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TOLL LIKE RECEPTORS IN DISEASES OF THE LUNG

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Abstract

The lung is in continuous contact with a diverse array of infectious agents, foreign antigens, and host-derived danger signals. To sample this expansive internal and external milieu, both resident myeloid and stromal/structure cells of the lung express a full complement of toll like receptors (TLRs) which recognize pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs). TLRs play a vital role in immune host defense against bacterial, mycobacterial, fungal, and viral pathogens of the lung. Additionally, TLRs contribute to disease pathogenesis in non-infectious pulmonary disorders, including airways disease, acute lung injury, and interstitial lung disease. In this review, TLR biology in the context of experimental infectious and non-infectious lung disease is discussed, and correlates to human lung disease, including therapeutic implications of these findings, are defined.

Keywords

pathogen recognition receptors; immunity; PAMPs; DAMPs; lung

Introduction

The lung is uniquely positioned to interact continuously with the ambient external environment and the entire blood circulation. As a consequence, the lung is bombarded by an array of infectious agents, foreign antigens, and host-derived danger signals. To sample this expansive internal and external milieu, the lung expresses a complete repertoire of pathogen recognition receptors (PRRs) to recognize both exogenous pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs) [1]. Most prominent in this process are toll-like receptors (TLR), which are present both at the cell surface (e.g. TLR1-6, 10) and intracellularly within endosomes (e.g. TLR3, TLR7/8, TLR9). Alveolar macrophages represent the major resident macrophage population in the lung, and are the sentinel phagocytic cells to come in contact with foreign substances that reach the terminal airways and airspaces. These cells express all mammalian TLRs, most prominently TLR2, TLR3, TLR4, TLR5, and TLR6 [2]. Similarly, both plasmacytoid and myeloid dendritic cells (DC) are ideally situated within the respiratory epithelium and interstitium. While these cells express a multitude of TLRs, plasmacytoid DC preferentially express TLR7/8 and TLR9, whereas myeloid DC express robust quantities

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of TLR2, TLR3, and TLR4 and TLR9 [3, 4]. Finally, stromal/structural cells of the lung actively participate in TLR-mediated immune responses, including airway and alveolar epithelial cells, fibroblasts and pulmonary endothelium [5-7]. Expression of TLRs on myeloid and stromal cells can be substantially modified by both microbial and non-infectious insults.

TLRs in infectious challenge of the lung

Given the lung's repeated exposure to a diverse group of infectious microorganisms, TLRs play a critical role in host defense of the respiratory tract and alveolus. In addition to TLRs, lung cells express several cytosolic pathogen recognition receptors (PRRs), including nucleotide binding oligomerization domain (NOD)-like receptors (NLRs) and the RNA helicase retinoic acid-inducible gene I (RIG-I). Lung TLRs and putative ligands are summarized in Figure 1.

TLRs in lung host defense against extracellular and intracellular bacteria

Bacteria are frequent causes of serious lung infection. Gram-positive bacteria, including *Streptococcus pneumoniae* and *Staphylococcus aureus*, represent the most common causes of community-acquired and nosocomial pneumonia, respectively. Peptidoglycan and lipoteichoic acid are prominent cell wall components of most Gram-positive organisms, and serve to activate TLR2-mediated inflammatory cytokine and chemokine release by alveolar macrophages in-vitro [8, 9]. However, while TLR2-deficient mice display a modest impairment in early inflammatory responses during pneumococcal pneumonia, clearance of bacteria and survival in these mice is unaltered [9]. Similarly, TLR4 mediates inflammatory cytokine release in response to the pneumococcal toxin hemolysin, but plays no role in lung clearance of *S. pneumoniae*. TLR9 is a MyD88-dependent intracellular TLR that recognizes unmethylated CpG motifs found in abundance in microbial, but not human DNA. Importantly, TLR9 deficient mice are more susceptible to intrapulmonary challenge with *S. pneumoniae*, which occurs in association with reduced alveolar macrophage activation and impaired lung bacterial clearance [10]. These findings suggest that TLR9, rather than TLR2 or 4, may be a major mediator of protective innate immunity during pneumococcal pneumonia. Although TLR2 has been shown to mediate inflammatory responses to the staphylococcal toxin Panton-Valentine Leukocidin, neither TLR2, TLR4, nor MyD88 is required for effective anti-staphylococcal host immunity during respiratory infection [11, 12]. Recently, the NLRs NOD1 and NOD2, which recognize the peptidoglycan component muramyl dipeptide (MDP), have been shown to be important in inflammatory cytokine release and bacterial eradication in a murine *S. aureus* skin infection model [13, 14]. Importantly, mice deficient in RIP2, the shared NOD1/2 adaptor molecule, are considerably more susceptible to intrapulmonary challenge with *S. aureus* than wildtype mice, an effect which is dependent on downstream activation of inflammasome-caspase-1-dependent IL-1 β release (unpublished observations, J. Deng). These later observations suggest that NLRs, rather than TLRs, are the predominant contributors to anti-staphylococcal immunity in the lung.

