



Full Length Article

The role of endosomal toll-like receptors in asthma

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ABSTRACT

Asthma is a heterogeneous inflammatory disease caused by association of genetic and environmental factors and its incidence has significantly increased over the latest years. The clinical manifestations of asthma are the result of airway hyper-reactivity to a variety of triggers such as aeroallergens, viral and bacterial components. Toll-like receptors (TLRs) are pathogen associated molecular pattern receptors, which are also expressed in the lung tissue as well as in several cells of the innate and adaptive immune system. Ligation of TLRs results in alterations in the expression of several inflammatory and anti-inflammatory mediators, which are known to be involved in the pathogenesis of asthma. The endosomal TLRs have been shown to be associated with the induction of asthmatic inflammation (TLR3), and with disease exacerbations (TLR7, TLR8 and TLR9). Targeting these receptors seems to be an effective choice for suppressing airway inflammation, eosinophilia and airway hyperresponsiveness in asthmatic patients. In this review we provide information regarding endosomal TLRs and their role in the pathogenesis of asthma as well as their potential use as targets for the development of novel treatments for the therapy of asthma.

1. Introduction

Asthma is a heterogeneous inflammatory disease, characterised by a history of respiratory symptoms including wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity usually related to variable expiratory flow limitation (Global Initiative for Asthma, 2014). The disease is more common in industrialised and urbanised areas and its prevalence has increased worldwide over the last decades (Mannino et al., 2002; Masoli et al., 2004; James et al., 2010). It is estimated that asthma affects approximately 300 million people of all ages, races and geographic origins whereas it is believed that over 100 million more people will be affected by 2025 (Masoli et al., 2004).

The clinical manifestations of asthma are the result of airway hyper-reactivity to several stimuli. This hyper-reactivity is associated to inflammatory infiltration of the airways by neutrophils, monocytes and eosinophils, leading to excessive mucus production and hyperplasia of the airway smooth muscle, which result in airflow limitation (Busse and Lemanske, 2001).

It is now well established that the main immunological pathway in the development of allergic asthma includes infiltration and accumula-

tion of polarised CD4 T helper 2 (Th2) cells in the airway mucosa (Kay, 2001). These cells, produce and secrete several inflammatory cytokines, such as interleukin (IL) 4, IL5, IL9, and IL13, which are related to the fundamental pathologic features of asthma (Robinson et al., 1992). In general, Th2 cells contribute to the pathology of allergic inflammation in asthma via two different mechanisms. The first includes IL4 production by the Th2 cells which stimulates B lymphocytes to produce allergen-specific immunoglobulin (Ig)E. IgE is subsequently bound to specific receptors (FcεR) expressed on cell surface of mast cells and this reaction leads to their degranulation resulting in the allergic response and airway hyperresponsiveness (Platts-Mills, 2001). The second mechanism through which Th2 cells result to airway hyperresponsiveness in asthmatic patients is by recruiting eosinophils to the airways mainly through production of IL5 (Platts-Mills, 2001). Following their recruitment, eosinophils produce and release several mediators which contribute to the development of airway hyperresponsiveness (Hogan et al., 1997).

It is widely supported that Th1 cytokines have the ability to downregulate Th2 responses (Mosmann and Coffman, 1989). Since Th2 cells and their mediators have principal role to the pathophysiology of allergic asthma it has been suggested that the enhanced number

