



Critical role of Toll-like receptors in pathophysiology of allergic asthma

Seyyed Shamsadin Athari^{a,b,c}, Seyyede Masoume Athari^d, Fateme Beyzay^c, Masoud Movassaghi^e, Esmaeil Mortaz^{f,g,*}, Mehdi Taghavi^h

^a Research Center for Food Hygiene and Safety, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

^b Health policy Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

^c Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

^d Department of Biology, Faculty of Basic Sciences, Maragheh University, Maragheh, Iran

^e Department of Pathology and Laboratory Medicine, University of California, Los Angeles (UCLA), Los Angeles, CA, USA

^f Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

^g Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

^h Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

ARTICLE INFO

Keywords:

TLRs
Immune system
Asthma

ABSTRACT

Allergic asthma is an airway disease, characterized by reversible bronchoconstriction, chronic inflammation of the airway, and thickness of smooth muscle in the respiratory tract. Asthma is orchestrated by an excessive Th2-adaptive immune response, in which innate immunity plays a key role. Recently TLRs have received more and more attention as they are central to orchestrate the innate immune responses. TLRs are localized as integral membrane or intracellular glycoproteins with those on the cell surface sensing microbial antigens and the ones, localized in intracellular vesicles, sensing microbial nucleic acid species. Having recognized microbial antigens, TLRs conduct the immune response towards a pro- or anti-allergy response. As a double-edged sword, they could initiate either harmful or helpful responses by the immune system in case of allergic asthma. In the current review, we will describe the role of TLRs and their signaling pathways in allergic asthma.

1. Introduction

Allergic asthma is a complicated chronic airway inflammatory disorder with a globally increasing rate of prevalence and occurrence over the past decades (Lambrecht and Hammad, 2012; Wu et al., 2014). Asthma is characterized by reversible bronchoconstriction, chronic inflammation of the airway, infiltration of eosinophils, hyper-responsiveness, and thickness of smooth muscle cell layer in the airway. The chronic inflammation in asthma is usually eosinophilic and is orchestrated by a complex network of different inflammatory cytokines with positive or negative feedback on each other. It leads to hypertrophy of smooth muscle cells, hyperplasia of mucus-secreting goblet cells, angiogenesis, and fibrosis (Athari and Athari, 2014; Lambrecht and Hammad, 2012; Wu et al., 2014).

Th2 response plays a key role in asthmatic patients as it both causes the immune cells increase and releases inflammatory mediators within the respiratory tract. IgE-producing B cells are the main cells responsible for pathophysiology of asthma (Lambrecht and Hammad, 2015; Wu et al., 2014). Asthma is handled by complex mechanisms. Although innate immunity plays a key role in asthma, excessive amount of Th2-

adaptive immune responses lead to a subsequent development of pathogenesis in the disease (Hilvering et al., 2015; Lambrecht and Hammad, 2015; Szefer, 2013).

Thanks to specific Pattern-Recognition Receptors (PRRs) such as the Toll-Like Receptors (TLRs), innate immune system can recognize some antigenic molecules from microbes, known as Pathogen-Associated Molecular Patterns (PAMP) (Akira and Takeda, 2004). TLRs and Nucleotide-binding oligomerization domain-like receptors (NLRs) are two species of PRRs (Akira and Takeda, 2004; Kawai and Akira, 2010). NF- κ B is the main controlling factor to signal the pathway of the TLRs that can get involved in the production of many pro-inflammatory cytokines and chemokines. Some of the most important TLRs are integral membrane glycoproteins, which have leucine-rich repeat motifs in the recognizing domain. TLRs are divided into two groups: 1) TLR1, 2, 4, 5, 6, 10, and 11 are expressed on the cell surface, recognizing proteins, lipoproteins, and polysaccharides in the bacteria and 2) TLR3, 7, 8, and 9 are localized within intracellular vesicles where they sense different types of microbial nucleic acids (Akira and Takeda, 2004; Kawai and Akira, 2010; Tesse et al., 2011).

Up to now, 12 TLRs and more than 20 NLRs have been identified

* Corresponding author at: Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
E-mail address: e.mortaz@uu.nl (E. Mortaz).

<http://dx.doi.org/10.1016/j.ejphar.2016.11.047>

Received 30 January 2016; Received in revised form 21 November 2016; Accepted 25 November 2016

Available online xxxx

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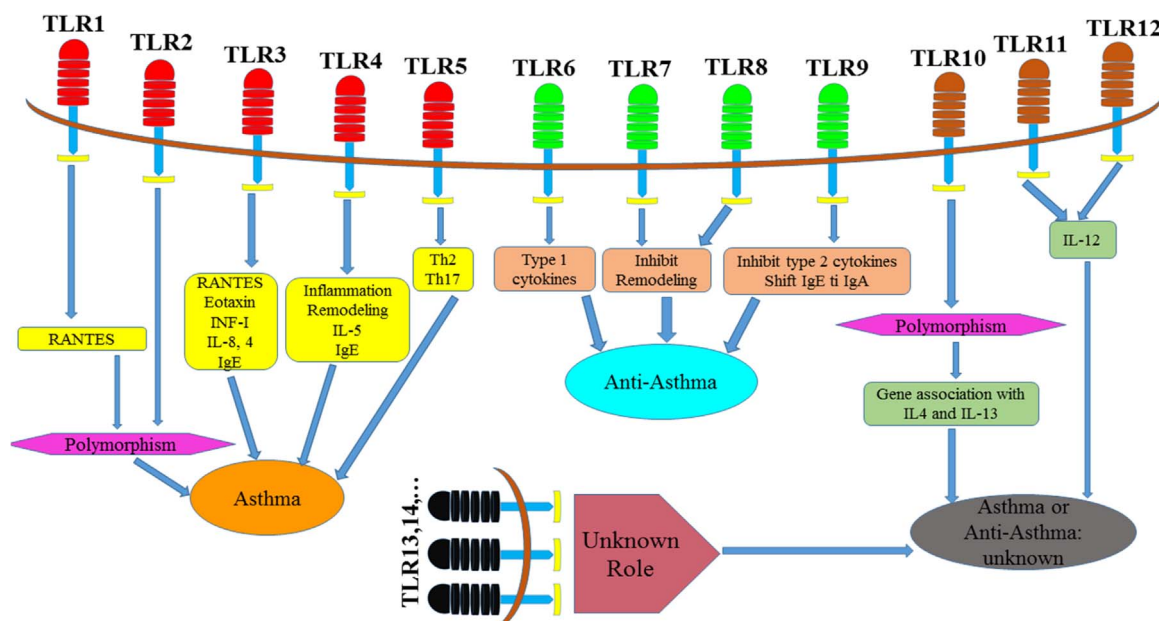


Fig. 1. Toll like receptors signaling through the various pathways with in collaboration with of internal adaptors proteins. Each TLR may induces or influences different functions in allergic asthma.

(Akira and Takeda, 2004; Akira et al., 2006; Tesse et al., 2011). Ligands bind to TLRs through Toll/IL-1 receptor domain (TIR) (Akira et al., 2006; Kawai and Akira, 2010). Most TLRs have been expressed on airway epithelial cells and respiratory track, playing an important role not only to regulate of innate and adaptive immune systems but also to maintain the aseptic condition in the airways (Akira et al., 2006). TLRs can shift immune response towards pro- or anti-allergy responses after successfully recognizing microbial antigens (Akira et al., 2006; Gon, 2008).

As a result, the present review has focused on the role of TLRs in the pathophysiology of allergic asthma and the possibility of using TLRs as a therapy for asthma (Fig. 1). Thus, in the following part we will describe important members of TLRs that have been linked to the pathophysiology of asthma.