In contrast to responses to Gram-positive bacterial pathogens, multiple TLRs participate in lung host immunity against Gram-negative bacteria. For example, TLR4 recognizes the lipid A moiety of LPS, and is the major TLR mediating early innate responses and clearance of non-flagellated Gram-negative organisms, including *Klebsiella pneumoniae* and *Hemophilus influenza* [13-15]. Both MyD88- and TIR domain-containing adaptor inducing IFN- β (TRIF)-dependent TLR4 pathways contribute to anti-*Klebsiella* responses, and requires TLR4 expression by bone marrow-derived cells rather than structural cells [16-19]. In addition, mice deficient in TLR9 display impaired dendritic cell mediated responses

during experimental *Klebsiella pneumoniae*, culminating in reduced lung bacterial clearance and decreased survival [4]. Innate responses to the flagellated extracellular bacteria *Pseudomonas aeruginosa* are mediated by several MyD88-dependent TLRs, predominantly TLR4 and TLR5 [11, 20, 21]. For instance, TLR4 or TLR5 single-deficient mice are not hyper-susceptible to intrapulmonary *P. aeruginosa* challenge, whereas mice doubly-deficient in TLR4 and TLR5 (TLR4/5^{-/-}) are considerably more susceptible than wildtype or single-deficient mice, suggesting a redundant role for TLR4 and TLR5 in anti-pseudomonal immunity [21]. Interestingly, both bone marrow-derived and stromal cells contribute to MyD88-dependent innate responses to *P. aeruginosa* in the lung [20].

Toll like receptors also participate in host responses to intracellular bacterial pathogens of the lung. *Legionella pneumophila* is a flagellated, obligate intracellular Gram-negative pathogen that causes severe pneumonia in both immunocompromised and immunocompetent hosts. In comparison to LPS from other Gram-negative bacteria, the lipid A component of *L. pneumophila* LPS preferentially activates TLR2 as opposed to TLR4. In vivo, TLR2^{-/-}, but not TLR4^{-/-} mice are moderately hyper-susceptible to *Legionella pneumoniae* [22]. Other MyD88-dependent TLRs involved include TLR5, which contributes to early influx of PMN and TNF- α production, and TLR9, which is required for mobilization of DC and classical rather than alternative activation of lung macrophages [23-25]. In addition, NLRs are important contributors to immunity against *L. pneumophila*. As an example, the NLR-associated proteins NAIP5 and IPAF are intracellular sensors of *Legionella* flagellin, and macrophages from mice with defects in NAIP5 dependent signaling are highly permissive for intracellular growth of *L. pneumophila* [26]. *Chlamydia pneumoniae* is another obligate intracellular bacterial that activates multiple TLR and NLR pathways. Chlamydial heat shock protein (HSP)-60 is recognized by TLR4, whereas cell wall peptidoglycans are recognized by TLR2 [27]. In vivo, defects in early inflammation and late bacterial clearance are observed in TLR2^{-/-}, but not TLR4^{-/-} mice, whereas a marked defect in clearance and increased mortality is observed in TLR2/4 double deficient mice, indicating some cooperativity in TLR2 and TLR4 mediated responses [28]. Increased susceptibility to Chlamydial pneumonia has recently been reported in NOD-1^{-/-}, NOD-2^{-/-}, and RIP2^{-/-} mice, which was linked to impaired macrophage effector responses, evidence that supports a meaningful role for NLRs in *C. pneumoniae* respiratory infections [29]. Like host innate responses to *Chlamydia*, TLR2 is required for effective clearance of *Mycoplasma pneumoniae* [30].

