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Toll-like receptors in the pathogenesis of pulmonary fibrosis

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ABSTRACT

Pulmonary fibrosis (PF) constitutes the end stage of a broad range of heterogeneous interstitial lung diseases, characterized by the destruction of the pulmonary parenchyma, deposition of extracellular matrix and dramatic changes in the phenotype of both fibroblasts and alveolar epithelial cells. More than 200 causes of pulmonary fibrosis have been identified so far, yet the most common form is idiopathic pulmonary fibrosis (IPF). IPF is a lethal lung disorder of unknown etiology with a gradually increasing worldwide incidence and a median survival of 3–5 years from the time of diagnosis. Despite intense research efforts, the pathogenesis remains elusive and no effective treatment is available. Accumulating body of evidence suggests an abnormal wound healing response followed by extracellular matrix deposition, destruction of lung architecture, ultimately leading to respiratory failure. The contribution of immune system in lung fibrogenesis had been largely underscored due to the absence of response to immunosuppressive agents; however, the premise that lung fibrosis has an immunologic background has been recently revived. Toll-like receptors (TLRs) are pattern recognition receptors (PRRs), which link innate and adaptive immune response and regulate wound healing. TLRs promote tissue repair or fibrosis in many disease settings including lung fibrosis, albeit with profound differences depending on the cellular microenvironment. This review summarizes the current state of knowledge regarding the mechanistic implications between TLRs and lung fibrosis and highlights the therapeutic potential of targeting TLR signaling at the ligand or receptor level.

1. Introduction

Pulmonary fibrosis (PF) constitutes the end stage of a broad range of heterogeneous interstitial lung diseases, characterized by the destruction of the pulmonary parenchyma, deposition of extracellular matrix and dramatic changes in the phenotype of both fibroblasts and alveolar epithelial cells (Wuyts et al., 2013). More than 200 causes of pulmonary fibrosis have been identified so far including genetic disorders, autoimmune diseases, environmental and occupational exposures to toxins, drugs and radiation, yet the most common form is idiopathic pulmonary fibrosis (IPF) which is pathologically indistinguishable from other forms, especially at the later stages of the disease (Travis et al., 2013). IPF affects approximately 5 million people worldwide and despite extensive research efforts its pathogenesis remains elusive reflecting to a significant health burden and an unmet therapeutic need (Raghu et al., 2015). The median survival without transplant in IPF is close to 3 years making it the non-lung cancer disease with the gravest prognosis (Spagnolo et al., 2015). Current pathogenetic theories suggest that the fibrotic lung shares many common features with the aging lung including genomic instability, telomere attrition, cellular senescence, mitochondrial dysfunction and immune dysregulation (Blackwell et al., 2013; Selman et al., 2008). The evidence that several anti-inflammatory agents were proven to be ineffective in IPF clinical trials (Idiopathic Pulmonary Fibrosis Clinical Research et al., 2012; King et al., 2009) severely underscored the premise that inflammation is necessary for fibrotic development and progression (Tzouvelekis et al., 2015). Nonetheless, the role of immune system in the pathogenesis of lung fibrosis has been recently revived. Currently, there is abundant evidence supporting the role of both innate and adaptive immunity in disease pathogenesis both in the experimental model and human lung fibrosis (Gross and Hunninghake, 2001; Strieter, 2005). Toll-like receptors (TLRs) are pattern recognition receptors (PRRs), which link innate and adaptive immune system and can be considered as master regulators of the structural and functional integrity of the tissue (Schnare et al., 2001). While the impact of TLRs in sensing pathogens is well established, accumulating body of evidence supports the notion that TLRs may act as regulators of immune responses in sterile inflammatory disorders including

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chronic fibrotic disorders such as pulmonary fibrosis (Huebener and Schwabe, 2013). This review aims to summarize the current state of knowledge regarding the role of TLRs in the regulation of wound healing responses and lung fibrogenesis and highlights the therapeutic potential of targeting TLR signaling for the prevention or treatment of the maladaptive responses to chronic lung injury.

2. Toll-like receptors, signaling and wound healing

Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) that recognize discrete molecular components for pathogens, called pathogen-associated molecular patterns (PAMPs) as well as endogenous ligands, termed damage-associated molecular patterns (DAMPs) (Medzhitov et al., 1997). The TLR ligands are generally categorized into three types: proteins, nucleic acids and lipid-based elements derived from a wide variety of bacteria, viruses, fungi or damaged cells. To date, 10 functional TLRs (TLR1-TLR10) have been identified in humans and 12 in mice. They are either intracellular (TLR3, TLR7-9 or TLR11-13) or extracellular (TLR1, 2, TLR4-6 and TLR10) (Kawasaki and Kawai, 2014). TLRs can be also subdivided into two categories based on the relative similarity of the PAMPS recognized. For example TLR1, TLR2, and TLR6 recognize lipid, whereas TLR3 and TLR7-9 recognize nucleic acids. TLRs are type 1 integral transmembrane glycoproteins consisting of an ectodomain comprising leucine-rich repeats, a transmembrane domain and an intracellular domain (TIR) that is homologous to IL-1 receptor. Binding to distinct biochemical components TLRs become activated through recruitment and interaction of the TIR domain with specific adaptor proteins including myeloid differentiation factor 88 (MyD88), MyD88 adaptor like (MAL), TIR-domain-containing adaptor-inducing interferon-β (TRIF) and TRIF adaptor molecule (TRAF). MyD88 is the most common adaptor molecule involved in most TLR signaling, with the exception of TLR3, which uses TRIF/TRAM to activate the IFN pathway (Dasu and Isseroff, 2012). TLR4 has been shown to use all four described TIR-containing adaptors. This interaction couples downstream protein kinases including JNK and MAPK leading to activation of key transcription factors such as NF-κB and the interferon regulatory factor (IRF) and induction of type 1 IFN and other proinflammatory cytokines gene transcription to counter invading pathogens and regulate immune responses (Kawasaki and Kawai, 2014; Kluwe et al., 2009).

