

Toll-like Receptors in the Respiratory System: Their Roles in Inflammation

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Allergic airway inflammation develops in the context of innate immune cells that express Toll-like receptors (TLRs). TLRs recognize microbial components and evoke diverse responses in immune and other respiratory cells through distinct signaling pathways. Bacterial and viral infection in the airway modulates the extent of allergic inflammation. TLR stimulation controls T helper (Th) 1, Th2, and Th17 cell differentiation, cytokine production in mast cells, and activation of eosinophils via direct and indirect pathways. TLR signals in dendritic cells increase expression of major histocompatibility complex proteins and T-cell coreceptors, resulting in greater T-cell activation with Th1 bias. TLR signals in mast cells increase their release of IL-5, and TLR signals in airway epithelial cells enhance airway generation of proallergic cytokines. Although these responses play an important protective role in infection, they may exacerbate allergic inflammation. Under some conditions, TLR stimulation, especially via TLR9, reduces Th2-dependent allergic inflammation through induction of Th1 responses. Therefore, understanding the regulatory role of TLRs in the pathogenesis of allergic airway inflammation may shed light on improving inflammation control in asthmatic patients.

Introduction

Toll-like receptor (TLR) is important in the recognition of various pathogens. TLRs are expressed in a variety of innate immune cells in the airway including epithelial cells, mast cells, and dendritic cells (DCs). Pathogens entering the airway may encounter these cells, and then are recognized by the expressed TLRs. The activation of the signaling cascades downstream of TLRs induces

coreceptor expression and cytokine secretion while also initiating the development of specific immune responses to eliminate the pathogens [1–3].

The essence of the hygiene hypothesis is that the increased incidence of allergic diseases in developed countries is explained by the decreased chances of infection during childhood [4,5]. The exposure to pathogens during bacterial and viral infection may drive the maturation of the immune system in infancy or childhood toward a T helper (Th) 1 phenotype, and away from a Th2 phenotype associated with allergic diseases. Although an attractive hypothesis, no definitive evidence supports the idea. On the other hand, polymorphisms in CD14, an associated protein of TLR4, and in TLR2, have been correlated with the risk of allergic diseases [4–6]. In addition, bacterial and viral infections in the airway provide diverse ligands for TLRs and thereby exacerbate airway inflammation in asthmatic patients. Therefore, the cells expressing TLRs are not only crucial for the detection of pathogens but also play an important role in the development of allergic diseases in humans. This review summarizes the current knowledge on the role of TLRs in allergic airway inflammation.

Immunologic Mechanisms Underlying Th2-driven Allergic Airway Inflammation

The cardinal features of acute asthma in humans are airway inflammation, mucus hypersecretion, and airway hyperreactivity [7,8]. Asthmatic patients' airways show the infiltration of eosinophils, degranulated mast cells, and lymphocytes, as well as hyperplasia of goblet cells. First, inhaled allergens are recognized by DCs beneath the airway epithelial cell layer. Next, these airway DCs migrate into the draining lymph nodes, where they present antigenic peptides of the inhaled allergens to naive CD4 T cells and induce Th1- and Th2-cell differentiation. The direction of Th1/Th2-cell differentiation is influenced by cytokines present in the environment. IL-12 is crucial for the differentiation of interferon (IFN)- γ -producing Th1 cells, while interleukin (IL)-4 is required for Th2 cells that produce IL-4, IL-5, and IL-13. DCs are the main source of IL-12 production.

