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Expression of toll-like receptor 2 and 4 is increased in the respiratory epithelial cells of chronic idiopathic interstitial pneumonia patients

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Summary
Background: Idiopathic interstitial pneumonia (IIP) is characterized by chronic interstitial inflammation and fibrosis. Although mounting evidence has suggested that toll-like receptor (TLR) 2 and TLR4 are involved in the pathogenesis of non-infectious lung injury *in vitro* and in mouse models, their roles in human IIP remain unknown.
Methods: To address this issue, we investigated the expression patterns of TLR2 and TLR4 by immunohistochemistry in resected lung tissues from patients with usual interstitial pneumonia (UIP) or nonspecific interstitial pneumonia (NSIP).
Results: Type II pneumocytes, bronchial epithelial cells (BECs), and alveolar macrophages accounted for the majority of TLR2- and TLR4-expressing cells in both UIP and NSIP. The numbers of TLR2 and TLR4-positive respiratory epithelial (RE) cells, including type II pneumocytes and BECs, were significantly greater in UIP and NSIP than in the control. In particular, the numbers of TLR2-positive RE cells were much greater in UIP than in NSIP. The intensities of TLR2 and

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TLR4 expression in type II pneumocytes were also significantly stronger in UIP and NSIP than in the control. A comparison of the TLR expression patterns between the fibroblastic and fibrotic areas in UIP indicated that the numbers TLR2 and TLR4-positive RE cells were similar in fibroblastic areas, whereas the TLR2-positive RE cells outnumbered the TLR4-positive RE cells in the fibrotic areas.

Conclusions: This study demonstrates that RE cells over-express TLR2 and TLR4 in the lungs of IIP patients. These findings suggest that high expression of TLRs may contribute to the pathogenesis of human IIP.

Introduction

Chronic diffuse or interstitial lung disease (ILD) includes a spectrum of non-infectious inflammatory conditions that typically evolves over weeks, months, or years [1]. Idiopathic interstitial pneumonia (IIP) is a subtype of ILD characterized by interstitial inflammation and a variable degree of fibrosis. Usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP) are the major histologic patterns in human IIP. The most unique and diagnostic characteristics of UIP are heterogeneous appearances at a low magnification. Thus, areas of fibrosis with scarring and honeycomb changes are interspersed with areas of less-affected or nearly normal parenchyma [2]. In addition to the advanced fibrotic areas, scattered fibroblastic foci of proliferating fibroblasts or myofibroblasts are observed in UIP. In contrast, NSIP is characterized by the spatially and temporally homogeneous involvement of the pulmonary parenchyma with inflammatory cell infiltration and/or fibrosis in the interstitium [3]. In general, UIP is associated with the idiopathic pulmonary fibrosis (IPF) that is characterized by the worst prognosis, including the progressive destruction of the lung and the decline of pulmonary function [4]. Several hypotheses have been suggested to explain the mechanisms of pulmonary fibrosis, for example, the loss of integrity of the alveolar-capillary barrier basement membrane; cytokines, such as TGF-β; continuous stimulus of antigen; epithelial-to-mesenchymal transition; and recruitment of bone marrow-derived fibrogenic progenitor cells [5]. However, the pathogenesis of human IPF remains unknown.

Toll-like receptors (TLRs) are a family of pattern-recognition receptors that trigger innate immune responses that are involved in the first line of host defense against microbial infection. These receptors also modulate adaptive immune responses. In addition to sensing the pathogen-associated molecular patterns (PAMP), TLRs also recognize various host-derived endogenous ligands [i.e., damage-associated molecular patterns (DAMP)] associated with tissue injury. TLRs are, thus, involved in the regulation of inflammatory or reparative processes and the remodeling of tissue during non-infectious tissue injury. TLRs are expressed in non-hematopoietic cells, including endothelial and epithelial cells, as well as immune cells [6]. Their expression patterns and functions vary as a function of the cell types, tissues, and forms of infectious or non-infectious stimuli [7,8]. Among TLRs, TLR2 and TLR4 have been demonstrated to play crucial roles in regulating non-infectious lung injury, although the results have been inconsistent [7—12]. TLR2 and TLR4 on alveolar macrophage and lung epithelial cells recognize hyaluronan, an extracellular matrix degradation product generated during lung tissue injury. A study using both TLR2- and TLR4-deficient mice suggested that these receptors promote inflammatory response and recovery from acute lung injury [7,12]. TLR4 was reported by others to promote the resolution of inflammation and fibrosis after acute or chronic lung injury [10]. In contrast to TLR4, blocking TLR2 attenuated bleomycin-induced pulmonary fibrosis, which suggested that TLR2-mediated signals contributed to pulmonary fibrosis [11]. Moreover, we recently demonstrated that TLR2, expressed by respiratory epithelial (RE) cells rather than immune cells, promoted bleomycin-induced pulmonary fibrosis (BIPF) by increasing the producing IL-27 and chemokines by these cells [9]. These findings suggest that TLRs expressed on RE cells may play a crucial role in the regulation of human IIP. Therefore, a comprehensive investigation of the expression patterns of TLRs in the lung tissues from IIP patients should be helpful for understanding the TLR-mediated regulation of IIP. The cellular expression of TLRs in chronic human IIP has not previously been described, although studies using cell lines, bronchoalveolar lavage (BAL) fluid, or animal tissues have been reported. TLR2 expression was elevated in BAL fluid from IPF patients compared to control [13]. However, there have been no reports of the TLR expression patterns in individual cell types in lung tissues from patients with IPF. Therefore, we investigated the expression patterns of TLR2 and TLR4 using surgically resected lung tissues from UIP or NSIP patients in comparison with control tissues.

Materials and methods

Patient selection

We selected 65 patients who underwent lung wedge resections under the clinical and radiological suspicion of IPF or NSIP at Seoul National University Hospital between 2005 and 2011. Among them, 36 cases were histologically confirmed to be UIP, and 29 cases were diagnosed as NSIP based on the diagnostic guidelines [1,3,14]. In addition, normal or mildly inflamed lung parenchyma was obtained from 18 patients who underwent lobectomy for primary pulmonary adenocarcinomas, and control tissues were taken from regions at least 5 cm away from the tumors. The clinical data were obtained from the medical record, and are summarized in Table 1. This study followed the World