2. Toll-like receptor and possible role in pathogenesis of asthma

Activated TLR2 initiates Th2-immune response, thus playing a critical role in asthma (Bezemer et al., 2012; Hussein et al., 2012). Genetic variations in TLRs affect the pattern of immune responses against environmental antigens. Polymorphism in the TLR2 gene is associated with abnormal immune response, making it more susceptible to immune-related diseases such as asthma (Bezemer et al., 2012; Eder et al., 2004; Hussein et al., 2012; Martinez and Holt, 1999). However, TLR2 signaling can also induce the expansion of Treg cells, reducing asthma exacerbations (Liu et al., 2006). TLR2 ligands (Table 1) are a wide spectrum of PAMPs ranging from the molecules on cell membrane of gram-positive and negative bacteria, mycoplasma, mycobacteria, yeasts, and parasites (Bezemer et al., 2012; Hussein et al., 2012; Martinez and Holt, 1999).

TLR2 Arg677Trp polymorphism seems to be a protective factor against replication of *Cytomegalovirus* (CMV) (Brown et al., 2009; Wu et al., 2001); therefore, polymorphisms in TLR genes have been associated with susceptibility to the disease. The heterozygous genotype of the TLR2 with Arg677Trp polymorphism occurs more frequently in CMV-seronegative adult patients than in CMV-infected ones, hence the heterozygous genotype is a protective factor in them. Regarding epidemiological evidence, there is a relationship between respiratory viral infections and asthma exacerbation. As a respiratory

pathogen, Murine CMV is capable of altering Th1/Th2 cytokine balance as well as increasing mucus production in allergic airway diseases. On the other hand, it can also activate TLRs and Th1 responses that help regulating viral infection and airway inflammation. However, polymorphisms in receptor genes could be a risk factor for other diseases (Brown et al., 2009; Wu et al., 2001).

Variants of TLR2 are associated with asthma and increased level of IL-9 (and other type 2 cytokines) (Eder et al., 2004; Lee et al., 2011). It is possible that distinct polymorphisms could increase viral replication and inflammation. Chronic inflammation may trigger asthma, leading at the same time to airway remodeling (Bezemer et al., 2012; Eder et al., 2004; Lee et al., 2011); therefore, TLR2 activation by agonists or infectious agents result in allergic asthma conditions.

3. Toll-like receptor 1 (TLR1) and 3 (TLR3) and their role in pathogenesis of asthma

RANTES synthesis is an imminent consequence of TLR1 activation (Lin et al., 1999; O'Neill, 2006). TLR3 stimulation, however, causes the production of type I interferons, IL-8, and RANTES. RANTES production, triggered by TLR3, is lower than the one by TLR1 (Yamamoto et al., 2003). TLR1 recognizes triacyl lipoproteins of bacteria and TLR3, the viral replicative intermediate double-stranded RNA (dsRNA) (Takeda et al., 2003).

In the trachea, TLR3 ligands induce airway responsiveness to bradykinin (Bachar et al., 2004). Activated TLR3 induces pro-inflammatory cytokines, eotaxin, and RANTES, leading to eosinophilic inflammation in the airway (Morris et al., 2005, 2006; Sukkar et al., 2006). Moreover, as a positive feedback, the cytokine pattern, induced by inflammatory cells, enhances the TLRs expression level in the respiratory system (Cooper et al., 2009; Kvarnhammar et al., 2013; Niimi et al., 2007). Rhinoviruses can also increase TLR3 expression in bronchial epithelial cells, triggering exacerbation of the allergic asthma (Hewson et al., 2005; Torres et al., 2010; Wang et al., 2009). As a result, TLR3 plays a critical role in allergic asthma. Long-term activation of TLR3 can cause lung inflammation (Meng et al., 2011; Stowell et al., 2009). TLR3 may increase IL-4 and IgE levels in serum by modulating Th2 immune response, though TLR3 does not alter the total number of cells in broncho-alveolar lavage fluid (Meng et al., 2011; Torres et al., 2010).

Table 1
Ligands of TLRs and their activators.

TLR type	Activators and ligands	References
TLR1	Bacteria: Triacyl lipopeptides	(Bachar et al., 2004; Morris et al., 2006; Kvarnhammar et al., 2013)
TLR2	Bacteria: Peptidoglycan, porins, Lipoarabinomannan, Lipoprotein/lipopeptide, Lipoteichoic acid, Fungi: Phospholipomannan, Zymosan Protozoa: tGPI-mutin Viruses: Hemagglutinin protein, Viral envelop proteins of measles virus, cytomegalovirus, and herpes simplex virus type I Host: Glycoinositolphospholipids, Heat shock proteins, Hyaluronan	(Sukkar et al., 2006; Morris et al., 2005; Kvarnhammar et al., 2013)
TLR3	Viruses: Double-stranded RNA (dsRNA)	(Sukkar et al., 2006; Niimi et al., 2007; Kvarnhammar et al., 2013; Cooper et al., 2009; Torres et al., 2010; Hewson et al., 2005; Stowell et al., 2009; Meng et al., 2011)
TLR4	Bacteria: Lipopolysaccharide (LPS) Fungi: Mannan Protozoa: Glycoinositolphospholipids Viruses: Envelope proteins, F protein of respiratory syncytial virus murine, retroviral envelope protein, Hyaluronic acid, Heparan sulfate Host: Heat shock proteins (Hsp) 60 and 70, fibrinogen, Fibronectin, Flavolipin, Taxol,	(Sukkar et al., 2006; Morris et al., 2005; Kvarnhammar et al., 2013)
TLR5	Bacteria: Flagellin	(Morris et al., 2006; Shi et al., 2011)
TLR6	Bacteria: Diacyl lipopeptides, lipoteichoic acid Fungi: zymosan	(Morris et al., 2006; Shi et al., 2011; Tantisira et al., 2004)
TLR7,8	Viruses: Single-stranded RNA (ssRNA), Imidazoquinoline	(Morris et al., 2006; Kvarnhammar et al., 2013; Meng et al., 2011; Tabeta et al., 2006)
TLR9	Bacteria: CpG-DNA Viruses: DNA	(Bauer et al., 2001; Bourke et al., 2003; Tabeta et al., 2006)
TLR11	Bacteria: Unknown Protozoa: Profilin-like molecule	(Bezemer et al., 2012; Yarovinsky et al., 2005; Plattner et al., 2008)
TLR12	Bacteria: Profilin	(Lee et al., 2013)
TLR10, 13	Unknown	(Shi et al., 2011; Lee et al., 2013; Kim et al., 2006; Oldenburg et al., 2012)

Single Nucleotide Polymorphisms (SNPs) of TLR1, such as rs4833095 and rs5743618, have been largely associated with high serum levels of IL-6, TNF- α , and IL-1 β in response to ligand (Hammad et al., 2009; Kormann et al., 2008; Mikacenic et al., 2013). These TLR1 SNPs, namely rs5743595 and rs4833095, reduce the risk of allergic asthma as they trigger Th1 responses and reduce IL-4 secretion. On the other hand, some genotypes of TLR1 induce Th2 cytokine in allergic asthma (Kormann et al., 2008; Mikacenic et al., 2013).

The genotype rs4833095 in TLR1 is correlated with lower serum IgE levels in allergic patients as on the whole IgE serum levels are considered to be correlating with SNPs (Mikacenic et al., 2013; Verheijden et al., 2012; Yang and Chiang, 2015). TLR3 agonist stimulates the production of type 1 cytokines (especially IFN- γ) that results in reduced airway inflammation. TLR3 also regulates the production of IL-6 as an inflammatory cytokine; therefore, TLR3 activation in the airway enhances inflammatory cytokines that act as a stimulus for allergic asthma.

4. Role of Toll-like receptor 4 (TLR4) in pathogenesis of asthma

TLR4 both mediates innate immune responses and controls the immune responses against Respiratory Syncytial Virus (RSV), which is a common cause for bronchiolitis and an important risk factor in the development of asthma (Caramori et al., 2006, 2009).