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and function of these Th2 cells is related to a decreased activation of Th1 cells probably caused by environmental factors (Camateros et al., 2006). This is the basis of the so-called hygiene hypothesis, which suggests that a lack of exposure to infective microorganisms during childhood increases susceptibility to allergic diseases and asthma due to deficient maturation of the immune system (Strachan, 2000; Romagnani, 2007). This hypothesis has been based on the presence of evidence that overcrowding unhygienic conditions, exposure to animals and infections (e.g. hepatitis A, measles) or immunisation with BCG result in a lower prevalence of atopic manifestations (Shaheen et al., 1996; Matricardi et al., 1997; Strachan, 1997; Douwes et al., 2007; Simpson et al., 2008). According to that, it seems that since bacteria and viruses elicit Th1 immunologic responses, insufficient exposure to Th1 stimulators, results to insufficient down-regulation of Th2 immune responses and to the development of allergic reactions (Folkerts et al., 2000). However, it is a fact that the majority of asthma exacerbations in both children and adults with asthma are triggered by infectious organisms such as respiratory viruses, which are known to induce a Th1 response mediated by interferon- γ (IFN- γ) (Johnston et al., 1995) and this observation leads to the conclusion that not all infections are protective.

It is generally accepted that the development of asthma is the result of the interaction between genetic predisposition and environmental factors. In the latest years there is a dramatic change on two of the most important environmental factors. The first is the decreased incidence of infection, as a result of vaccination programs, sterile food preparations and the widespread use of antibiotics and the second is the alterations in diet especially regarding the consumption of saturated and unsaturated fats (Phipps et al., 2007). These two major alterations in the environmental conditions are believed to result in the underdevelopment of immunoregulatory mechanisms resulting in abnormal maturation of the adaptive immune system and to a shift towards Th2 response (Holt et al., 2005). Although there is still lack of full understanding of the generation of Th2 cell responses, there is emerging evidence showing that inappropriate stimulation of specific Toll-like receptors (TLRs) may play a role (Phipps et al., 2007). It is thus believed that microbial exposures may activate innate immune pathways through expression of TLRs and suppress Th2 cell immunity (Braun-Fahrlander et al., 2002; Ege et al., 2006).

2. Toll-like receptors

The first line response of an organism to identify and react to an invading pathogen is innate immunity, with several different mechanisms, pathways, proteins and/or cells included in this process. One of the most significant families of proteins that are able to identify and activate antigen presenting cells (APCs) are pattern recognition receptors (PRRs). They recognise conserved pathogen-associated molecular patterns (PAMPs, or microbe-associated molecular patterns (MAMPs)) and danger-associated molecular patterns (DAMPs) (Iwasaki and Medzhitov, 2010). One of the transmembrane PRRs is the TLR family of proteins (Cook et al., 2003).

The TLR family includes 10 different proteins that are either located in the cell membrane or in endosomes (Takeda and Akira, 2005) (Table 1). The first member of the family was identified in *Drosophila* (Hashimoto et al., 1988). Later on its antimicrobial potency was described (Lemaitre et al., 1996) and only shortly after, the identification of a human homolog was announced (Medzhitov et al., 1997). Discovery of TLRs has dramatically improved our knowledge of the molecular mechanisms involved in innate immunity, while there are increasing data suggesting their involvement not only in innate but also in activation and transition to acquired immunity (Iwasaki and Medzhitov, 2010).

TLRs are distributed in different cellular sites. More specifically TLR1, TLR2, TLR4, TLR5, TLR6 are mainly located in the cell membrane, while TLR3, TLR7, TLR8 and TLR9 have been shown to

Table 1
TLRs expression, PAMPs, DAMPs and signaling pathways (Werling and Jungi, 2003; Takeda and Akira, 2005; Cervantes et al., 2012).