Clinical observations made in patients with certain TLR genetic polymorphisms generally support findings obtained in animal models. For example, critically ill patients with simultaneous polymorphisms for TLR4 and the TLR4 adaptor protein TIRAP/Mal, or homozygous for the TIRAP/Mal polymorphism, display reduced monocyte cytokine release ex-vivo and are at higher risk for severe infections [31]. Moreover, a reduced function TLR5 polymorphism, occurring in approximately 10% of the general population, has been linked to increased susceptibility to pneumonia caused by *L. pneumophila* [32].

TLRs in lung host defense against *Mycobacterium tuberculosis*

Host defense against *Mycobacterium tuberculosis* (Mtb) requires both a vigorous innate and cellular immune response. Multiple mycobacterial components activate TLR2 and/or TLR4 signaling cascades, including the glycolipid phosphatidylinositol mannoside, lipoarabinomannan (LAM), and mycobacterial lipoproteins [33]. In vivo, TLR2^{-/-} mice infected with aerosolized Mtb form granulomas poorly and are unable to control infection. Similarly, mice with defective TLR4 signaling succumb to infection earlier than wildtype mice, although the defect in clearance is not as great as that observed in TLR2^{-/-} mice. TLR2/4 double mutant mice are more susceptible than single mutant mice, whereas mice

deficient in MyD88 die quickly of acute necrotizing pneumonia due to uncontrolled infection [34]. These studies indicate both cooperative interactions of TLR2 and TLR4, as highlight the importance of other yet to be defined MyD88-dependent TLRs. A possible candidate is TLR9, as mice deficient in TLR9 are unable to form granuloma in responses to several microbial antigens, including mycobacterial PPD [35]. Intracellular Mtb infection in macrophages also promotes activation of the NOD2/RIP2 pathway, leading to induction of type I interferon production [36]. The functional significance of the NOD2/RIP2 pathway in pulmonary Mtb infection in-vivo remains to be clarified.

TLRs in lung host defense against fungi

Fungal organisms present an array of PAMPs that vary considerably depending upon the fungal species, the morphotype of the fungal cells, and the site of infection. For example, cell walls of fungal yeast or spores contain complex sugar polymers which are recognized by TLR2 in combination with Dectin-1 (β -glucan), TLR4 (mannose, cryptococcal glucuronoxylomannan), TLR2/6 heterodimers (zymosan), and TLR9 (DNA from *Candida* and *Cryptococcus* spp.) [37, 38]. In general, inflammatory cell influx, cytokine/chemokine production, and clearance is dramatically impaired in MyD88^{-/-} mice administered either systemic or intrapulmonary fungal organisms, including *C. albicans*, *Aspergillus fumigatus*, and *C. neoformans* [37-39]. Of the MyD88-dependent TLRs, TLR4 is primarily responsible for chemokine-dependent clearance during systemic *C. albicans* infection, whereas TLR2 and TLR9 were required for optimal TNF- α and IL-12 production, respectively, but are dispensable for candidal clearance [40, 41]. Likewise, although both TLR4 and TLR2 contributed to early PMN influx in response to intrapulmonary *A. fumigatus* spores, only mice deficient in TLR4, but not TLR2 or TLR9, showed defects in fungal clearance [40]. Interestingly, only the spore form of *A. fumigatus* was capable of activating TLR4, whereas hyphal forms were not recognized by this TLR [42]. By comparison, TLR2 appears to be the predominant TLR responsible for clearance of *C. neoformans* in a systemic infection model, as TLR2^{-/-}, but not TLR4^{-/-} mice were hyper-susceptible, which occurred in association with reduced expression of relevant type 1 cytokines [39]. We have recently demonstrated an important role for TLR9 in pulmonary cryptococcal infection, as infected TLR9^{-/-} mice displayed skewing toward a detrimental Th2 cytokine response, rather than a protective Th1 response observed in infected wildtype mice [43]. Finally, although TLR4 is responsible for a portion of the innate immune response to *Pneumocystis jirovecii* (formally *P. carinii*), as evidenced by reduced cytokine production in TLR4^{-/-} mice [44], TLR2 plays a fundamental role in the regulation of inflammatory responses and microbial clearance in mice [45].