Activation of TLR4 pathways may primarily cause chronic inflammation and oxidative stress to eliminate invading pathogens; however, uncontrolled inflammation can result in bronchial remodeling in the respiratory system (Caramori et al., 2006; Hammad et al., 2009; Verheijden et al., 2012). TLR4 signaling increases IL-5 secretion by mast cells, causing the generation of allergic type 2 cytokines by airway epithelial cells (Caramori et al., 2009; Koppelman, 2006). Polymorphism in TLR4 gene is associated with excessive production of IgE against aeroallergens, bronchial hyperresponsiveness, and pathophysiology of allergic asthma (Caramori et al., 2009; Koppelman, 2006). SNPs in TLR4, including Asp299Gly and

Thr399Ile (also known as rs4986790 and rs4986791, respectively), are associated with airway hyperresponsiveness to inhaled allergens and allergic asthma, yet only in cases of exposure to endotoxin (Eder et al., 2004; Kulig et al., 1999; Werner et al., 2003). Airway inflammation as a result of TLR4 depends on the nature of the endotoxin and exposure conditions (Hollingsworth et al., 2004). Brief exposure to endotoxin, induces similar responses to allergen in both TLR4-deficient and wild-type mice (Hollingsworth et al., 2006).

Some researchers have found that in stress-caused asthma TLR stimulation in the presence of stress, results in pro-inflammatory cytokines, produced by mast cells. Activation of TLR4 pathway is followed by the production of TNF- α and IL-6. TLR4, also triggering the production of pro-allergic cytokines, thymic stromal lymphopoietin, GM-CSF, IL-9, IL-25, and IL-33 (Kleijung et al., 2003; Pockley, 2003; Vabulas et al., 2001). Inhaling a TLR4 antagonist suppresses bronchial hyperreactivity and asthma; therefore, TLR4 antagonists can be used as efficient drugs for asthma treatment, though more studies are required to do so.

5. Possible role of Toll-like receptor 5 (TLR5) in pathogenesis of asthma

Some microbial products can prime allergic asthma by activating TLR5 pathway signaling. Maruoka et al. (2011) tested allergenicity of bacterial flagellin in asthma model, finding out that bacterial protein flagellin primed Th2 and Th17 responses in allergic asthma models. Sensitized mice displayed severe asthma symptoms, such as airway eosinophilic inflammation, goblet cell hyperplasia, mucus hyper-secretion, airway obstruction, and airway hyperresponsiveness. The authors also emphasized that major adjuvant in house dust is bacterial flagellin; therefore, both adjuvants and allergens are present in house, being in turn sensed by TLR5, thus promoting allergic asthma. TLR5 is a known sensor of bacterial flagellin. Asthmatic people have higher serum levels of bacterial flagellin-specific antibodies, as compared to non-asthmatic ones (Maruoka et al., 2011; Wilson et al., 2012).

Shikhagaie et al. (2013) presented the immunostimulatory role of

TLR5 in the airway epithelium and smooth muscle, though not in patients with severe asthma, where TLR5 expression is less than healthy controls (Shikhagaie et al., 2013).

6. Role of Toll-like receptor 6 (TLR6) in pathogenesis of asthma

TLR6 is a highly conserved member of PRRs (Tantisira et al., 2004). TLR6 is upregulated in fungal allergic asthma, though what role it plays exactly with regards to this disease still remains a moot point (Kurup et al., 2005). A heterodimer of TLR6 and TLR2 recognize diacyl lipopeptides, zymosan, and lipoteichoic acid of the fungi. Moreover, triacyl lipopeptides are recognized by heterodimers of TLR2 and TLR1 too and signaling through both mentioned heterodimers leads to not only NF- κ B activation but cytokine production (Hornig et al., 2002; Ozinsky et al., 2000). TLR6 provides protection by regulating IL-6 and TGF- β and promoting Th17 responses during allergic asthma process (Moreira et al., 2011).

Some studies have indicated that TLR6 SNPs play a protective role in allergic asthma by generating type-1 cytokines. These studies have also showed that defective expression and/or function of TLR6 increases the frequency of allergic asthma occurrence (Hoffjan et al., 2005; Kormann et al., 2008; Tantisira et al., 2004). Moreover, TLR6 is one of the important immunoregulators in fungal allergic asthma and the absence of its function leads to inflammation and bronchial remodeling (Kormann et al., 2008). Agonists of TLR2/6 heterodimer can be employed as therapeutic tools to attenuate fungal allergic asthma (Weigt et al., 2005).

In animal models for allergic asthma, TLR6 activation induce Th17 responses and produce IL-23, leading to a protective response by reducing inflammatory responses in fungal-induced asthma (Hoffjan et al., 2005; Moreira et al., 2011; Weigt et al., 2005). Thus, targeting TLR6 can stop the inflammatory responses and result in therapeutic effects in clinical asthma.

7. Role of Toll-like receptor 7 (TLR7) and 8 (TLR8) in pathogenesis of asthma

Stimulated TLR7 inhibits asthma exacerbations and airway remodeling (Møller-Larsen et al., 2008; Roponen et al., 2010). Synthetic ligands of TLR7 can be used as a treatment for asthma, hence TLR7 may play an important role in pathophysiology and asthma treatment. TLR7 polymorphisms have been associated with asthma (Møller-Larsen et al., 2008; Roponen et al., 2010). TLR7 is expressed in bronchial epithelial cells, airway smooth muscle (Cherfils-Vicini et al., 2010; Ekman et al., 2011), eosinophils, and B lymphocytes (Caron et al., 2005; Gorden et al., 2005; Hart et al., 2005).

The immunomodulatory potential of TLR7 makes it an appealing candidate for therapeutic-target in allergic asthma (G Drake et al., 2012). With an inhibitory effect on IgE level (Meng et al., 2011), TLR7 prevents Th2-mediated airway inflammation in asthma, but simultaneously it stimulates the production of such Th1 cytokines as IL-12 and IFN- γ (Drake et al., 2012; Kaufman and Jacoby, 2012). Eotaxin is a strong eosinophil chemotactic factor, the level of which is reduced by TLR7 (Pham Van et al., 2011), at which time both eosinophilic infiltration and inflammation in the airways are decreased. TLR7 also prevents irreversible airway remodeling in chronic allergic asthma (Camateros et al., 2007).

TLR7 agonists come with some side effects such as fever, myalgia, and sepsis (Fidock et al., 2011; Pockros et al., 2007). To reduce these, TLR7 stimulation may be limited to local tissues only, which in case of asthma it should be administered by inhalation.

Genome-Wide Association Studies (GWAS) have revealed a strong link between susceptibility to allergic asthma and Xp22 sex chromosome, which contain TLR7 and TLR8 loci (Brasch-Andersen et al., 2008; Haagerup et al., 2004). TLR8 is highly homologous to TLR7,

both localized in the endosomal compartment (Diebold et al., 2004; Jurk et al., 2002). Some studies have suggested that TLR8 may regulate TLR7 function (Demaria et al., 2010). Synthetic TLR7/TLR8 ligand prevents development of allergic asthma by inhibiting inflammatory responses in the airways in response to allergens (Camateros et al., 2007; Moisan et al., 2006; Sel et al., 2007). TLR7 activation decreases Th2 response and acts as a bronchodilator, making it an attractive option for the treatment of allergic asthma.

8. Role of Toll-like receptor 9 (TLR9) in pathogenesis of asthma

TLR9 is the best sensor of bacterial DNAs such as those containing short sequences of unmethylated CpG oligodesoxynucleotide (ODN) motifs (Zhang et al., 2005). TLR9 stimulates some alterations in cellular redox balance and peroxynitrite formation, activating NF- κ B (Bauer et al., 2001; Manna et al., 2006; Wagner, 1999). In immune cells, CpG DNA activates TLR9 signaling (Ahmad-Nejad et al., 2002; Krieg, 1999). Neutrophils are one of such immune cells which constitutively express TLR9 (Kokkinopoulos et al., 2005; Kool et al., 2011; Parker et al., 2005).