	Cell distribution	PAMPs	DAMPs	Signal effector	Secreted Cytokines/ Chemokines	Alternate name	GeneID
TLR1	Cell surface Mo, M ϕ , DC, B, EC	Triacylated lipopeptides peptidoglycans LPS	HSP60, HSP70 Gp96, HMGB1, proteoglycans	TIRAP, MyD88, Mal	ILs	CD281, TIL, TIL, LPRS5, rsc786	7096
TLR2	Cell surface Mo, M ϕ , DC, B, EC, SMC	Triacylated lipopeptides peptidoglycans, LPS	HSP60, HSP70 Gp96, HMGB1, proteoglycans	TIRAP, MyD88, Mal	ILs	CD282, TIL4	7097
TLR3	Endosomes B, T, NK, EC DC, SMC	dsRNA, tRNA, siRNA	mRNA tRNA	TRIF	ILs, type I IFNs	CD283, IIAE2	7098
TLR4	Cell surface and endosomes Mo, M ϕ , DC, MC, EC, SMC	LPS palmitate Flagellin	HSP22, HSP60, HSP70, HSP72, Gp96, HMGB1, proteoglycans fibronectin, tenascin-C Unknown	TRAM, TRIF TIRAP, MyD88 Mal MyD88	ILs, type I IFNs	ARMD10, CD284, TLR -4, TOLL	7099
TLR5	Cell surface Mo, M ϕ , DC, EC				IC	MELIOS, SLE1, SLEB1, TIL3	7100
TLR6	Cell surface Mo, M ϕ , MC, B	Diacylated lipopeptides ssRNA, imidazoquinolines, guanosine analogs	HSP60, HSP70 Gp96, HMGB1, proteoglycans ssRNA	TIRAP, MyD88, Mal MyD88	IC	CD286	10333
TLR7	Endosomes Mo, M ϕ , DC, B				ILs, type I IFNs	TLR7-like	51284
TLR8	Endosomes Mo, M ϕ , DC, MC	ssRNA, imidazoquinolines	ssRNA	MyD88	ILs, type I IFNs	CD288	51311
TLR9	Endosomes Mo, M ϕ , DC, B, T, EC	CpG DNA, CpG ODNs	Chromatin IgG complex	MyD88	ILs, type I IFNs	CD289	54106
TLR10	Endosomes Mo, M ϕ , DC	Profilin-like proteins	Unknown	MyD88	ILs	CD290	81793

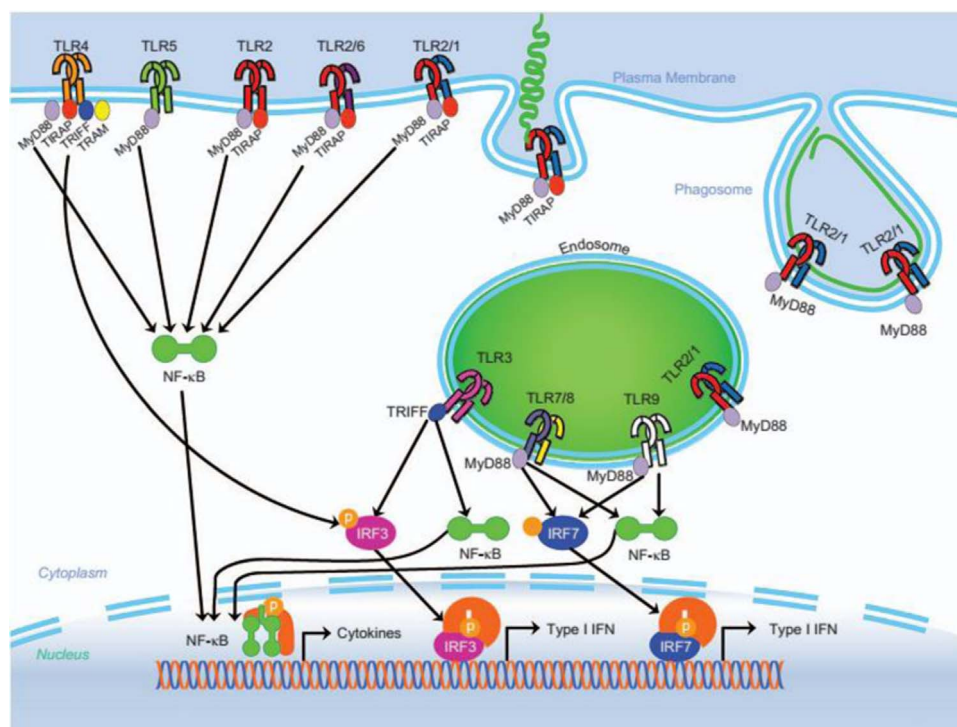


Fig. 1. Toll-like receptor cell localisation and signaling pathway reproduced with permission from (Cervantes et al., 2012).