TLRs in host defense against respiratory viruses

Viral infection of the respiratory tract is common, and TLRs play a crucial role in both viral clearance and disease pathogenesis during infection. Several intracellular RNA-sensing TLRs are particularly prominent, including TLR7 and TLR8, which recognize single stranded (ss) RNA present in viruses such as influenza A virus (IAV); and TLR3, which recognizes double stranded (ds) RNA found in respiratory viruses such as respiratory syncytial virus (RSV) or presumably during viral replication of ssRNA viruses. The RNA helicase RIG-I is a dual system to sense and respond to intracellular dsRNA [46-48]. During IAV infection, both TLR7 and RIG-I are required for maximal antiviral IFN- α/β responses, with TLR7 mediating IFN production from plasmacytoid DC, and RIG-I necessary for conventional DC IFN responses. In general, maximal induction of innate cytokines and viral clearance during IAV infection is dependent on both MyD88 dependent TLRs (presumably TLR7/8) and RIG-I, whereas adaptive responses require MyD88 alone [46-51]. By comparison, TLR3 appears to mediate many of the detrimental host effects during IAV

infection, as TLR3^{-/-} mice have modest impairment in viral clearance, but decreased inflammatory cytokine/chemokine expression, CD8⁺ T cell influx, and reduced lung injury, resulting in improved survival [52]. TLR3-mediated effects are believed to be mediated by lung epithelial cells, which highly express this TLR [7]. All three viral sensors (TLR3, TLR7/8, and RIG-I) contribute to innate cytokine and interferon responses during RSV infection, although their individual contribution to clearance and pathology has not been completely defined [46, 50]. Finally, certain viruses (IAV, RSV) encode glycoproteins that activate both TLR2/6 and TLR4, which also contribute to inflammatory cytokine/chemokine production, PMN influx, and viral clearance [53].

TLRs in Non-infectious Lung Disease

In addition to their essential function in infection, emerging evidence exists to implicate a vital role for TLRs in non-infectious disease processes involving the lung, including airway disease, acute lung injury (ALI), and interstitial lung disease (ILD).

TLRs in Airway Disease

Multiple TLRs have been implicated in the regulation of allergic airway inflammation. Airway administration of large doses of LPS can promote Th1 cytokine responses that dampen allergic airway inflammation. However, LPS in low concentrations (such as that present with common allergens such as house dust mite allergen) can potentiate asthma in a fashion that is dependent on TLR4. In a murine asthma model, i.n. administration of ovalbumin containing low concentrations of LPS induced an inflammatory response by eosinophils and Th2-type T cells [54]. This allergic response was considerably blunted in TLR4-deficient mice. Moreover, Hollingsworth and colleagues showed that wildtype mice exposed to low-dose LPS developed exuberant airflow obstruction in response to methacholine bronchoprovocation, as compared with TLR4 knockout mice [55]. Recent data suggests that TLR4-mediated cytokine responses by airway stromal cells, rather than bone marrow derived cells, promotes the maturation of Th2-polarized lung DC (DC₂) [56]. These findings suggest that LPS in “physiological” doses can potentiate allergic responses to inhaled antigens. Like TLR4, stimulation of TLR3 via virus-derived dsDNA results in Th2 cytokine production and influx of eosinophils, myeloid DCs and inflammatory T cells in TRIF-dependent manner [57]. Functionally, this correlates with increased airway hyperreactivity. In contrast to TLR3 and -4, other TLRs have been shown to suppress asthmatic inflammation and therefore may represent potential therapeutic targets. For instance, TLR7 agonists act as potent bronchodilators in animal models of allergic asthma, primarily by suppressing Th2 responses [58-60]. Similarly, TLR9 agonists (e.g. CpG) can promote DC polarization toward DC₁ rather than DC₂ to allergens [61], which may be due, in part, to induction of CD4⁺CD25⁺regulatory T cells (T_{reg}), which are known to inhibit allergic inflammation [62]. In animal models, the use of CpG oligodeoxynucleotides (CpG-ODN) as TLR9 agonists with or without concurrent immunotherapy decreases airway inflammation, bronchial hyperreactivity, and IgE levels [63, 64]. Although human studies of CpG-ODN administration for treatment of allergic rhinitis have been promising, a large trial of nebulized CpG-ODN in asthma patients failed to show any significant difference in FEV₁, sputum eosinophil count, or Th2 cytokine gene expression [65]. Nonetheless, the evidence of benefit of CpG ODN in humans with other allergic and atopic diseases compels further human trials.