Several studies have demonstrated that TLR9 activation by CpG ODN prevents allergic airway inflammation and bronchial hypersensitivity in asthma. Consequently, activation of TLR9 signaling pathway has a potential therapeutic application in allergic asthma (Silverman and Drazen, 2003).

TLR9 agonist restores Th2/Th1 balance in the immune responses against allergic asthma (Humbert et al., 2005) by stimulating the production of neutralizing monoclonal antibodies against pro-inflammatory Th2 mediators (Agler et al., 2011; Corren et al., 2010; Haldar et al., 2009; Parker et al., 2011). TLR9 activation also induces regulatory T cells (Tregs) which inhibits Th2 responses (Ito et al., 2007).

TLR9 ligands can directly induce B cells to switch from IgE to IgA phenotype and block IL-4, an important cytokine in the development of allergic asthma (Shen et al., 2008). TLR9 ligands (unmethylated CpG-ODNs) can also prevent allergen-induced eosinophilic inflammation in asthmatic patients' airways (Hopp, 1999). Moreover, the ligand can reduce IgE levels circulation and bronchial hyperreactivity. Activation of TLR9 by CpG-ODNs can prevent the development of airway remodeling (Jain et al., 2002, 2003; Kline et al., 2002), inducing strong Th1-like cytokines patterns (Kline et al., 1999). Mammalian chromosomes contain TTAGGG hexamers as repetitive stretches in telomeric ends. Synthetic ODNs that contain these motifs as TLR9 agonist are tested in clinical trials to treat allergic asthma (Sato et al., 2008). TLR7 and TLR9 agonists induce CXCL8 release from monocytes. It has been shown that CXCL8 level was higher in healthy volunteers, compared to asthmatic patients. TNF- α and IL-6 production is also stimulated by TLR9 agonists (Camateros et al., 2007; Jain et al., 2003; Meng et al., 2011; Shen et al., 2008).

9. Role of Toll-like receptor 10 (TLR10) in pathogenesis of asthma

TLR10 gene includes 3269 bases on the short arm of chromosome 4 which encodes 811-amino acid protein, approximately 50% identical to TLR1 and TLR6. TLR10 contains multiple leucine-rich repeats, cysteine-rich, transmembrane domain, and a cytoplasmic TOLL interleukin-1 receptor domain; however, specific functions of TLR10 are not currently known (Chuang and Ulevitch, 2001). In SNP studies, high levels of polymorphisms have been found in the TLR10 gene in the respiratory system (Lazarus et al., 2002); therefore, genetic variations of TLR10 gene can contribute to asthma susceptibility.

TLR10 mRNA is expressed predominantly in B lymphocytes and is increased, following the engagement of B cell antigen receptor. TLR10 is expressed in lung tissue where it can be involved in the regulation of

innate immune responses allergens (Bourke et al., 2003; Sebastiani et al., 2003; Wang et al., 1998).

In genetic joint analyses, joint influences have been found between some genes. In this regard, a gene-to-gene interaction has been reported between IL-13 and α -chain of IL-4-receptor (Battle et al., 2007; Chan et al., 2006; Kim et al., 2006). On the other hand, it has been found that there is a joint association between IL-13 and TLR-10 in allergic asthma. The functional importance of these joint associations as well as their action during asthma pathogenesis has not yet been fully understood (Kim et al., 2006; Lee et al., 2004).

10. Role of Toll-like receptor 11 (TLR11) and TLR12 in pathogenesis of asthma

TLR11 is primarily expressed in epithelial cells of urinary tract and lung epithelial cells (Tamura et al., 2008; Zhang et al., 2004).

TLR11 induces IL-12 in response to the parasite (especially *Toxoplasma gondii*) (Yarovinsky et al., 2005), playing a major role in IL-12-dependent immune system response which is characterized by the reaction of CD8+ dendritic cells against the parasite (Plattner et al., 2008). TLR11 recognizes the parasite in a complex with TLR12 at which both TLRs directly bind to parasite. Both TLR11 and 12 appear to be intracellular TLRs (Barton and Kagan, 2009). TLR11/TLR12 complex initiates the MyD88 signaling cascade via the TLR11 Toll/IL-1R domain. Nevertheless, TLR11, and not TLR12, is bound to MyD88 (Lee et al., 2013; Plattner et al., 2008). The role of these two receptors in allergic asthma is still unknown but it seems that due to their role in stimulating eosinophilic responses in parasitic infections, they may also trigger the development of pathophysiologic responses in asthma by attracting and activating eosinophils. To better understand the role of these TLRs in allergic asthma, more studies are needed to use animal models for asthma.

11. TLR13

TLR13 is involved in cell activation (Kawai and Akira, 2011; Tabeta et al., 2006), being a bacterial single-stranded RNA (ssRNA) sensor. It is also able to recognize vesicular stomatitis virus (Shi et al., 2011). TLR13 is an important bacteria sensor, capable of detecting the peptidyl transferase loop of bacterial 23S rRNA (D'Costa et al., 2011; Deshmukh et al., 2011). It is a novel member of the TLR family. Murine TLR13 gene transcription is regulated through three cis-acting elements with NF- κ B acting as an inhibitor of TLR13 transcription (D'Costa et al., 2011; Oldenburg et al., 2012; Sagar et al., 2013; Tabeta et al., 2006). The role of TLR13 and the TLRs, discovered later, such as TLR14, 15, 16 etc., in humans is still unknown; therefore, the role of these receptors in allergic asthma cannot be judged. Further studies should be planned and conducted to clear their exact role and pathways.

12. Conclusion and future directions

Asthma is a multifactorial disease and a significant global public health problem. TLRs are involved in the development of allergic asthma and may contribute to the development of immune response or tolerance to the antigens (Fig. 1). TLRs are involved in recognizing microbial products and handling immune defense against pathogens. TLRs' signaling can shift the inflammatory responses towards either pathologic or protective pathways that result in tissue injury or regeneration, respectively. Hence, based on the type of the TLRs along with the activated signaling pathway, TLR stimulation could either repair an injured tissue or damage a normal one. In order to achieve therapeutic goals for controlling and treating allergic asthma, it is necessary to evaluate the exact mechanism of action and estimate respective contributions of individual receptor. Current knowledge concerning the TLRs provides novel approaches for development of

TLR-based therapies. TLR ligands can be applied as drugs to treat allergic asthma. For instance, TLR9 is a powerful inducer of Th1 immune responses and agonists of TLR9 have been used for patients in clinical trials for the treatment of allergic asthma. Moreover, manipulation of Tregs function or Th1/Th2 balance by applying TLR ligands could be an efficient approach to control or prevent allergic asthma. TLR ligands can be administered locally, hence the harmful side-effects, which may appear in systemic use, are inhibited. TLRs give us new intervening strategies and are potential to become novel therapeutics for the prevention, control, and treatment of allergic asthma; however, the goal is not easily achieved as the signaling pathways of TLRs and TLR-mediated cytokines have a very complex network of connections among one another, in which any inattentive intervention can end disastrously. Therefore, future studies targeting these receptors will need more precise examinations and deeper information on the exact mechanisms of their actions. However, TLRs are good candidates to overcome the abnormal immune responses-derived diseases such as asthma, though this approach needs more studies in animal and human models in the future.