be localized in the endosomal compartment (Fig. 1) (Nishiya and DeFranco, 2004). TLRs are transmembrane proteins with extra-membrane leucine-rich repeats (LRRs) and an intra-membrane highly conserved region called Toll/IL1 receptor (TIR). In line with their localisation, membrane TLRs identify molecules of the microbe cell surface, while endosomal TLRs recognise breakdown products; either single or double stranded, DNA or RNA sequences. Even though TLRs were identified as key members of the innate immunity system, they are highly specific, while surprisingly they usually are specific for more than one molecule (Table 1). TLRs are highly expressed in tissues involved with immune responses and tissues exposed to pathogens (Zarembler and Godowski, 2002; Takeda and Akira, 2005).

2.1. TLR signaling

The TLR signaling cascade aims in initiation of biological and physiological responses towards a recognised pathogen. The initial events of this cascade require interactions between TLRs and adaptor molecules that link the receptor complex to downstream signaling molecules, mediated by modular homophilic domains present in both Toll-like and IL-1 receptors (Cook et al., 2003). After the identification of the pathogen, TLRs homo- or hetero-dimerise in order to initiate the downstream signaling cascade (Takeda and Akira, 2004; Kawai and Akira, 2006).

Two major pathways have been identified (Fig. 1); the first involves the TIR domain of TLRs and MyD88 dependent phosphorylation of the IL-1 receptor-associated kinase (IRAK). IRAK associates with tumor necrosis factor receptor associated factor 6 (TRAF6), leading to the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), c-Jun N-terminal kinases (JNKs) and activator protein 1 (AP1). The second pathway is MyD88 independent and involves the activation of the interferon regulatory factor 3 (IRF-3) via the TIR domain-containing adaptor inducing IFNβ (TRIF), resulting in induction of several IFN-inducible genes. However, this pathway seems to have an induction delay and impaired inflammatory cytokine production. Several other TIR containing domain molecules have been identified, including Toll-interacting protein (Tollip/Mal), TIRAP and

TRAM that can provide alternate pathways for activation or modulation of activation (Takeda and Akira, 2004; Kawai and Akira, 2006), though mainly for cell membrane TLRs.

There is growing evidence that TLRs may trigger responses not only as homo- or hetero-dimers but also as a synergistic manner in which TLR-TLR interactions facilitate responses to viral and bacterial pathogens (Kawai and Akira, 2011). Synergistic effects have been observed between endosomal TLRs when monocyte derived macrophages and dendritic cells (DCs) are co-stimulated with TLR8, TLR3 and TLR4 ligands and between TLRs and other PRRs including the nucleotide oligomerisation domain receptor (NOD) to induce inflammatory cytokine production (Makela et al., 2009; Saiga et al., 2011).

2.2. TLR role in innate and adaptive immunity

The innate immunity system is based on pattern recognition of germ-line encoded PAMPs of specific classes of microorganisms, while the adaptive immune systems is based on highly specific clonally expressed receptors and it is their combination, that renders the immune system of mammals highly effective (Palm and Medzhitov, 2009).

TLR cascade pathway causes the production of a vast array of cytokines/chemokines that are produced by APCs, DCs, T and B lymphocytes, macrophages (MΦs) and plasmacytoid dendritic cells (pDCs). TLR signaling can lead to the production of key inflammatory chemokines connected to microbial infection including type I IFNs (α, β), ILs (6, 8, 10, 12), TNF, CXCL1, MCPs 1-4) and CCLs (Iwasaki and Medzhitov, 2004). Furthermore, TLRs have been suggested to induce activation of several effector classes of adaptive immune responses including antibody production (IgM, IgA, IgG) and T cell responses (Th1, Th17, cytotoxic T cells) (Iwasaki and Medzhitov, 2004; 2010). Finally, TLRs have been connected with antibody production regulation and B cell maturation and activation (Bernasconi et al., 2003; Hua and Hou, 2013).