3. Toll-like receptors in chronic lung diseases

The respiratory tract is constantly accessible to environmental elements and therefore represents the first line of defense of the human body for inhaled environmental or infectious particles. To this end and to maintain tissue homeostasis and function, both structural and immune lung cells are equipped with diverse cell- and stimulus-specific TLRs that can initiate an appropriate immune response to endogenous or exogenous ligands (Lafferty et al., 2010). Abundant evidence supports the role of TLRs in lung remodeling following chronic lung injury and leading to several chronic lung diseases, including chronic obstructive pulmonary disease (COPD), asthma and pulmonary hypertension.

3.1. COPD

COPD represents a typical disease paradigm where the interplay between bacterial infections and host-immune response is fundamental for disease development and progression. TLR4 deficiency has been linked to spontaneous emphysema due to augmented generation of oxidants and aberrant elastolytic activity (Zhang et al., 2006). Translational relevance to these findings emerged from genotypic analysis of patients with COPD correlating TLR4 functional variants with either worse or favorable prognosis depending on TLR4 expression levels (Sabroe et al., 2004). Similarly, TLR9 transcription variants have been linked to increased COPD susceptibility (Pabst et al., 2013) and TLR2 expression has been found significantly downregulated in COPD macrophages (Droemann et al., 2005). The latter evidence underlines TLRs not only as promising therapeutic targets but also as reliable biomarkers of COPD progressiveness and treatment responsiveness (Bezemer et al., 2012; Mortaz et al., 2009; Pace et al., 2011).

3.2. Asthma

Based on the “hygiene hypothesis” the increased prevalence of asthma in the developed world over the past 50 years represents the result of a decreased load in infectious organisms and therefore activation of the immune system through TLRs (Strachan, 1989, 2000). An emerging body of evidence emphasizes the significant contribution of TLRs activation by either microorganisms, dietary lipids or damaged cells in regulating adaptive immunity that orchestrate the pathogenesis of asthma (Shi et al., 2006). Airway epithelial, dendritic, mast and mesenchymal cells as well as eosinophils represent the master mediators of TLR-associated aberrant immune responses leading to perpetuated Th2 inflammation, airway remodeling and constriction and consequently to airflow obstruction and decreased airway caliber (Phipps et al., 2007). Activation of TLRs by either bacterial (TLR2, TLR9) or viral (TLR3, TLR8) products has been proposed to enhance the release of thymic stromal lymphopoietin (TSLP), an IL-7-like cytokine, by airway epithelial cells than activates resident dendritic cells to prime Th2 immune responses (Han et al., 2012; Lee and Ziegler, 2007). Aberrant interactions of TLRs (TLR2, TLR3) with bacterial (peptidoglycan) or viral (rhinovirus) components has been shown to drive degranulation of mast cells and release of muscle-constricting mediators such as leukotrienes and histamine as well as increased production of Th2 cytokines, ultimately resulting in airway narrowing and sustained inflammation (Varadaradjalou et al., 2003). Possible dual roles of TLRs in asthma development and progression have been also proposed by showing that TLR2 and TLR4 are upregulated in blood cells of farmer's children compared to non-farmers' children rendering them resistant to atopy development (Lauener et al., 2002). Suppression of Th2 immune responses through induction of nitric oxide synthase activity by TLR4 activation has been suggested as a relevant mechanism (Rodriguez et al., 2003). In line with this, functional TLR variants have been associated with either increased or decreased asthma susceptibility (Kormann et al., 2008; Lazarus et al., 2004; Moller-Larsen et al., 2008). On the basis of the dual-role of TLRs, caution should be taken while designing TLR-based therapies for patients with asthma (Klaassen et al., 2013).

3.3. Pulmonary hypertension

TLRs have also been associated with pulmonary hypertension, a confounding factor of IPF mortality. Recent experimental data implicate TLR2, TLR3 and TLR4 in the pathogenesis of pulmonary hypertension. TLR2/NF-κB signaling mediates endothelial inflammation in pulmonary artery endothelial cells (Tan et al., 2014). TLR3 stimulation has been shown to induce the production of vasoconstrictive and pro-inflammatory agents including endothelin-1, IP10 and IL-8 in human pulmonary artery smooth muscle cells (George et al., 2012). TLR4 overexpression has been detected on monocytes of patients with primary pulmonary hypertension (Raychaudhuri et al., 2002). TLR4 knockout mice were significantly protected from hypoxia-induced pulmonary