References

- Agler, A.H., Kurth, T., Gaziano, J.M., Buring, J.E., Cassano, P.A., 2011. Randomised vitamin E supplementation and risk of chronic lung disease in the Women's Health Study. *Thorax* 66, 320–325.
- Ahmad-Nejad, P., Häcker, H., Rutz, M., Bauer, S., Vabulas, R.M., Wagner, H., 2002. Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur. J. Immunol.* 32, 1958–1968.
- Akira, S., Takeda, K., 2004. Toll-like receptor signalling. *Nat. Rev. Immunol.* 4, 499–511.
- Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. *Cell* 124, 783–801.
- Athari, S.S., Athari, S.M., 2014. The importance of eosinophil, platelet and dendritic cell in asthma. *Asian Pac. J. Trop. Dis.* 4, S41–S47.
- Bachar, O., Adner, M., Uddman, R., Cardell, L.O., 2004. Toll-like receptor stimulation induces airway hyper-responsiveness to bradykinin, an effect mediated by JNK and NF- κ B signaling pathways. *Eur. J. Immunol.* 34, 1196–1207.
- Barton, G.M., Kagan, J.C., 2009. A cell biological view of Toll-like receptor function: regulation through compartmentalization. *Nat. Rev. Immunol.* 9, 535–542.
- Battle, N.C., Choudhry, S., Tsai, H.J., Eng, C., Kumar, G., Beckman, K.B., Naqvi, M., Meade, K., Watson, H.G., LeNoir, M., 2007. Ethnicity-specific gene–gene interaction between IL-13 and IL-4R α among African Americans with asthma. *Am. J. Respir. Crit. Care Med.* 175, 881–887.
- Bauer, S., Kirschning, C.J., Häcker, H., Redecke, V., Hausmann, S., Akira, S., Wagner, H., Lipford, G.B., 2001. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc. Natl. Acad. Sci. USA* 98, 9237–9242.
- Bezemer, G.F., Sagar, S., Van Bergenhenegouwen, J., Georgiou, N.A., Garssen, J., Kraneveld, A.D., Folkerts, G., 2012. Dual role of Toll-like receptors in asthma and chronic obstructive pulmonary disease. *Pharmacol. Rev.* 64, 337–358.
- Bourke, E., Bosio, D., Golay, J., Polentarutti, N., Mantovani, A., 2003. The toll-like receptor repertoire of human B lymphocytes: inducible and selective expression of TLR9 and TLR10 in normal and transformed cells. *Blood* 102, 956–963.
- Brasch-Andersen, C., Möller, M.U., Haagerup, A., Vestbo, J., Kruse, T.A., 2008. Evidence for an asthma risk locus on chromosome Xp: a replication linkage study. *Allergy* 63, 1235–1238.
- Brown, R.A., Gralewski, J.H., Razonable, R.R., 2009. The R753Q polymorphism abrogates toll-like receptor 2 signaling in response to human cytomegalovirus. *Clin. Infect. Dis.* 49, e96–e99.
- Camateros, P., Tamaoka, M., Hassan, M., Marino, R., Moisan, J., Marion, D., Guiot, M.-C., Martin, J.G., Radzioch, D., 2007. Chronic asthma-induced airway remodeling is prevented by toll-like receptor-7/8 ligand S28463. *Am. J. Respir. Crit. Care Med.* 175, 1241–1249.
- Caramori, G., Ito, K., Contoli, M., Di Stefano, A., L Johnston, S., M Adcock, I., Papi, A., 2006. Molecular mechanisms of respiratory virus-induced asthma and COPD exacerbations and pneumonia. *Curr. Med. Chem.* 13, 2267–2290.
- Caramori, G., Oates, T., Nicholson, A.G., Casolari, P., Ito, K., Barnes, P.J., Papi, A., Adcock, I.M., Chung, K.F., 2009. Activation of NF- κ B transcription factor in asthma death. *Histopathology* 54, 507–509.
- Caron, G., Duluc, D., Frémaux, I., Jeannin, P., David, C., Gascan, H., Delneste, Y., 2005. Direct stimulation of human T cells via TLR5 and TLR7/8: flagellin and R-848 up-regulate proliferation and IFN- γ production by memory CD4+ T cells. *J. Immunol.* 175, 1551–1557.
- Chan, I.H., Leung, T.F., Tang, N.L., Li, C.Y., Sung, Y.M., Wong, G.W., Wong, C.K., Lam, C.W., 2006. Gene-gene interactions for asthma and plasma total IgE concentration in Chinese children. *J. Allergy Clin. Immunol.* 117, 127–133.
- Cherfils-Vicini, J., Platonova, S., Gillard, M., Laurans, L., Validire, P., Caliendo, R., Magdeleinat, P., Mami-Chouaib, F., Dieu-Nosjean, M.C., Fridman, W.H., 2010. Triggering of TLR7 and TLR8 expressed by human lung cancer cells induces cell survival and chemoresistance. *J. Clin. Invest.* 120, 1285–1297.
- Chuang, T.H., Ulevitch, R.J., 2001. Identification of hTLR10: a novel human Toll-like