Moreover, signaling from TLRs induces the upregulation of costimulatory molecules on the specialised DCs and may serve to their resistance to immunosuppression by T regulatory cells (TREGs) (Berk

et al., 2014). DC maturation, is essential for the induction of pathogen-specific adaptive immune responses, indicating that TLRs may serve as a link between innate and adaptive immunity (Iwasaki and Medzhitov, 2004). Furthermore, TLRs, especially those expressed in endosomes, seem to be crucial in antigen presentation through MHC type II molecules (Blander and Medzhitov, 2006). Cellular migration to inflamed tissue is controlled mainly through diffusible chemotactic factors and cell adhesion molecules (Laudanna et al., 2002), some of which are produced via TLR signaling (Huang et al., 2001).

2.3. TLRs in asthma pathogenesis

Asthma is a disease with a defined clinical manifestation, in which the underlying physiological and molecular mechanisms are heterogeneous (Anderson, 2008; Lotvall et al., 2011). TLRs have been linked to autoimmune responses (Stetson, 2009), allograft rejection (Goldstein et al., 2003) and pulmonary diseases (Phipps et al., 2007; Bezemer et al., 2012).

Initial studies in asthma focused on polarised CD4 T helper 2 (Th2) cells in the airway mucosa (Kay, 2001) and linked the inflammatory cytokines produced by them (IL4, IL5, IL9, and IL13) to the fundamental pathologic features of asthma (Robinson et al., 1992). Th2 type reaction has been linked mainly with humoral reactions to extracellular infections involving production of IgE from B lymphocytes, stimulation and sequential degranulation of mast cells, resulting in the allergic response and airway hyperresponsiveness (Platts-Mills, 2001). Furthermore, Th2 cell polarisation results eosinophil recruitment in the airways, mainly through production of IL5, leading to further production and release of several mediators, which contribute to airway hyperresponsiveness (Hogan et al., 1997; Platts-Mills, 2001). However, there seem to be many more types of cells contributing in the pathogenesis of asthma and the different endotypes described (Anderson, 2008; Lotvall et al., 2011; Lambrecht and Hammad, 2015).

TLRs are not only expressed in the residing and recruited immune cells of the airway epithelium (Iwasaki and Medzhitov, 2004) but also in the pulmonary epithelial cells (ECs), airway smooth muscle cells (ASMCs) (Table 1) (Akbari et al., 2003; Lambrecht and Hammad, 2003; Phipps et al., 2007) and the most recently described innate lymphoid cells (ILCs) (Hazenberg and Spits, 2014).

The plausible connection of TLRs in asthma involves deregulation of the innate immunity response to an identified MAMP and a shift to a Th2 type immune response. This would include activation of residential mast cells in acute response, and activation of Th2 cells and recruitment of eosinophils in late response. These two events combined lead to airway hyperreactivity and airflow obstruction (Barnes, 2008). Furthermore, TLR signaling has been connected to activation of lung DCs, and resistance to immunosuppression by TREGs (Berk et al., 2014) that seem to be involved in both the induction and maintenance of asthmatic phases (Akbari et al., 2003). Moreover, TLR3 has been linked to a shift to a Th2 response to inhaled antigens, including smoke (Bezemer et al., 2012). There is also supporting evidence that mast cell degranulation may be influenced by the TLR signaling cascade (Kulka et al., 2004) and that TLR overexpression in eosinophils is a significant mechanism against viral infection and exacerbation of allergic inflammation (Nagase et al., 2003).

