Toll-Like Receptor 7-Targeted Therapy in Respiratory Disease

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Summary
Allergic asthma and allergic rhinitis are inflammatory diseases of the respiratory tract characterized by an excessive type-2 T helper cell (Th2) immune response. Toll-like receptor 7 (TLR7) is a single-stranded viral RNA receptor expressed in the airway that initiates a Th1 immune response and has garnered interest as a novel therapeutic target for treatment of allergic airway diseases. In animal models, synthetic TLR7 agonists reduce airway hyperreactivity, eosinophilic inflammation, and airway remodeling while decreasing Th2-associated cytokines. Furthermore, activation of TLR7 rapidly relaxes airway smooth muscle via production of nitric oxide. Thus, TLR7 has dual bronchodilator and anti-inflammatory effects. Two TLR7 ligands with promising pharmacologic profiles have entered clinical trials for the treatment of allergic rhinitis. Moreover, TLR7 agonists enhance influenza vaccine efficacy and also reduce viral titers when given during an active airway infection. In this review, we examine the current data supporting TLR7 as a therapeutic target in allergic respiratory diseases.

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Introduction
Human airways are repeatedly exposed to invading pathogens. To combat these threats, the airways mount a variety of innate and adaptive immune responses. Microbes are first detected in the airway by several different classes of pattern recognition receptors, one of which is Toll-like receptors (TLRs). Ten different TLRs have been identified in humans, each recognizing different viral, bacterial, fungal or protozoan microbial ligands. TLR ligation triggers the production of pro-inflammatory cytokines and interferons. In this review we focus on TLR7, first recognized as a receptor for viral single-stranded RNA. We examine its potential as a therapeutic immunomodulatory target in asthma, its recently identified function as a bronchodilator, and its role in vaccine development.

TLR7 Expression, Signaling and Ligands

TLR7 is located in endosomes [1] and recognizes single-stranded viral RNA [2, 3]. Viral entry into endosomes occurs via endocytosis and autophagy [4]. Plasmacytoid dendritic cells constitutively express high levels of TLR7 and are the primary source of interferon alpha (IFN-α) following TLR7 activation [5]. B cells express low levels of TLR7 [6] and undergo proliferation and class-switching in response to TLR7 agonists [7]. Monocytes [8], eosinophils [9], NK cells [10], CD8+ T cells [11], and CD4+ T cells [12] also express TLR7. Activation of TLR7 in leukocytes initiates a MyD88-NFκB signaling cascade and robust production of type-1 T helper cell(Th1)-related antiviral and pro-inflammatory cytokines, including IFN-α, tumor necrosis factor alpha (TNF-α), interleukin 12 (IL-12), and IL-6 [2, 3].

TLR7 also expressed by airway epithelial cells in a peri-nuclear distribution [13], and they release IFN-β and IFN-γ when exposed to the TLR7 agonist resiquimod [14]. Airway nerves express TLR7; however, the localization within neurons is distinct from other cell types. TLR7 is expressed both within the sensory nerve dorsal root ganglion cell body [15, 16] and along parasympathetic and sensory nerve axons [17] (fig. 1). TLR7 has also recently been described on the cell surface of B cells [18], and it is likely that axons express TLR7 on the cell surface as well. Stimulation of neu-
Neuronal TLR7 triggers a MyD88-independent pathway and subsequent activation of nociceptors [19]. Neuronal TLR7 activation also leads to the release of nitric oxide (see section below). It is disputed whether or not airway smooth muscle express TLR7 [17, 20, 21]; however, the majority of studies suggest airway smooth muscle does not express TLR7.

TLR7 classically recognizes single-stranded, GU-rich viral RNA. In addition to viral RNA, Hornung et al. [22] showed that some siRNAs activate TLR7, which may complicate the interpretation of studies using siRNA. Endogenous miRNAs such as Let7b [19], Let7c and miR21 [23] also activate TLR7. The ability to distinguish self-versus non-self-RNAs may hinge on endosomal localization of TLR7 [24] as well as structural features of the specific RNA [25–27]. A host of synthetic TLR7 ligands also exist. The antiviral and immunomodulating properties of imidazoquinolines were therapeutically recognized before their TLR7 agonist properties were discovered [28]. Imiquimod (R837, S26308) is a TLR7 agonist approved for treatment of anal and genital warts, actinic keratosis, and basal cell carcinoma [29]. Resiquimod (R848, S28463) is a derivative of imiquimod that activates both TLR7 and TLR8 (another viral single-stranded RNA receptor) [30] and more potently induces TNF-α and IL-12 secretion [29] when compared with imiquimod. Gardiquimod [31], 3M-019 [32], CL097 [33], S-27609 [34], MEDI9197 (clinicaltrials.gov NCT02556463), and 852A [35] are also imidazoquinoline TLR7 agonists.
agonists, while the guanosine analogues Loxoribine [36], and ANA773 [37] as well as the adenine analogues SM-324405 [38], AZ12441970 [38], GSK2245035 [39], AZD8848 [40], and GS-9620 [41] also activate TLR7. Three synthetic oligonucleotide TLR7 antagonists are currently in preclinical and clinical trials: DV1179 (dynavax.com), IMO-8400 and IMO-3100 [42].

**Targeting TLR7 in Asthma**

Asthma is characterized by airflow limitation due to excessive Th2-related inflammation including airway eosinophilia and elevated production of IL-4, IL-5, and IL-13. TLR7 agonists activate dendritic cells (DCs), airway epithelial cells (AECs), and Th1 cells to induce Th1 immune polarization that reduces airway hyper-reactivity and airway remodeling. Airway nerves express TLR7 and upon activation release nitric oxide (NO) to cause airway smooth muscle relaxation and bronchodilation.