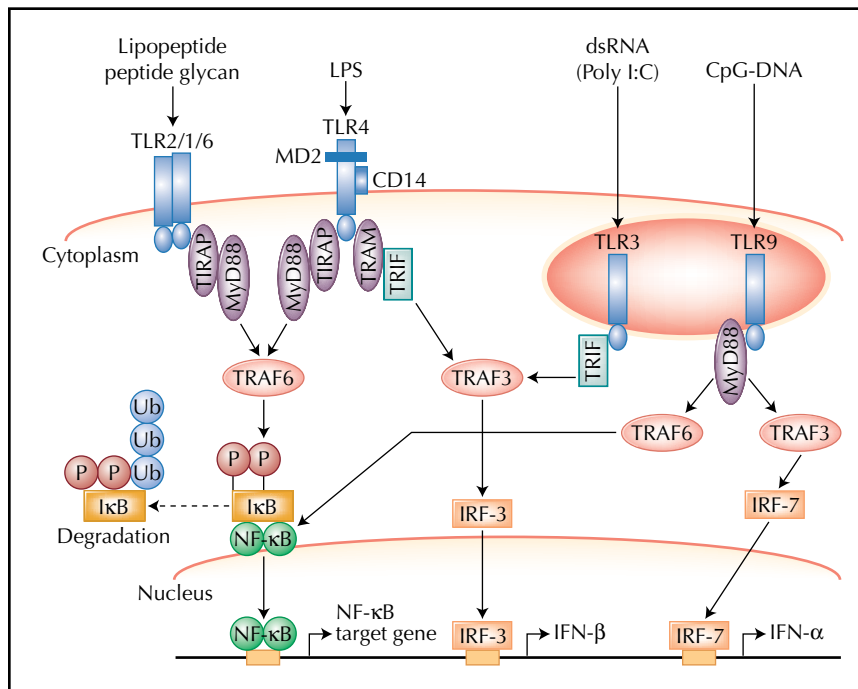


Figure 1. Toll-like receptors (TLRs) and signaling. The major intracellular signaling pathways downstream of TLRs are illustrated. IFN—interferon; NF-κB—nuclear factor-κB. (Adapted from Takeda and Akira [2] and Trinchieri and Sher [3].)

IL-4 and IL-13 produced from Th2 cells initiate the synthesis of antigen-specific immunoglobulin (Ig) E from B cells. IgE antibodies circulate in the blood and bind to the high-affinity IgE receptor (FcεRI) on the surface of mast cells. When allergens are bound to the receptor-bound IgE molecules, then mast cells are activated and release cytokines, chemokines, and chemical mediators such as histamine, prostaglandin, and leukotrienes, which induce airway smooth muscle contraction and increased vascular permeability. In epithelial cells, IL-4 and IL-13 also induce goblet cell hyperplasia [9] and production of chemokines, including thymus and activation-regulated chemokine (TARC), which attracts Th2 cells [10], and eotaxin, which attracts eosinophils [11]. IL-5 induces terminal differentiation in eosinophils and thereby recruits them to inflammatory lesions [12]. The mature eosinophils have intracellular granules containing major basic protein, eosinophil-derived neurotoxin, eosinophil peroxidase, and eosinophil-cationic proteins. The release of these proteins directly damages the airway epithelium, enhances airway hyperreactivity, and initiates the degranulation of mast cells.

TLR Ligands and Signals

The TLR-mediated activation of immune cells leads to the production of type 1-IFN, proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1, and IL-6 [2,3] (Fig. 1). TLRs use adaptor molecules such as myeloid differentiation factor (MyD) 88, TIR-containing adaptor protein (TIRAP), TIR-containing adaptor inducing IFN-β (TRIF), and TRIF-related adaptor molecule (TRAM) for signal transduction. The

ligation of all TLRs except TLR3 leads to the MyD88/TRAF6/nuclear factor (NF)-κB pathway. The endotoxin lipopolysaccharide (LPS), a compound of the outer cell membrane of gram-negative bacteria, is recognized by TLR4. TLR2 recognizes other components of the cell wall found in gram-negative and gram-positive bacteria, such as lipoproteins or peptidoglycan (PGN) by forming a heterodimer with TLR1 or TLR6. TLR9 recognizes unmethylated CpG dinucleotides in bacterial genomic DNA. Vertebrate DNA is not stimulatory due to the high level of methylation at CpG dinucleotides. TLR9 stimulation activates the MyD88/TRAF6/NF-κB pathway to induce proinflammatory cytokines while also inducing IFN-α production via the activation of interferon regulatory factor (IRF)-7 [13,14]. TLR3 and TLR4 activate the MyD88-independent pathway, in which TRIF, TRAF3, and IRF-3 are involved. The TRAF3/IRF-3 pathway induces IFN-β production (Fig. 1). Double-stranded (ds) RNA is a ligand for TLR3 [15]. dsRNA is generated as an intermediate during the replication cycle of single-stranded (ss) RNA or DNA viruses. Therefore, TLR3 is believed to be an antiviral receptor.

