inflammation and tissue destruction, followed by production of fibrogenic cytokines and interstitial fibrosis [30], whereas inhibition of IL-1β alleviates fibrosis [31]. Interleukin 4 (IL-4) is also an important molecule in fibrogenesis, since it acts on fibroblast receptors to regulate proliferation, collagen synthesis, and differentiation into myofibroblasts [32-34]. Expression of IL-4 receptors increases in bleomycin-induced lung injury [35]. TNF may also play a significant role in TGF-β production and PF [36], for example, bleomycin administration caused significantly less PF in TNF-deficient mice than in wild-type mice [37]. Vagotomy can also attenuate PF by suppressing the production of fibrogenic cytokines (TGF-β and IL-4), which stimulate fibroblast differentiation into myofibroblasts [17].

## Fibrogenic cells

In the adaptive immune response, various $CD4^+$ T cell subsets are distinct. For example, $CD4^+$ progenitor T cells can be further categorized into $Th_1$, $Th_2$ and regulatory T (Treg) cells. Up-regulation of $Th_2$ cytokines and down-regulation of Treg cytokines are associated with fibrosis. IPF is usually characterized by a shift from $Th_1$ to $Th_2$ cytokine expression [38]. Much like the $Th_1/Th_2$ dichotomy, there are also two major types of macrophages. $M_1$ macrophages are pro-inflammatory (via LPS, INF-γ, and $Th_1$ associated cytokines), whereas $M_2$ macrophages are pro-fibrotic (via glucocorticoids, TGF-β, and $Th_2$ associated cytokines) [39]. Neuropeptides, vasoactive intestinal polypeptide, and pituitary adenylate cyclase-activating polypeptide are all potent immune-modulators. They induce a switch from $Th_1$ to $Th_2$ immune responses, which then leads to the differentiation of monocytes into pro-fibrotic $M_2$ macrophages [39]. Modulation of macrophage phenotypes can prevent PF in bleomycin-induced or TGF-β-upregulated murine PF models [40]. For example, treatment with serum amyloid protein mitigates fibrosis by decreasing the number of $M_2$ macrophages and fibrocytes. Vagotomy also decreases the number of fibrogenic cells (myofibroblasts and $M_2$ macrophages) in the lung tissue and bronchoalveolar lavage fluid, supporting the hypothesis that the vagus nerve may promote the proliferation of fibrogenic cells [17].

## Conclusion

Neuro-immune interaction influences the pathophysiological processes of various diseases, yet, is still an evolving area of research. In this article, we have illustrated that the interaction occurs at different levels (systemic, cellular, and molecular) and provided evidence to support the hypothesis that the NVS plays an important role in the pathogenesis of PF. More specifically, PF develops as the number and size of NEBs, pro-fibrogenic cytokines, and cells also increase, as evidenced in mice intravenously injected with bleomycin. This pro-fibrotic process can be attenuated by interrupting the NVS. Further delineation of the underlying mechanisms will potentially lead to novel strategies to treat PF.

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