- receptor preferentially expressed in immune cells. *Biochim. Biophys. Acta (BBA)-Gene Struct. Expr.* 1518, 157–161.
- Cooper, P.R., Lamb, R., Day, N.D., Branigan, P.J., Kajekar, R., San Mateo, L., Hornby, P.J., Panettieri, R.A., 2009. TLR3 activation stimulates cytokine secretion without altering agonist-induced human small airway contraction or relaxation. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* 297, L530–L537.
- Corren, J., Busse, W., Meltzer, E.O., Mansfield, L., Bensch, G., Fahrendholz, J., Wenzel, S.E., Chon, Y., Dunn, M., Weng, H.H., 2010. A randomized, controlled, phase 2 study of AMG 317, an IL-4R α antagonist, in patients with asthma. *Am. J. Respir. Crit. Care Med.* 181, 788–796.
- D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., 2011. Antibiotic resistance is ancient. *Nature* 477, 457–461.
- Demaria, O., Pagni, P.P., Traub, S., De Gassart, A., Branzk, N., Murphy, A.J., Valenzuela, D.M., Yancopoulos, G.D., Flavell, R.A., Alexopoulou, L., 2010. TLR8 deficiency leads to autoimmunity in mice. *J. Clin. Invest.* 120, 3651–3662.
- Deshmukh, S.D., Kremer, B., Freudenberg, M., Bauer, S., Goldenbock, D.T., Henneke, P., 2011. Macrophages recognize streptococci through bacterial single-stranded RNA. *EMBO Rep.* 12, 71–76.
- Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., e Sousa, C.R., 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303, 1529–1531.
- Drake, M.G., Kaufman, E.H., Fryer, A.D., Jacoby, D.B., 2012. The therapeutic potential of Toll-like receptor 7 stimulation in asthma. inflammation & allergy-drug targets. *Former. Curr. Drug Targets-Inflamm. Allergy* 11, 484–491.
- Eder, W., Klimecki, W., Yu, L., von Mutius, E., Riedler, J., Braun-Fahrlander, C., Nowak, D., Martinez, F.D., Team, A.S., 2004. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J. Allergy Clin. Immunol.* 113, 482–488.
- Ekman, A.K., Adner, M., Cardell, L.O., 2011. Toll-like receptor 7 activation reduces the contractile response of airway smooth muscle. *Eur. J. Pharmacol.* 652, 145–151.
- Fidock, M., Souberbielle, B., Laxton, C., Rawal, J., Delpuech-Adams, O., Corey, T., Colman, P., Kumar, V., Cheng, J., Wright, K., 2011. The innate immune response, clinical outcomes, and ex vivo HCV antiviral efficacy of a TLR7 agonist (PF-4878691). *Clin. Pharmacol. Ther.* 89, 821–829.
- Gon, Y., 2008. Toll-like receptors and airway inflammation. *Allergol. Int.* 57, 33–37.
- Gorden, K.B., Gorski, K.S., Gibson, S.J., Kedl, R.M., Kieper, W.C., Qiu, X., Tomai, M.A., Alkan, S.S., Vasilakos, J.P., 2005. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. *J. Immunol.* 174, 1259–1268.
- Haagerup, A., Borglum, A., Binderup, H., Kruse, T., 2004. Fine-scale mapping of type I allergy candidate loci suggests central susceptibility genes on chromosomes 3q, 4q and Xp. *Allergy* 59, 88–94.
- Haldar, P., Brightling, C.E., Hargadon, B., Gupta, S., Monteiro, W., Sousa, A., Marshall, R.P., Bradding, P., Green, R.H., Wardlaw, A.J., 2009. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N. Engl. J. Med.* 360, 973–984.
- Hammad, H., Chieppa, M., Perros, F., Willart, M.A., Germain, R.N., Lambrecht, B.N., 2009. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat. Med.* 15, 410–416.
- Hart, O.M., Athie-Morales, V., O'Connor, G.M., Gardiner, C.M., 2005. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN- γ production. *J. Immunol.* 175, 1636–1642.
- Hewson, C.A., Jardine, A., Edwards, M.R., Laza-Stanca, V., Johnston, S.L., 2005. Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. *J. Virol.* 79, 12273–12279.
- Hilvering, B., Tak, T., Tesselaar, K., Koenderman, L., 2015. Similar activation of sputum granulocytes in eosinophilic and non-eosinophilic asthma. *Clin. Transl. Allergy* 5, 1.
- Hoffjan, S., Stemmler, S., Parwez, Q., Petrasch-Parwez, E., Arinir, U., Rohde, G., Reinitz-Rademacher, K., Schultze-Werninghaus, G., Bufer, A., Epplen, J.T., 2005. Evaluation of the toll-like receptor 6 Ser249Pro polymorphism in patients with asthma, atopic dermatitis and chronic obstructive pulmonary disease. *BMC Med. Genet.* 6, 1.
- Hollingsworth, J.W., Cook, D.N., Brass, D.M., Walker, J.K., Morgan, D.L., Foster, W.M., Schwartz, D.A., 2004. The role of Toll-like receptor 4 in environmental airway injury in mice. *Am. J. Respir. Crit. Care Med.* 170, 126–132.
- Hollingsworth, J.W., Whitehead, G.S., Lin, K.L., Nakano, H., Gunn, M.D., Schwartz, D.A., Cook, D.N., 2006. TLR4 signaling attenuates ongoing allergic inflammation. *J. Immunol.* 176, 5856–5862.
- Hopp, R., 1999. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *Pediatrics* 104, 373–374.
- Hornig, T., Barton, G.M., Flavell, R.A., Medzhitov, R., 2002. The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature* 420, 329–333.
- Humbert, M., Beasley, R., Ayres, J., Slavin, R., Hebert, J., Bousquet, J., Beeh, K.M., Ramos, S., Canonica, G., Hedegcock, S., 2005. Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 60, 309–316.
- Hussein, Y.M., Awad, H.A., Shalaby, S.M., Ali, A.S.A., Alzahrani, S.S., 2012. Toll-like receptor 2 and Toll-like receptor 4 polymorphisms and susceptibility to asthma and allergic rhinitis: a case-control analysis. *Cell. Immunol.* 274, 34–38.
- Ito, T., Yang, M., Wang, Y.H., Lande, R., Gregorio, J., Perng, O.A., Qin, X.F., Liu, Y.J., Gilliet, M., 2007. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J. Exp. Med.* 204, 105–115.
- Jain, V.V., Businga, T.R., Kitagaki, K., George, C.L., O'Shaughnessy, P.T., Kline, J.N., 2003. Mucosal immunotherapy with CpG oligodeoxynucleotides reverses a murine model of chronic asthma induced by repeated antigen exposure. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* 285, L1137–L1146.
- Jain, V.V., Kitagaki, K., Businga, T., Hussain, I., George, C., O'Shaughnessy, P., Kline, J.N., 2002. CpG-oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. *J. Allergy Clin. Immunol.* 110, 867–872.