TLRs on DCs Control Th1, Th2, and Th17 Cell Differentiation

DCs contribute to adaptive immune responses by presenting antigenic peptides to naive T cells and thus secreting specific cytokines. DCs express different TLRs depending on the DC subset [16] (Table 1). TLR engagement following exposure to allergen contaminants or to infectious agents modulates DC functions. Immature DCs in the peripheral tissues recognize pathogens by TLRs,

Table 1. TLR expression on human innate immune cells

Study	Cell type	TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR9	TLR10
Homma et al. [43], Sha et al. [44]	Airway epithelial cell	+	++	++	++	++	++	-	-	+	-
Wong et al. [50]	Eosinophil	+	++	-	+	+	+	+	-	+	-
Kulka et al. [39]	Mast cell	+	+	+	+	+	+	+	-	+	+
Iwasaki and Medzhitov [16]	mDC	++	++	++	-	+	++	+/-	++	++	+
Iwasaki and Medzhitov [16]	pDC	+	-	-	-	-	++	++	-	++	+
Iwasaki and Medzhitov [16]	Monocyte- derived DC	++	++	++	++	+/-	++	-	++	-	ND

+, ++, and - indicate the relative mRNA or protein expression. +/- indicates conflict results.
DC—dendritic cell; ND—not determined; TLR—Toll-like receptor.

leading to the regulation of cell-surface expression of costimulatory molecules (CD80 and CD86) and major histocompatibility class (MHC) class II molecules. Concomitantly, captured pathogens or allergens are processed and presented to T cells by the upregulated antigen-MHC class II complexes. The induction of CD80/86 on the antigen-presenting cell (APC) by TLRs leads to the efficient activation of T cells specific for pathogens. TLR stimulation also induces expression of cytokines, such as IL-12, chemokines, and their receptors. IL-12 contributes to the differentiation of naive T cells into Th1 cells. Therefore, TLR signals in DCs are critically involved in determining the Th1/Th2 balance.

Using mice deficient in MyD88, investigators demonstrated that immunization of mice with adjuvants containing TLR ligands such as LPS and CpG DNA results in Th1 responses accompanied by the induction of Th1-dependent antibody isotypes [17]. In addition, every TLR ligand for TLR2, TLR3, TLR4, TLR7, and TLR9 has also been reported to induce Th1 responses [18–21]. These results suggest that TLR stimulation activates the Th1 responses while also inhibiting Th2-driven allergic inflammation. However, the triggering of TLR2 results in Th2 cell differentiation [22]. Furthermore, stimulation of lung DCs with low doses of inhaled LPS induces Th2 responses and allergic airway inflammation such as eosinophilia in the lung, an increase in the serum IgE titer, and the production of Th2 cytokines, whereas a high dose of LPS in the same conditions induces Th1 responses [23,24•]. Physiologically, a low dose of LPS presumably corresponds to the amounts available after the inhalation of LPS associated with environmental antigens, whereas a high dose may correspond to the amount present during infections with gram-negative bacteria. The TLR signal may induce both Th1 and Th2 responses and the dose of TLR ligands determines the Th1/Th2 balance via DCs [25,26].