Consistent with animal models, polymorphisms in a variety of TLRs may influence the development of allergic asthma and atopy in humans [66]. For instance, some, but not all investigators have observed that the TLR4 polymorphism, Arg299Gly is linked to an increased prevalence of asthma and reduced LPS responsiveness [67]. A polymorphism of

CD14, a membrane-bound protein that facilitates LPS presentation to TLR4, has been associated with allergic and atopic diseases. In addition to TLR4, polymorphisms of TLRs 2, and 6-11 have been associated with an increased likelihood of atopic asthma (Table 1).

As with asthma, links between TLRs and chronic obstructive pulmonary disease (COPD) have been proposed. In particular, TLR4 appears to play a complex and at times paradoxical role in COPD. Activation of TLR4 by cigarette smoke results in increased BAL levels of inflammatory cytokines, including IL-1 [68] and IL-8 [69]. Moreover, an increase in TLR4-positive cells has been found in the BAL fluid of COPD patients, which correlates with increased BAL neutrophils and reduced neutrophil apoptosis [70]. Finally, cigarette smoke generates reactive oxygen species in the lung, which can promote lung injury in a TLR4-dependent fashion [71, 72]. Conversely, other studies in both animals and humans suggest the TLR4 may function to prevent the development of emphysema. TLR4-deficient mice develop pulmonary emphysema with age due to an upregulation of Nox3, which in turn generates an increase in reactive oxygen species [73]. In smokers, the presence of the reduced function TLR4 polymorphism, TLR4-T399I, is associated with an increased likelihood of developing COPD [74]. Moreover, reduced expression of TLR4 in the nasal epithelium has been observed in patients with severe COPD, as compared to those with less severe disease [75]. These investigators went on to show that exposure to cigarette smoke extract abrogated TLR4 expression in human airway epithelial cells in a dose-dependent manner. Collectively, these studies suggest that while TLR4 may contribute to airways inflammation in COPD, the absence or reduced expression of TLR4 may promote abnormal breakdown of alveolar architectures, perhaps due to the loss of pro-survival effects of TLR4 signaling on alveolar epithelium.

Patients with COPD are highly susceptible to recurrent viral and bacterial respiratory infections, and these infections worsen COPD severity. Mechanisms accounting for dysregulated antimicrobial and inflammatory TLR responses in these patients have not been clearly defined [76]. Stimulation of TLR9 induces expression of a variety of host defense genes, including IFN- α/β required for antimicrobial immunity. However, exposure of cells to cigarette smoke extract suppresses this effect *in vitro* [77]. Paradoxically, cigarette smoke potentiates TLR9-mediated IL-8 expression and neutrophil influx, suggesting that ongoing smoking may not only impair airway host defenses, but also potentiate detrimental airway inflammation in response to infection.