In non-immune cells, ECs from asthmatics have shown overproduction of mucin and heat shock proteins (HSPs) as well as increased activation of NF- κ B, AP-1 and other transcription factors of the TLR signaling pathway (Holgate et al., 2003). Even though, microbe infection of ECs is recognised to lead to production of pro-inflammatory mediators, damage of the epithelium from environmental stimuli and pathogens may affect TLR signaling pathways and promote aberrant inflammatory responses (Monick et al., 2003). Furthermore, ECs with their stromal cells have been shown to produce thymic stromal lymphopoietin (TSLP), an epithelial-derived cytokine that has recently been implicated in both asthma and atopic dermatitis

by activating DCs to prime Th2 cell responses (Soumelis et al., 2002). TSLP has been shown to be expressed after the activation of TLRs via their MAMPs (Takai et al., 2014).

More specifically, for endosomal TLRs, TLR3 ligands have been connected with TSLP-mediated activation of DCs and promotion of Th17 differentiation and production of the highly inflammatory cytokines IL-17 and IL-25. These cytokines have been implicated in pathogenesis of inflammatory disorders including severe asthma (Bezemer et al., 2012). Furthermore, long-term activation of TLR3 has been shown to induce inflammation and impair respiratory function in mice (Stowell et al., 2009). TLR7 and TLR8 have been linked to neutrophilic activation and possible disease exacerbations, while TLR7 and TLR9 have been linked to the activation of eosinophils during viral respiratory infections, suggesting their possible contribution to allergic exacerbations. Furthermore, TLR9 agonists seem to activate Th1 responses, attenuating the Th2 response primarily involved in the allergic phenotype of asthma (Bezemer et al., 2012). Finally, defects in the production of TLR7 have been linked to increased susceptibility to viruses and a virus-induced asthma phenotype in mice (Kaiko et al., 2013).

Several polymorphisms have been identified in TLR genes, that have been shown to correlate with reduced or increased risk of asthma development, but are mostly focused on membrane TLRs (Kormann et al., 2008; Kanagaratham et al., 2011; Medvedev, 2013) or TLR7/8 (Moller-Larsen et al., 2008).

3. Targeting endosomal TLRs for the treatment of asthma

The most common triggers in the development of asthmatic exacerbations are viral infections, and rhinovirus is one of the commonest causes of the development of wheezing especially in atopic children (Bacharier et al., 2007). Since endosomal TLRs are able to recognise the genomic material of invading pathogens, and to trigger antiviral responses, they can be considered as important targets for the development of novel treatments for asthmatic exacerbations (Table 2), especially for those of viral etiology (Aryan et al., 2014).

Studies in animal models have shown that TLR3 activation can alert the immune response and inflammation, mimicking a viral infection that leads to AHR and bronchospasm (Morishima et al., 2008; Shiraishi et al., 2008). Targeting TLR3, several inhibitors have been used to reduce AHR; these include capsazepinoids, single-stranded DNA oligodeoxynucleotides (ssDNA-ODNs) and resveratrol (Aryan et al., 2014). Capsazepinoids are able to suppress the production of inflammatory cytokines such as TNF- α and IL-8 in airway epithelial cells from asthmatic subjects and they contribute to ASMC relaxation (Mahmutovic-Persson et al., 2012). ssDNA-ODNs seem to inhibit the production of pro-inflammatory cytokines triggered by TLR3 activation in PBMCs (Skold et al., 2012). When administered intranasally they seem to be able to prevent Th2 airway inflammation in non-human primates (Skold et al., 2012; Aryan et al., 2014). Finally, resveratrol, a polyphenol found in red grapes, red wine, blueberries and many other plants, seems to have a protective effect in asthmatic inflammation caused by infection from RSV by preventing upregulation of TLR3 expression (Zang et al., 2011).

Surprisingly, although TLR3 activation has been shown to induce inflammatory immunological responses, one study has shown that a TLR3 agonist, polyinosinic: polycytidylic acid (Poly(I: C)) was able to reduce AHR, inflammation and IgE production in animal models with asthma, possibly via the induction of IL-10 and IL-12 (Sel et al., 2007).