Until recently, effector CD4 T cells were divided into several functional subpopulations: Th1, Th2, and T regulator

cells (Treg) cells. Recently, researchers identified a distinct proinflammatory T-cell lineage, called Th17 cells, which produce IL-17A, a cytokine that induces IL-8 and recruits neutrophils [27–29]. The differentiation of Th17 cells is induced by transforming growth factor (TGF)- β together with IL-6 [30,31] and ROR γ t expression in naive CD4 T cells [32]. Veldhoen et al. [33•] showed that the differentiation of Th17 cells requires a DC factor elicited by TLR4 and MyD88-dependent signals in addition to TGF- β . Furthermore, TLR3 and TLR9 signals also effectively induce the DC factors that act in concert with TGF- β to differentiate Th17 cells [33•]. An analysis of the DC-derived factors that act in concert with TGF- β led to the identification of IL-6 as a critical co-factor. IL-6- and TGF- β -induced Th17 cells are crucially involved in the pathogenesis of certain autoimmune diseases [34]. Because the IL-17A mRNA level in sputum is significantly elevated in asthmatic patients in comparison with healthy controls, it may also enhance the development of allergic airway inflammation [35]. However, Schnyder-Candrian et al. [36] showed that exogenous IL-17 reduces established airway inflammation, airway hyperreactivity, and hyper mucus production in ovalbumin (OVA)-sensitized mice. Their findings suggest that IL-17 induces DC activation and antigen uptake, thus leading to reduction of T-cell activation and reduced production of IL-4, IL-5, and IL-13, thus resulting in reduced allergic responses.

TLR Stimulation Enhances IL-5 Production but not Degranulation in Mast Cells

Mast cells play an important role in anaphylactic reactions by the multivalent binding of allergens to receptor-bound IgE, and release inflammatory mediators such as histamine, prostaglandins, and leukotrienes. In addition, mast cells regulate the levels of allergic airway inflammation by producing cytokines (eg, IL-4, IL-5, IL-6, IL-10, IL-13, and TNF- α), which are important in the pathogenesis of various allergic reactions [37,38]. Mast cells express all TLRs except TLR8 [39] (Table 1). Our study showed