Cystic Fibrosis (CF) is a common genetic disorder characterized by chronic airway inflammation and persistent/recurrent infections due primarily to Gram-negative bacteria such as *P. aeruginosa*. It has been speculated that alterations in TLR pathways may increase the likelihood of chronic bacterial colonization. In support of this notion, TLR4 expression is diminished in CF bronchial epithelial cells, resulting in reduced TLR4 signaling in response to LPS *in vitro* [78]. Likewise, Hauber and associates found a decreased number of TLR4-positive airway epithelial cells in CF patients compared to controls [79], whereas no statistically significant difference was noted in the number TLR2-positive cells. This suggests that the lack of TLR4-expressing cells at the epithelial interface contributes to the impaired host response to LPS. While some studies have replicated these results [80], others do not find differences in TLR4 expression and signaling in CF patients [81]. As previously discussed, bacterial flagellin is the primary agonist for TLR5 and is abundantly expressed by *P. aeruginosa*. In contrast to TLR4, TLR5 expression is up-regulated on airway neutrophils in CF patients [82], and exposure to flagellin results in increased pro-inflammatory cytokine production in patients with CF [83]. These findings raise the possibility that TLR5 may contribute, in part, to deleterious inflammatory responses that characterize the CF airway.

TLRs in acute lung injury

Acute lung injury (ALI) is a clinical syndrome characterized by injury to the alveolar-capillary membrane and non-cardiogenic pulmonary edema, culminating in refractory hypoxemia and high mortality. There are a variety of causes of ALI, including infectious pneumonia, sepsis, and non-infectious lung insults. In animal models, TLRs have been linked to both the initiation and immunomodulation of ALI. Ample data indicates that TLR2 and TLR4 promote lung inflammation in response to both infectious (e.g. LPS, whole bacteria) and non-microbial (e.g. bleomycin, ozone) stimuli [1, 84]. TLR4 activation can also promote lung injury in response to ozone exposure [85]. However, activation of TLR4 alone or in conjunction with TLR2 has been shown to reduce lung injury in other models of ALI, due in part to promotion of pro-survival effects on alveolar epithelium. For example, the i.t. administration of bleomycin to wildtype mice resulted in a prominent alveolar inflammatory response, which was substantially reduced in TLR2/TLR4 double-knockout mice. Despite reduced inflammation, there was more severe disruption of the alveolar capillary membrane, as manifest by enhanced permeability and alveolar epithelial cell (AEC) apoptosis, as well as increased mortality in TLR2/TLR4 mutant mice [86]. In this model, the extracellular matrix component hyaluronan was responsible for activating TLR2 in conjunction with TLR4, and lung epithelial cell specific expression of hyaluronan protected against lung injury. Mice deficient in TLR4 are also more susceptible to hyperoxia-induced lung injury due to increased apoptosis of both pulmonary endothelial and epithelial cells, an effect attributable to reduced expression of the cytoprotective gene product heme oxygenase-1 [72, 84]. We have recently observed increased lung injury and AEC apoptosis in mice with defective TLR4 signaling during experimental Gram-negative pneumonia, which occurred in association with reduced lung expression of GM-CSF and was prevented by reconstitution of this cytokine in TLR4 mutant mice during pneumonia (U. Bhan, submitted). Collectively, these studies indicate a vital protective role for TLR4 and TLR2 in both infectious and non-infectious lung injury.

As previously mentioned, hyaluronan fragments and other matrix components function as TLR2/4 agonists in bleomycin and possibly hyperoxia induced lung injury. Another candidate TLR2/4 agonist in ALI is high mobility group box-1 (HMGB-1). HMGB-1 is a nuclear protein which regulates gene transcription, but when passively released into the extracellular environment from dead or dying cells can facilitate TLR2 and TLR4 activation by bridging stereotypic interactions between these TLRs and their respective PAMPs and DAMPs [87, 88]. Conversely, endogenous factors uniquely present in the lung can block TLR interactions with agonists. Specifically, surfactant protein A (SP-A) can bind to and block TLR2 and TLR4 responses, which modify TLR-mediated inflammatory and cell survival responses [89, 90]. Hence, SP-A may function to dampen injurious TLR-mediated inflammatory responses within the lung.