In recent years, it has been shown that TLR7 stimulation seems to prevent Th2-mediated airway inflammation in animal models of asthma (Kaufman and Jacoby, 2012), thus, targeting TLR7 has gained extensive interest in the treatment of asthma (Drake et al., 2012, 2013). Synthetic TLR7 agonists that have been developed include imidazoquinolines imiquimod (R837), resiquimod (R848) guanine nucleoside analogs and the 2-substituted-8-hydroxyadenine derivative (SA-2).

Table 2

Compounds targeting TLRs and their possible therapeutic potential in asthmatic inflammation.

Target molecule or molecule	Target and function	Action	Reference
Poly(I:C)	TLR3 agonist	Reduction of AHR, inflammation and IgE production via induction of IL10 and IL12	(Sel et al., 2007)
Capsazepinoids	TLR3 inhibitor	They suppress the production of TNF- α and CXCL-8 in airway epithelial cells from asthmatic subjects and they contribute to airway smooth muscle cell relaxation	(Mahmutovic-Persson et al., 2012)
Single-stranded DNA oligonucleotides	TLR3 inhibitor	They inhibit production of pro-inflammatory cytokines triggered by TLR3 activation in PBMCs and prevent Th2 airway inflammation in non-human primates	(Skold et al., 2012)
Resveratrol	TLR3 inhibitor	Prevents upregulation of TLR3 expression	(Zang et al., 2011)
Imiquimod (R837)	TLR7 agonist	Reduces the production of inflammatory cytokines (IL-4, IL-5 and IL-13) and eosinophilic inflammation in animal models Reduces alveolar macrophages, B cells and TNF- α and increases the number of dendritic cells and natural killer cells in lung tissue and IL-10 production in mice. Has significant bronchodilator effects	(Moisan et al., 2006; Meng et al., 2011; Drake et al., 2012). (Hackstein et al., 2012)
Resiquimod (R848)	TLR7 agonist	Reduces the production of inflammatory cytokines (IL-4, IL-5 and IL-13) and eosinophilic inflammation in animal models	(Kaufman et al., 2011; Drake et al., 2013). (Moisan et al., 2006; Meng et al., 2011; Drake et al., 2012).
2-substituted-8-hydroxyadenine derivative (SA-2).	TLR7/8 agonist	Suppresses airway hyperresponsiveness Prevents airway remodeling by reducing goblet cell hyperplasia and smooth muscle mass hypertrophy Reduces Th1 and Th2 cytokine protein expression in the lungs Reduces production of Th17/Th2 family cytokines, and airway hyperresponsiveness by enhancing the production of IL-10	(Quarcoo et al., 2004; Xirakia et al., 2010). (Camateros et al., 2007). (Vultaggio et al., 2011)
CpG-motif oligodeoxynucleotides (CpG ODN)	TLR9 agonists	Reduce IL-4, IL5 and IgE production and decrease airway eosinophilia, Enhance IL-10 production Reduce airway collagen deposition and goblet cell hyperplasia-metaplasia Inhibition of allergic inflammation in animal models	(Kline et al., 1998; Aryan et al., 2014). (Jain et al., 2002) (Ashino et al., 2008)
Allergen TLR9-ISS conjugate (AIC)	TLR9 agonist	Inhibits airway inflammation in sensitised animal models of asthma. Reduces Th2 responses (such as production of IL-5, CCL17, and CCL22) by increasing Th1 and T-regulatory cytokines (IFN- γ , CXCL9, and CXCL10).	(Santeliz et al., 2002) (Simons et al., 2004)
ObG10	TLR9 agonist	Reduces symptoms concomitant medication needs and increase the quality of life in patients with allergy	(Senti et al., 2009; Klimek et al., 2011)
Bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside (QbG10)	TLR9 agonist	Improves asthma control and preserves pulmonary function even after steroid withdrawal in patients with persistent allergic asthma compared to placebo	(Beeh et al., 2013)

R837 and R848 have been shown to reduce acutely the production of inflammatory cytokines, such as IL-4, IL-5 and IL-13 as well as eosinophilic inflammation in animal models (Moisan et al., 2006; Meng et al., 2011; Drake et al., 2012). Furthermore, R837 administration in mice resulted to the reduction of alveolar macrophages, B cells and TNF- α together with an increase in the number of DCs and natural killer cells in the lung tissue and IL-10 production. (Hackstein et al., 2012). Finally, studies have shown that R837 has significant bronchodilator effects in both animal models of AHR and in human airways in vitro, possibly by inducing the production of nitric oxide, prostaglandins, and large-conductance calcium-activated potassium channels (Kaufman et al., 2011; Drake et al., 2013).