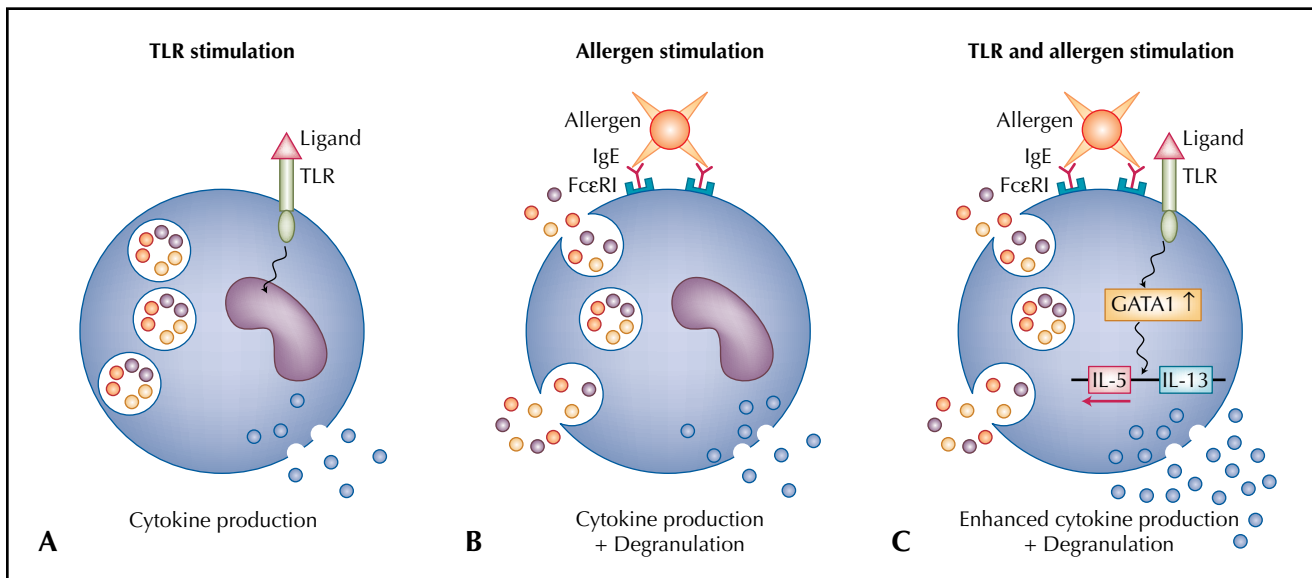


Figure 2. Toll-like receptors (TLRs) and allergen stimulation enhance cytokine production but not degranulation in mast cells. **A**, Stimulation of mast cells with TLR induces cytokine production, but does not release chemical mediators (degranulation). **B**, Allergen/immunoglobulin (Ig) E-mediated activation of mast cells induces release of cytokines and degranulation. **C**, Allergen and TLR stimulation enhances Th2 cytokine production with increased expression of GATA1, but does not enhance degranulation. FcεRI—high-affinity immunoglobulin E receptor.

that allergic airway inflammation is regulated through TLR4 stimulation on mast cells [40•]. The OVA-induced eosinophilic airway inflammation in the lung was dramatically increased by the intranasal administration of low-dose LPS (1 µg) in wild-type mice, whereas no such increase was observed in either mast cell-deficient mice (W/W^y) or TLR4-deficient mice. Therefore, the adoptive transfer of bone marrow-derived mast cell (BMMC) from wild-type, but not from TLR4-deficient, mice restores the increased eosinophilic inflammation in mast cell-deficient mice. Wild-type BMMC pretreated with LPS in vitro also reconstitute the eosinophilic inflammation. Moreover, an in vitro analysis revealed that the treatment of BMDCs with LPS results in NF-κB activation, a sustained up-regulation of GATA1 and GATA2 expression, and an increased capability to produce IL-5 and IL-13. Dramatic increases in the expression of IL-5 and IL-13 are detected in LPS-treated BMDCs after costimulation with LPS and IgE/antigen. An overexpression of GATA1 but not GATA2 in mast cell lines results in increased transcriptional activity of IL-4 and IL-5. Furthermore, levels of transcription of Th2 cytokines in BMDC are decreased by the introduction of small interfering RNA of GATA1. Therefore, mast cells appear to control allergic airway inflammation after their activation and modulation through TLR4-mediated induction of GATA1 and subsequent increase in Th2 cytokine production. Qiao et al. [41•] reported that TLR ligand fails to induce degranulation in mast cells. Other reports also showed that TLR stimulation fails to enhance degranulation [39,42]. Therefore, these studies indicate that TLR stimulation enhances cytokine production from mast cells, but it fails to enhance degranulation (Fig. 2).

Activation of Respiratory Epithelial Cells by TLRs Exacerbates Allergic Airway Inflammation

Respiratory epithelial cells lie at the interface between the host and the environment. They represent the initial site for bacteria to colonize in the respiratory tract. Recent studies have revealed that TLR1 through TLR6, and TLR9 are expressed on airway epithelial cells [43,44] (Table 1). A viral infection is known to cause an exacerbation of asthma in patients suffering from chronic airway inflammation or airway hyperreactivity [45]. Among the viruses that infect the airway, respiratory syncytial virus (RSV), influenza virus, and parainfluenza virus are pathogens that exacerbate asthma. They are RNA viruses and synthesize dsRNA during replication in infected cells. Moreover, dsRNA stimulation significantly increases the expression of cytokines (IL-1β, granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-6), chemokines (IFN-inducible protein [IP]-10, released on activation, normal T-cell expressed and secreted [RANTES], macrophage inflammatory protein [MIP]-1α, IL-8), and the intercellular adhesion molecule (ICAM)-1 [46]. Although these responses play a crucial role in the immune responses against viral infection in the airway, they may also contribute to an exacerbation of airway inflammation.

Thymic stromal lymphoprotein (TSLP) is an IL-7-like cytokine and is expressed mainly in epithelial cells [47,48]. TSLP activates immature DCs to produce eotaxin and TARC, and attracts eosinophils and Th2 cells, respectively. TSLP-activated DCs also express increased levels of OX40L, which preferentially triggers the differentiation of allergen-specific naive CD4 T cells into Th2 cells. The expression of

TSLP is induced in airway epithelial cells by stimulation with TLR3 ligand and Th2 cytokines [49•]. These data imply that respiratory viral infection and the recruitment of Th2 cytokine-producing cells may amplify allergic inflammation via the induction of TSLP in the asthmatic airway.