**Airway Inflammation**

TLR7’s immunomodulatory properties have been demonstrated in a variety of preclinical models. Specifically, TLR7 agonists’ potent induction of Th1 immunity attenuates the excessive Th2 phenotype in allergic asthma and allergic rhinitis (fig. 2). For example, resiquimod given prophylactically or therapeutically reduced inflammatory cell recruitment as well as IL-4, IL-5, IL-13 and IgE levels following allergen challenge in sensitized animals [47–50]. Resiquimod reversed airway reactivity [47, 48, 50] and prevented airway smooth muscle proliferation and goblet cell hyperplasia following chronic allergen challenge [49]. Thus, TLR7-targeted therapy may prevent both pathologic remodeling of the airway and airflow obstruction. In support of this approach, two recently completed clinical trials evaluated the tolerability and safety of TLR7 agonists for treatment of allergic rhinitis. In a phase IIa study, 60 μg of AZD8848 given once per week before allergen challenge reduced immediate post-challenge rhinitis symptoms as well as morning symptoms for 5 days following treatment [40]. In a randomized, double-blind, placebo-controlled study, 20 ng of GSK2245035 given intranasally was well tolerated by patients. A higher concentration of TLR7 agonist or more frequent dosing caused fevers, headache, myalgia, and nasal irritation [39], reinforcing the importance of characterizing the therapeutic range for these agonists in humans. A once-weekly dosing regimen maintained TLR7’s pharmacologic response while avoiding tolerance or excessive immune stimulation. Studies of TLR7 agonists in humans with asthma are also underway. A trial of TLR7 stimulation in patients with mild asthma has been completed (clinicaltrials.gov NCT01607372), and results are pending.

**Acute Bronchodilation**

Rapid bronchodilators remain a cornerstone of asthma care. TLR7 agonists acutely relax airway smooth muscle within seconds

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**Fig. 2.** Therapeutic effects of TLR7 agonists. **A** Asthma is characterized by excessive Th2-related inflammation including airway eosinophilia and elevated production of IL-4, IL-5, and IL-13. TLR7 agonists activate dendritic cells (DCs), airway epithelial cells (AECs), and Th1 cells to induce Th1 immune polarization that reduces airway hyper-reactivity and airway remodeling. **B** Airway nerves express TLR7 and upon activation release nitric oxide (NO) to cause airway smooth muscle relaxation and bronchodilation.
to minutes [17, 51], an effect distinct from their ability to inhibit Th2 inflammation. Relaxation is unaffected by eosinophilic airway inflammation and blocked by a nitric oxide synthase inhibitor (L-NMMA), indicating that TLR7 induces bronchodilation via the production of nitric oxide (fig. 2). TLR7-mediated nitric oxide release likely occurs in airway nerves since airway smooth muscle does not express TLR7 and airway epithelium was not required for TLR7-mediated bronchodilation. Physiologically, TLR7-mediated release of nitric oxide may serve a protective role in limiting airway constriction during early viral infection and is an attractive pharmacologic target as a novel bronchodilator.

Viral Infections

Respiratory virus infections exacerbate asthma. Rhinovirus, respiratory syncytial virus, and influenza account for the majority of viruses detected during an acute exacerbation [52]. However, prevention of viral infections and treatment options are limited. Annual influenza vaccination is currently the only routine antiviral preventative measure. This strategy has produced variable results depending on the specificity of the vaccine for a given year’s influenza strain [53]. This has led to the search for adjuvants that can augment the immune response to vaccine. In mice, imiquimod administered at the time of vaccination improved morbidity [54] and increased survival following influenza challenge [55]. This effect may be due to enhanced viral antigen uptake and presentation by dendritic cells in the presence TLR7 agonist [56]. Incorporating RNA or 3M-019 into the vaccine also promoted antibody production via the induction of isotype class-switching during influenza challenge [32, 54]. Furthermore, intranasal delivery of a dual TLR7/TLR8 imidazoquinoline agonist 3M-011, given either before influenza infection or after inoculation, reduced influenza virus titers in the lung of rats [57]. TLR7’s antiviral effects have potential for both prevention and treatment of influenza infection.

Safety Profile and Future Studies

TLR7 agonists’ dual activity as an acute bronchodilator coupled with chronic attenuation of Th2-related inflammation offers a therapeutic profile unique from current bronchodilators. However, the potential for TLR7 agonists to stimulate Th1 immune responses necessitates a thorough evaluation of their therapeutic dosing range in human studies. Furthermore, allergic airway diseases have heterogeneous phenotypes that will require careful classification in order to identify who will benefit from TLR7 agonist treatment. To this point, intranasal delivery of TLR7 agonists in non-allergic animal models is pro-inflammatory and causes airway neutrophilia, while TLR7 agonist suppresses airway eosinophilia without causing airway neutrophilia in allergic animals [48]. Similarly, a subset of healthy volunteers and patients with allergic rhinitis experienced transient elevation of blood neutrophil counts following intranasal TLR7 treatment [39]. Thus patients with eosinophilia and/or other markers of a Th2 phenotype should be selected for TLR7 trials, similar to an approach used for the recently approved anti-IL5 drug mepolizumab [58], with close attention paid to Th1-related side effects.

Conclusions

TLR7 is a clinically attractive therapeutic target as an acute bronchodilator, as a chronic anti-inflammatory mediator, and as an antiviral therapy against influenza. While TLR7 agonists require more thorough evaluation in humans, aerosolized TLR7 agonists with limited systemic absorption are promising treatment options for respiratory diseases.

Disclosure Statement

The authors of have no conflicts of interest to declare.

References