In comparison to protective roles for TLR2 and TLR4 in several lung injury models, TLR3 appears to promote deleterious lung inflammation and injury during hyperoxia-induced ALI [91]. AEC abundantly express TLR3, which is further upregulated during hyperoxia in-vitro. Interestingly, TLR3 deficient AEC were protected from hyperoxia induced apoptosis in-vitro, and TLR3^{-/-} mice developed less lung injury and prolonged survival when compared to their wildtype counterparts. Consistent with animal models, increased expression of TLR3 has been observed in patients with ALI/ARDS, with expression localized primarily to pulmonary epithelium.

TLRs in Interstitial Lung Disease

Interstitial lung diseases (ILD) are a heterogeneous group of disorders with the common end result of variable amounts of lung parenchymal inflammation and fibrosis. Many of these diseases are idiopathic in etiology, although there is some evidence to support the notion that respiratory infections, particularly viruses, contribute to tissue injury that triggers an exaggerated wound healing and repair response mediated by fibroblasts and myofibroblasts [92]. Experimental findings in animal models suggest that TLRs may initiate or modify the fibroproliferative response to fibrogenic stimuli. As discussed previously, TLR4 in conjunction with TLR2, appears to suppress fibrogenesis in i.t. bleomycin challenged animals, due primarily preventing epithelial injury. Similarly, Paun and colleagues found enhanced radiation-induced lung fibrosis in TLR2/TLR4 double-knockout mice, but not in either TLR2 or TLR4 single-knockout mice, consistent with a protective role for these TLRs in combination [93]. The ligand(s) responsible for TLR2 and 4 activation in this study were not characterized, but thought to be mediated by matrix components.

Conversely, studies in humans suggest that certain TLRs are associated with and may promote dysregulated fibrotic responses. As an example, enhanced expression of TLR9 has been described in surgical lung biopsies of patients with both usual interstitial pneumonitis/idiopathic pulmonary fibrosis (UIP/IPF) and non-specific interstitial pneumonitis (NSIP) [94]. TLR9 localized to the alveolar epithelium and lung interstitium. Furthermore, treatment of UIP/IPF fibroblasts with the TLR9 agonist CpG-ODN promoted myofibroblast differentiation, but failed to do so in normal primary fibroblasts. Importantly, TLR9 expression was greater in lung biopsies of UIP/IPF patients prone to rapid progression of fibrosis, as compared with those who progress slowly [95]. Taken together, these studies highlight a key role for TLR9 in the pathogenesis of at least some forms of fibrotic ILD. In addition to TLR9, increased levels of TLR3 have been described in BAL fluid of patients with UIP/IPF and connective tissue disease-associated pulmonary fibrosis, as compared with sarcoidosis patients [96]. The finding of increased TLR3 is of some importance, as treatment of fibroblasts with the TLR3 agonist Poly(I:C) has recently been shown to promote fibroblast-to-myofibroblast differentiation and matrix production [97]. There was also increased numbers of TLR7 and TLR9-positive cells in the BAL fluid of both UIP/IPF and sarcoidosis patients. The functional significance of TLR3, TLR7, and TLR9 as pathogenic mediators in experimental or clinical fibrotic lung disease requires further study.

Sarcoidosis is a granulomatous lung disease characterized by a predominant Th1 cellular response and often culminating in lung fibrosis. No ideal animal models exist, therefore there is limited data in experimental models to implicate TLRs in disease pathogenesis. In a murine model of Th1-mediated granulomatous responses to PPD, mice deficient in TLR9 were unable to form pulmonary granulomas, due largely to impaired DC-driven Th1 and Th17 cytokine responses [35]. Conversely, pulmonary granulomas are more extensive in TLR9^{-/-} mice in response to the Th2 antigen *Schistosoma mansoni*, suggesting a predominant Th2 response in the absence of TLR9 [98]. In human studies, Wikén and associated reported increased expression of TLR2 and TLR4 on peripheral blood monocytes of sarcoidosis patients, and increased proinflammatory cytokine production upon co-stimulation of TLR2 and NOD2 [99]. However, these investigators also found reduced TLR2 expression on cultured BAL cells from sarcoidosis patients, without differences in TLR4 or TLR9 expression [100]. Investigations of genetic mutations of TLRs and sarcoidosis susceptibility have produced conflicting results. Pabst and associates found an association between the TLR4 polymorphisms, Asp299Gly and Thr399Ile, and increased likelihood of sarcoidosis in a German population [101]. A follow up study could not confirm the association with these specific polymorphisms, but did find a significant linkage between markers on the TLR4 gene locus and sarcoidosis [102]. Still other studies have failed to