TLR Stimulation Induces Activation of Eosinophils

Eosinophils are the major effector cells in allergic inflammation. A significant expression of TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, and TLR9 in human peripheral blood eosinophils has been confirmed using Western blot and flow cytometry analyses [50] (Table 1). TLR ligands PGN, flagellin (TLR5 ligand), and Imiquimod R837 (TLR7 ligand) but not Poly I-C (synthetic dsRNA), LPS, ssRNA, or CpG can induce the release of IL-1 β , IL-6, IL-8, and GRO- α from eosinophils [50]. These TLR ligands can also upregulate cell surface molecules including ICAM and CD18, a component of CD11a and Mac-1, and induce degranulation in eosinophils for the release of toxic superoxides and eosinophil-cationic protein. Therefore, eosinophils may play a crucial role in the innate immune responses against infected bacteria and viruses and also in the initiation of allergic inflammation via the release of cytokines, chemokines, superoxides, and granular toxic proteins. Several investigators have reported that TLR stimulation enhances airway eosinophilia [22,51]. The exacerbation of airway inflammation with TLR stimulation is induced not only by the intensive accumulation of eosinophils but also the enhanced activation of eosinophils.

Possibility of Attenuating Airway Inflammation with TLR9 Stimulation

TLR9 is widely expressed in cells including mast cells, eosinophils, epithelial cells, and plasmacytoid DCs (Table 1). The TLR9 ligand CpG DNA is repeatedly shown to down-regulate OVA-induced allergic inflammation in murine models [52,53]. The mechanisms of CpG motifs and their potential roles and therapeutic abilities are thought to be that the CpG motif triggers DCs to produce IL-12 and subsequent IFN- γ production from Th1 cells. Furthermore, the coadministration of the CpG motif and an allergen is more effective than the administration of the CpG motif alone. Although only the administration of oligodeoxynucleotide-containing CpG (CpG-ODN) stimulates innate immune cells expressing TLR9, coadministration may induce allergen-specific Th1 cells more efficiently and result in the inhibition of antigen-specific Th2 cells. In mast cells, CpG-ODNs inhibit the secretion of IL-4 by IgE cross-linking and degranulation [52]. CpG-ODNs also inhibit IgE production by upregulating the T-bet expression in B cells [52]. Allergens conjugated with CpG-ODN can bind to allergen-specific B cells, and they may selectively inhibit the production of allergen-specific antibodies.

CpG-ODN is currently being tested in clinical trials with allergy patients [19]. Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate (AIC) is a novel therapeutic compound that consists of purified Amb a 1 obtained from short ragweed proteins linked to an immunostimulatory phosphorothioate oligodeoxyribonucleotide. Tulic et al. [54] showed that the subcutaneous administration of AIC to ragweed-sensitive patients before the onset of ragweed season decreases the nasal inflammatory response. Simons et al. [55] also showed that IL-4 production from ragweed-specific T cells in peripheral blood mononuclear cells decreases after AIC administration.

Conclusions

Although the administration of some TLR ligands may be effective for patients with rhinitis, microbial infection appears to undoubtedly exacerbate allergic asthma. Asthma is often considered to be a chronic but not a life-threatening disease. However, the World Health Organization estimates that asthma accounts for one of every 250 deaths worldwide. The genomes of microorganisms separated from patients who died from acute asthma were analyzed, and a correlation between viral infection and fatal asthma was suggested [56]. Therefore, TLR stimulation may worsen the inflammation in patients suffering from chronic asthma. More extensive study on the role of TLRs in patients with asthma is expected to lead not only to controlling the symptoms of asthma, but also to preventing death.

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Disclosure

No potential conflict of interest relevant to this article was reported.

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