R848 or S28463 is a TLR7/8 agonist, which has been shown to suppress airway hyperresponsiveness when administered either systemically or intranasally (Quarcoo et al., 2004; Xirakia et al., 2010). Furthermore, this compound was able to prevent the development of goblet cell hyperplasia and smooth muscle mass hypertrophy by inhibiting their proliferation, which leads to the prevention of the development of airway remodeling (Camateros et al., 2007). This inhibition was associated with a reduction of both helper T cell type 1 and type 2 cytokine protein expression in the lungs (Camateros et al., 2007). Finally, SA2, is a TLR7 stimulator, which has been shown to be able to reduce production of Th17/Th2 family cytokines, and airway hyperresponsiveness by enhancing the production of IL-10 in animal models of allergic asthma (Vultaggio et al., 2011).

Targeting TLR9 has been also proposed as an important target for

the skewing of inflammatory response towards Th1 direction and thus reducing allergic inflammation (Chen et al., 2011). Studies in animal models of asthma have shown that CpG-motif ODNs (CpG ODNs), which are synthetic TLR9 agonists reduced IL-4, IL5 and IgE production and decreased airway eosinophilia, while they enhanced production of IL-10 (Kline et al., 1998; Aryan et al., 2014). CpG-ODNs also seem to be effective in the reduction of markers of airway remodeling, such as airway collagen deposition and goblet cell hyperplasia-metaplasia (Jain et al., 2002). Administration of CpG-ODNs in animal models was able to inhibit allergic inflammation (Ashino et al., 2008).

Administration of allergen/immunostimulatory sequence ODN conjugate (AIC) in sensitised animal models of asthma resulted in the inhibition of airway inflammation (Santeliz et al., 2002). Studies in human subjects with allergy have also shown that AIC was able to reduce Th2 responses (such as production of IL-5, CCL17, and CCL22) by increasing Th1 and T-reg cytokines (IFN γ , CXCL9, and CXCL10) (Simons et al., 2004). However, clinical trials using AIC found that this compound was unable to reduce nasal symptoms in patients with allergic rhinitis (Tulic et al., 2004). Finally, ObG10 is an A-type CpG-ODN that has been shown to reduce symptoms concomitant medication needs and increase the quality of life in patients with allergy (Senti et al., 2009; Klimek et al., 2011).

Recently, QbG10 (bacteriophage Qbeta-derived virus-like particle with CpG-motif G10 inside), a novel TLR9 agonist has been developed. This agonist, packages into virus-like particles and stimulates the immune system toward a Th1-mediated protective response. In a

recent study has been shown that QbG10 administration to asthma patients improved their asthma control and preserved their pulmonary function even after steroid withdrawal (Beeh et al., 2013). Currently, novel TLR9 agonists are under development and results from clinical trials are expected in order to ascertain their possible use in the therapy of asthma (Aryan et al., 2014).

4. Conclusion

Studies have shown that TLRs play an important role in directing the interaction between innate and adaptive immunity and thus they might be an important link between environmental factors and asthma pathology. The use of TLRs as possible therapeutic targets may redirect the immune response away from the airways during an allergic response plausibly by enhancing tolerance to allergens and this might result to the modulation of disease progression. Agents that target TLRs seem to represent a potentially important etiologic treatment in patients with allergic airway diseases since they are able to suppress airway inflammation and reduce airway hyperresponsiveness. More studies are needed in order to demonstrate whether a modulation of the interaction between TLRs and their ligands will potentiate significant therapeutic effects.

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