confirm the associations with Asp 299Gly or Thr399Ile in either Greek [103] or Dutch [104] populations. Likewise, no associations with TLR9 polymorphisms and sarcoidosis have been discovered, although decreased IFN γ production has been seen in response to TLR9 stimulation in peripheral blood mononuclear cells of sarcoidosis patients [105]. While altered TLR function and/or expression may contribute to disease pathogenesis in sarcoidosis, there is a lack of compelling evidence to date that this is due to differences in TLR mutations.

Hypersensitivity pneumonitis (HP) is a lung disease characterized by non-necrotizing granulomatous inflammation and interstitial mononuclear infiltration, most notably CD8+ T cells [106]. The Th1 cytokine IFN γ , produced by T cells, NKT cells, and neutrophils is important in the establishment of granulomatous inflammation in HP [107, 108]. In an animal model of HP induced by *Saccharopolyspora rectivirgula* (the causative agent of farmer's lung) the early influx of neutrophils to the lung required MyD88, implicating a role for one or more of the MyD88-dependent TLRs [109]. In addition to Th1-type cytokines, recent studies have identified the importance of Th17 immune responses in the development of HP [110, 111]. Interestingly, TLR6 signaling is required for IL-17A expression in the *S. rectivirgula*-induced HP model [112]. Furthermore, research performed in our laboratory has identified a dominant role for TLR9 in the formulation of both Th1 and Th17 cytokine responses in a murine model of *Stachybotrys chartarum*-induced HP (unpublished data, U. Bhan). Although the vast majority of evidence suggests that Th1 and Th17 responses are critical for the initiation of the granulomatous inflammation seen in HP, there is also data to support a role for Th2 responses in the maintenance of inflammation and the development of fibrosis in later stages of HP [113, 114]. TLRs involved in these later stages of HP, or in human HP have not been identified.

Conclusions

Toll like receptors are integral components of lung immune function during health and disease. Their fundamental role in the initiation and propagation of innate and acquired immune responses to microbial invasion is well known. There is novel and exciting data emerging that clearly implicates a prominent role for TLRs in the pathogenesis of acute and chronic pulmonary diseases. While more research is necessary to more completely understand the nature of TLRs and ligands involved, cell specificity of TLR responses, and regulation of downstream TLR signal transduction pathways, this family of PRR may represent a promising target for therapeutic intervention in the treatment of acute and chronic lung disease.

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Figure 1.
Schematic depicting TLRs in the lung and their respective PAMPs and DAMPs.

Table 1

Selected TLR polymorphisms and their relationship with lung diseases

Receptor	Allele	Association
Pneumonia		
TLR4	Asp ²⁹⁹ Gly	- Associated with increased mycobacterial burden and
	Thr ³⁹⁹ Ile	more advanced disease in tuberculosis patients [115] -Associated with increased severity of RSV infection [116]
TLR5	TLR5 ^{392STOP}	- Increased susceptibility to <i>Legionella</i> pneumonia [25]
Asthma		
TLR2	rs4696480	- Reduced risk of hay fever and allergic asthma [67]
TLR4	Asp ²⁹⁹ Gly	- Increased risk of asthma [117]
		- No association with asthma [118]
TLR7	rs5743781	- Increased risk of asthma [67]
TLR8	rs5744077	- Increased risk of asthma [67]
TLR10	rs11096956	- Increased risk of asthma [67]
	rs4129009	
COPD		
TLR4	Asp ²⁹⁹ Gly	- Increased likelihood of COPD [74]
Sarcoidosis		
TLR4	Asp ²⁹⁹ Gly	- Associated with development of chronic progressive
	Thr ³⁹⁹ Ile	disease in sarcoidosis [101] - No association with sarcoidosis [102-104]
TLR9	rs187084	- No association with sarcoidosis [105]
	rs5743839	
	rs352139	
	rs352140	