- Jurk, M., Heil, F., Vollmer, J., Schetter, C., Krieg, A.M., Wagner, H., Lipford, G., Bauer, S., 2002. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nat. Immunol.* 3, 499, (499).
- Kaufman, E., Jacoby, D., 2012. Upping the antedrug: is a novel anti-inflammatory Toll-like receptor 7 agonist also a bronchodilator? *Br. J. Pharmacol.* 166, 569–572.
- Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* 11, 373–384.
- Kawai, T., Akira, S., 2011. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650.
- Kim, H.-B., Lee, Y.-C., Lee, S.-Y., Jung, J., Jin, H.-S., Kim, J.-H., Kim, B.-S., Kang, M.-J., Jang, S.-O., Kim, J., 2006. Gene-gene interaction between IL-13 and IL-13R α 1 is associated with total IgE in Korean children with atopic asthma. *J. Hum. Genet.* 51, 1055–1062.
- Kleinjung, T., Arndt, O., Feldmann, H.J., Bockmühl, U., Gehrmann, M., Zilch, T., Pfister, K., Schönberger, J., Marienhagen, J., Eilles, C., 2003. Heat shock protein 70 (Hsp70) membrane expression on head-and-neck cancer biopsy—a target for natural killer (NK) cells. *Int. J. Radiat. Oncol. Biol. Phys.* 57, 820–826.
- Kline, J.N., Kitagaki, K., Businga, T.R., Jain, V.V., 2002. Treatment of established asthma in a murine model using CpG oligodeoxynucleotides. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* 283, L170–L179.
- Kline, J.N., Krieg, A.M., Waldschmidt, T.J., Ballas, Z.K., Jain, V., Businga, T.R., 1999. CpG oligodeoxynucleotides do not require T H1 cytokines to prevent eosinophilic airway inflammation in a murine model of asthma. *J. Allergy Clin. Immunol.* 104, 1258–1264.
- Kokkinopoulos, I., Jordan, W., Ritter, M., 2005. Toll-like receptor mRNA expression patterns in human dendritic cells and monocytes. *Mol. Immunol.* 42, 957–968.
- Kool, M., GeurtsVanKessel, C., Muskens, F., Madeira, F.B., van Nimwegen, M., Kuipers, H., Thieleman, K., Hoogsteden, H.C., Hammad, H., Lambrecht, B.N., 2011. Facilitated antigen uptake and timed exposure to TLR ligands dictate the antigen-presenting potential of plasmacytoid DCs. *J. Leukoc. Biol.* 90, 1177–1190.
- Koppelman, G.H., 2006. Gene by environment interaction in asthma. *Curr. Allergy Asthma Rep.* 6, 103–111.
- Kormann, M.S., Depner, M., Hartl, D., Klopp, N., Illig, T., Adamski, J., Vogelberg, C., Weiland, S.K., von Mutius, E., Kabesch, M., 2008. Toll-like receptor heterodimer variants protect from childhood asthma. *J. Allergy Clin. Immunol.* 122, 86–92, e88.
- Krieg, A.M., 1999. Direct immunologic activities of CpG DNA and implications for gene therapy. *J. Gene Med.* 1, 56–63.
- Kulig, M., Bergmann, R., Klettke, U., Wahn, V., Tacke, U., Wahn, U., Group, M.A.S., 1999. Natural course of sensitization to food and inhalant allergens during the first 6 years of life. *J. Allergy Clin. Immunol.* 103, 1173–1179.
- Kurup, V.P., Raju, R., Manickam, P., 2005. Profile of gene expression in a murine model of allergic bronchopulmonary aspergillosis. *Infect. Immun.* 73, 4381–4384.
- Kvarnhammar, A.M., Tengroth, L., Adner, M., Cardell, L.O., 2013. Innate immune receptors in human airway smooth muscle cells: activation by TLR1/2, TLR3, TLR4, TLR7 and NOD1 agonists. *PLoS One* 8, e68701.
- Lambrecht, B.N., Hammad, H., 2012. The airway epithelium in asthma. *Nat. Med.* 18, 684–692.
- Lambrecht, B.N., Hammad, H., 2015. The immunology of asthma. *Nat. Immunol.* 16, 45–56.
- Lazarus, R., Vercelli, D., Palmer, L.J., Klimecki, W.J., Silverman, E.K., Richter, B., Riva, A., Ramoni, M., Martinez, F.D., Weiss, S.T., 2002. Single nucleotide polymorphisms in innate immunity genes: abundant variation and potential role in complex human disease. *Immunol. Rev.* 190, 9–25.
- Lee, B.L., Moon, J.E., Shu, J.H., Yuan, L., Newman, Z.R., Schekman, R., Barton, G.M., 2013. UNC93B1 mediates differential trafficking of endosomal TLRs. *Elife* 2, e00291.
- Lee, S.G., Kim, B.S., Kim, J.H., Lee, S.Y., Choi, S.O., Shim, J.Y., Hong, T.J., Hong, S.J., 2004. Gene-gene interaction between interleukin-4 and interleukin-4 receptor α in Korean children with asthma. *Clin. Exp. Allergy* 34, 1202–1208.
- Lee, S.O., Brown, R.A., Kang, S.H., Abdel-Massih, R.C., Razanable, R.R., 2011. Toll-like receptor 2 polymorphism and gram-positive bacterial infections after liver transplantation. *Liver Transpl.* 17, 1081–1088.
- Lin, R., Heylbroeck, C., Genin, P., Pitha, P.M., Hiscott, J., 1999. Essential role of interferon regulatory factor 3 in direct activation of RANTES chemokine transcription. *Mol. Cell. Biol.* 19, 959–966.
- Liu, H., Komai-Koma, M., Xu, D., Liew, F.Y., 2006. Toll-like receptor 2 signaling modulates the functions of CD4+ CD25+ regulatory T cells. *Proc. Natl. Acad. Sci. USA* 103, 7048–7053.
- Manna, S.K., Rangasamy, T., Wise, K., Sarkar, S., Shishodia, S., Biswal, S., Ramesh, G.T., 2006. Long term environmental tobacco smoke activates nuclear transcription factor-kappa B, activator protein-1, and stress responsive kinases in mouse brain. *Biochem. Pharmacol.* 71, 1602–1609.
- Martinez, F.D., Holt, P.G., 1999. Role of microbial burden in aetiology of allergy and asthma. *Lancet* 354, SII12–SII15.
- Maruoka, S., Wilson, R., Whitehead, G., Foley, J., Flake, G., Sever, M., Zeldin, D., Kraft, M., Garantziotis, S., Hashimoto, S., 2011. The toll-like receptor (TLR) 5 ligand, flagellin, promotes asthma by priming allergic responses to indoor allergens. *Eur. Respir. J.* 38, 4891.
- Meng, L., He, X., Zhu, W., Yang, X., Jiang, C., Sun, Q., Raza, A., Zhang, S., Xue, Q., Xie, X., 2011. TLR3 and TLR7 modulate IgE production in antigen induced pulmonary inflammation via influencing IL-4 expression in immune organs. *PLoS One* 6, e17252.
- Mikacenic, C., Reiner, A.P., Holden, T.D., Nickerson, D.A., Wurfel, M.M., 2013. Variation in the TLR10/TLR1/TLR6 locus is the major genetic determinant of interindividual difference in TLR1/2-mediated responses. *Genes Immun.* 14, 52–57.

- Moisan, J., Camateros, P., Thuraingam, T., Marion, D., Koohsari, H., Martin, P., Boghdady, M., Ding, A., Gaestel, M., Guio, M., 2006. TLR7 ligand prevents allergen-induced airway hyperresponsiveness and eosinophilia in allergic asthma by a MYD88-dependent and MK2-independent pathway. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* 290, L987–L995.
- Møller-Larsen, S., Nyegaard, M., Haagerup, A., Vestbo, J., Kruse, T.A., Børglum, A.D., 2008. Association analysis identifies TLR7 and TLR8 as novel risk genes in asthma and related disorders. *Thorax* 63, 1064–1069.
- Moreira, A.P., Cavassani, K.A., Ismailoglu, U.B., Hullinger, R., Dunleavy, M.P., Knight, D.A., Kunkel, S.L., Uematsu, S., Akira, S., Hogaboam, C.M., 2011. The protective role of TLR6 in a mouse model of asthma is mediated by IL-23 and IL-17A. *J. Clin. Investig.*, 121.
- Morris, G.E., Whyte, M.K., Martin, G.F., Jose, P.J., Dower, S.K., Sabroe, I., 2005. Agonists of toll-like receptors 2 and 4 activate airway smooth muscle via mononuclear leukocytes. *Am. J. Respir. Crit. Care Med.* 171, 814–822.
- Morris, G.E., Parker, L.C., Ward, J.R., Jones, E.C., Whyte, M.K., Brightling, C.E., Bradding, P., Dower, S.K., Sabroe, I., 2006. Cooperative molecular and cellular networks regulate Toll-like receptor-dependent inflammatory responses. *FASEB J.* 20, 2153–2155.
- Niimi, K., Asano, K., Shiraishi, Y., Nakajima, T., Wakaki, M., Kagyo, J., Takihara, T., Suzuki, Y., Fukunaga, K., Shiomi, T., 2007. TLR3-mediated synthesis and release of eotaxin-1/CCL11 from human bronchial smooth muscle cells stimulated with double-stranded RNA. *J. Immunol.* 178, 489–495.
- Oldenburg, M., Krüger, A., Ferstl, R., Kaufmann, A., Nees, G., Sigmund, A., Bathke, B., Lauterbach, H., Suter, M., Dreher, S., 2012. TLR13 recognizes bacterial 23S rRNA devoid of erythromycin resistance-forming modification. *Science* 337, 1111–1115.
- O'Neill, L.A., 2006. How Toll-like receptors signal: what we know and what we don't know. *Curr. Opin. Immunol.* 18, 3–9.
- Ozinsky, A., Underhill, D.M., Fontenot, J.D., Hajjar, A.M., Smith, K.D., Wilson, C.B., Schroeder, L., Adorem, A., 2000. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc. Natl. Acad. Sci. USA* 97, 13766–13771.
- Parker, J.M., Oh, C.K., LaForce, C., Miller, S.D., Pearlman, D.S., Le, C., Robbie, G.J., White, W.I., White, B., Molino, N.A., 2011. Safety profile and clinical activity of multiple subcutaneous doses of MEDI-528, a humanized anti-interleukin-9 monoclonal antibody, in two randomized phase 2a studies in subjects with asthma. *BMC Pulm. Med.* 11, 1.
- Parker, L.C., Whyte, M.K., Dower, S.K., Sabroe, I., 2005. The expression and roles of Toll-like receptors in the biology of the human neutrophil. *J. Leukoc. Biol.* 77, 886–892.
- Pham Van, L., Bardel, E., Gregoire, S., Vanoirbeek, J., Schneider, E., Dy, M., Thiebaut, N., 2011. Treatment with the TLR7 agonist R848 induces regulatory T-cell-mediated suppression of established asthma symptoms. *Eur. J. Immunol.* 41, 1992–1999.
- Plattner, F., Yarovsky, F., Romero, S., Didry, D., Carlier, M.F., Sher, A., Soldati-Favre, D., 2008. Toxoplasma profilin is essential for host cell invasion and TLR11-dependent induction of an interleukin-12 response. *Cell Host Microbe* 3, 77–87.
- Pockley, A.G., 2003. Heat shock proteins as regulators of the immune response. *Lancet* 362, 469–476.
- Pockros, P.J., Guyader, D., Patton, H., Tong, M.J., Wright, T., McHutchison, J.G., Meng, T.-C., 2007. Oral resiquimod in chronic HCV infection: safety and efficacy in 2 placebo-controlled, double-blind phase IIa studies. *J. Hepatol.* 47, 174–182.
- Roponen, M., Yerkovich, S.T., Hollans, E., Sly, P.D., Holt, P.G., Upham, J.W., 2010. Toll-like receptor 7 function is reduced in adolescents with asthma. *Eur. Respir. J.* 35, 64–71.
- Sagar, S., Verheijden, K.A., Georgiou, N.A., Garssen, J., Kraneveld, A.D., Vos, A.P., Folkerts, G., 2013. Differential regulation of inflammation and immunity in mild and severe experimental asthma. *Mediat. Inflamm.* 2013.
- Sato, T., Shimosato, T., Alvord, W.G., Klinman, D.M., 2008. Suppressive oligodeoxynucleotides inhibit silica-induced pulmonary inflammation. *J. Immunol.* 180, 7648–7654.
- Sebastiani, P., Lazarus, R., Weiss, S.T., Kunkel, L.M., Kohane, I.S., Ramoni, M.F., 2003. Minimal haplotype tagging. *Proc. Natl. Acad. Sci. USA* 100, 9900–9905.
- Sel, S., Wegmann, M., Sel, S., Bauer, S., Garn, H., Alber, G., Renz, H., 2007. Immunomodulatory effects of viral TLR ligands on experimental asthma depend on the additive effects of IL-12 and IL-10. *J. Immunol.* 178, 7805–7813.
- Shen, E., Lu, L., Wu, C., 2008. TLR7/8 ligand, R-848, inhibits IgE synthesis by acting directly on B lymphocytes. *Scand. J. Immunol.* 67, 560–568.
- Shi, Z., Cai, Z., Sanchez, A., Zhang, T., Wen, S., Wang, J., Yang, J., Fu, S., Zhang, D., 2011. A novel Toll-like receptor that recognizes vesicular stomatitis virus. *J. Biol. Chem.* 286, 4517–4524.
- Shikhagaie, M., Bergqvist, A., Andersson, C., Mori, M., Krohn, K., Dahl, R., Hoffman, H.J., Björner, L., Erjefält, J., 2013. Altered distribution patterns of TLR5 and TLR7 in the central airways of severe asthmatics (P4427). *J. Immunol.* 190, 206.202–206.202.
- Silverman, E.S., Drazen, J.M., 2003. Immunostimulatory DNA for asthma: better than eating dirt. *Am. J. Respir. Cell Mol. Biol.* 28, 645–647.
- Stowell, N.C., Seideman, J., Raymond, H.A., Smalley, K.A., Lamb, R.J., Egenolf, D.D., Bugelski, P.J., Murray, L.A., Marsters, P.A., Bunting, R.A., 2009. Long-term activation of TLR3 by poly (I:C) induces inflammation and impairs lung function in mice. *Respir. Res.* 10, 1.
- Sukkar, M.B., Xie, S., Khorasani, N.M., Kon, O.M., Stanbridge, R., Issa, R., Chung, K.F., 2006. Toll-like receptor 2, 3, and 4 expression and function in human airway smooth muscle. *J. Allergy Clin. Immunol.* 118, 641–648.
- Szefer, S.J., 2013. Advances in pediatric asthma in 2012: moving toward asthma prevention. *J. Allergy Clin. Immunol.* 131, 36–46.
- Tabeta, K., Hoebe, K., Janssen, E.M., Du, X., Georgel, P., Crozat, K., Mudd, S., Mann, N., Sovath, S., Goode, J., 2006. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. *Nat. Immunol.* 7, 156–164.
- Takeda, K., Kaisho, T., Akira, S., 2003. Toll-like receptors. *Annu. Rev. Immunol.* 21, 335–376.
- Tamura, T., Yanai, H., Savitsky, D., Taniguchi, T., 2008. The IRF family transcription factors in immunity and oncogenesis. *Annu. Rev. Immunol.* 26, 535–584.
- Tantisira, K., Klimecki, W., Lazarus, R., Palmer, L., Raby, B., Kwiatkowski, D., Silverman, E., Vercelli, D., Martinez, F., Weiss, S., 2004. Toll-like receptor 6 gene (TLR6): single-nucleotide polymorphism frequencies and preliminary association with the diagnosis of asthma. *Genes Immun.* 5, 343–346.
- Tesse, R., Pandey, R., Kubesch, M., 2011. Genetic variations in toll-like receptor pathway genes influence asthma and atopy. *Allergy* 66, 307–316.
- Torres, D., Dieudonné, A., Ryffel, B., Vilain, E., Si-Tahar, M., Pichavant, M., Lassalle, P., Trottein, F., Gosset, P., 2010. Double-stranded RNA exacerbates pulmonary allergic reaction through TLR3: implication of airway epithelium and dendritic cells. *J. Immunol.* 185, 451–459.
- Vabulas, R.M., Ahmad-Nejad, P., da Costa, C., Miethke, T., Kirschning, C.J., Häcker, H., Wagner, H., 2001. Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J. Biol. Chem.* 276, 31332–31339.
- Verheijden, K., Kraneveld, A., Georgiou, N., Garssen, J., Folkerts, G., Sagar, P.S., 2012. Different Toll-Like receptors (tlrs) and nod-like receptors (nlrs) expression profiles in lung tissue during mild and severe experimental asthma. *Am. J. Respir. Crit. Care Med.* 185, A4183.
- Wagner, H., 1999. Bacterial CpG DNA activates immune cells to signal infectious danger. *Adv. Immunol.* 73, 329–368.
- Wang, D.G., Fan, J.B., Siao, C.J., Berno, A., Young, P., Sapolsky, R., Ghandour, G., Perkins, N., Winchester, E., Spencer, J., 1998. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 280, 1077–1082.
- Wang, Q., Nagarkar, D.R., Bowman, E.R., Schneider, D., Gosangi, B., Lei, J., Zhao, Y., McHenry, C.L., Burgens, R.V., Miller, D.J., 2009. Role of double-stranded RNA pattern recognition receptors in rhinovirus-induced airway epithelial cell responses. *J. Immunol.* 183, 6989–6997.
- Weigt, H., Nassenstein, C., Tschernig, T., Mühlradt, P.F., Krug, N., Braun, A., 2005. Efficacy of macrophage-activating lipopeptide-2 combined with interferon- γ in a murine asthma model. *Am. J. Respir. Crit. Care Med.* 172, 566–572.
- Werner, M., Topp, R., Wimmer, K., Richter, K., Bischof, W., Wjst, M., Heinrich, J., 2003. TLR4 gene variants modify endotoxin effects on asthma. *J. Allergy Clin. Immunol.* 112, 323–330.
- Wilson, R.H., Ahmuka, S., Whitehead, G.S., Foley, J.F., Flake, G.P., Sever, M.L., Zeldin, D.C., Kraft, M., Garantziotis, S., Nakano, H., 2012. The Toll-like receptor 5 ligand flagellin promotes asthma by priming allergic responses to indoor allergens. *Nat. Med.* 18, 1705–1710.
- Wu, C.A., Puddington, L., Whiteley, H.E., Yamouyiannis, C.A., Schramm, C.M., Mohammad, F., Thrall, R.S., 2001. Murine cytomegalovirus infection alters Th1/Th2 cytokine expression, decreases airway eosinophilia, and enhances mucus production in allergic airway disease. *J. Immunol.* 167, 2798–2807.
- Wu, D., Zhou, J., Bi, H., Li, L., Gao, W., Huang, M., Adcock, I.M., Barnes, P.J., Yao, X., 2014. CCL11 as a potential diagnostic marker for asthma? *J. Asthma* 51, 847–854.
- Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., 2003. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* 301, 640–643.
- Yang, C.A., Chiang, B.L., 2015. Toll-like receptor 1 N248S polymorphism affects T helper 1 cytokine production and is associated with serum immunoglobulin E levels in Taiwanese allergic patients. *J. Microbiol. Immunol. Infect.*
- Yarovinsky, F., Zhang, D., Andersen, J.F., Bannenberg, G.L., Serhan, C.N., Hayden, M.S., Hieny, S., Sutterwala, F.S., Flavell, R.A., Ghosh, S., 2005. TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 308, 1626–1629.
- Zhang, D., Zhang, G., Hayden, M.S., Greenblatt, M.B., Bussey, C., Flavell, R.A., Ghosh, S., 2004. A toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 303, 1522–1526.
- Zhang, X., Shan, P., Qureshi, S., Homer, R., Medzhitov, R., Noble, P.W., Lee, P.J., 2005. Cutting edge: TLR4 deficiency confers susceptibility to lethal oxidant lung injury. *J. Immunol.* 175, 4834–4838.