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REVIEW

Interaction between Helminths and Toll-Like Receptors: Possibilities and Potentials for Asthma Therapy

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ABSTRACT

Toll-like receptors (TLRs) are essential components of the innate immune system. They play an important role in the pathogenesis of allergic diseases, especially asthma. Since TLRs significantly orchestrate innate and adaptive immune response, their manipulation has widely been considered as a potential approach to control asthma symptoms. It is well established that helminths have immunoregulatory effects on host immune responses, especially innate immunity. They release bioactive molecules such as excretory-secretory (ES) products manipulating TLRs expression and signaling. Thus, given the promising results derived from preclinical studies, harnessing helminth-derived molecules affecting TLRs can be considered as a potential biological therapy for allergic diseases. Prospectively, the data that are available at present suggest that, in the near future, it is possible that helminth antigens will offer new therapeutic strategies and druggable targets for fighting allergic diseases. This review describes the interactions between helminths and TLRs and discusses the potential possibilities for asthma therapy. In this opinion paper, the authors aimed to review the updated literatures on the interplay between helminths, TLRs, and asthma with a view to proposing helminth-based asthma therapy.

ABBREVIATIONS: AAM: alternative activated macrophage; AMs: alveolar macrophages; APCs: antigen presenting cells; BAL: bronchoalveolar lavage; CA: crude antigen; CIA: collagen-induced arthritis; CTL: C-type lectins; DCs: dendritic cells; DDCs: dermal dendritic cells; DTH: delayed hypersensitivity reaction; DUSPs: dual specificity phosphatases; ES: excretory-secretory; GRAIL: gene related to anergy in murine lymphocytes; HDEs: house dust extracts; HDM: house dust mites; IBD: inflammatory bowel disease; IFN: interferon; ILC: innate lymphoid cells; ILC2: type 2 innate lymphoid cells; iNKT: invariant NKT; i.p.: intraperitoneal; LCs: Langerhans cells; LPMCs: lamina propria mononuclear cells; MAPK: mitogen-activated protein kinase; MGL: macrophage galactose C-type lectin; MHC: major histocompatibility complex; MR: mannose receptor; NF- κ B: nuclear factor B; OVA: ovalbumin; PAMP: pathogen-associated molecular pattern; PBMCs: peripheral blood mononuclear cells; PRRs: pattern recognition receptors; SEA: *Schistosoma* egg antigen; sFLA: standard Flagellin; Th2: T helper2; TLR: Toll-like receptor; TNF α :

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tumor necrosis factor α ; Tregs: regulatory T cells; TSLP: thymic stromal lymphopoietin

Introduction

It has been estimated that more than 300 million people suffer from asthma worldwide and the numbers are increasing. Asthma is an intricate disease with high morbidity and mortality, characterized by airway inflammation, mucus hypersecretion, recurrent episodes of airway obstruction, and wheezing [1].

It is well established that the prevalence of allergic diseases such as asthma and rhinitis in developing countries is less than developed societies. On the basis of a hypothesis known as “hygiene hypothesis,” one possible explanation for this apparent inconsistency is elimination of infectious diseases, thanks to higher hygiene standard in developed countries [2]. A growing body of evidence implies that bacteria and even worms in the digestive tract offer protection against a variety of allergic and autoimmune disorders [3]. Commensal bacteria and parasitic helminths play a central role in immune system development [2]. The inhibitory effects of bacterial infections on allergic responses have widely been reported, as it is thought that lack of exposure to microorganisms in infancy is associated with susceptibility to allergic reactions [4]. Apart from bacteria, worms have also gained great interest owing to their substantial capability for immunoregulation and dampening of host immune responses. A cross-sectional study suggested that there is an inverse relationship between *Ascaris lumbricoides* infection and prevalence of asthma within the population of rural endemic area [5]. Likewise, a randomized-controlled trial study reported that the majority of inflammatory bowel disease (IBD) patients after infection with *Trichuris suis*, went into remission [6]. In this regard, several clinical trials of helminth therapy have been conducted in patients with multiple sclerosis (MS) and IBD; some of them have provided a degree of success [7]. Helminths secrete bioactive molecules known as excretory-secretory (ES) products that surprisingly modulate DCs [8], induce regulatory T cells (Treg) and modify the expression of pattern recognition receptors (PRRs), especially TLRs. Indeed, helminths have developed these multifarious mechanisms to evade and suppress host immune system.

Despite the high prevalence of asthma worldwide, extensive healthcare costs, and immense economic burden, asthma is poorly controlled [9]. In addition, the exact pathophysiological mechanism underlying asthma, as a heterogeneous disease with various phenotypes, has not yet been recognized. Available major treatment for asthma consists of corticosteroids, but these drugs are not curative and symptoms return soon. Moreover, severe types of asthma are relatively resistant to the therapeutic effect of corticosteroids and bronchodilators [10]. Up to now, several biological therapies such as monoclonal antibodies against IgE, Th2-related cytokines, and their receptors have been developed [11]. Recently, helminthic therapy as a novel biological therapy has shown promising results against a wide range of allergic, autoimmune, and inflammatory diseases in experimental model.

This review describes the interactions between helminths and TLRs and discusses the implications for novel therapeutic modality against asthma. TLRs are implicated in the pathogenesis of asthma, thus modifying them by helminth-derived molecules may be an approach to treatment. This article aims to review the literatures on the interplay between helminths, TLRs, and asthma with a view to proposing helminth-based asthma therapy.

Immunopathology of Th2-based asthma

Asthma is an intricate and heterogeneous disease caused by multiple factors. There are different forms of asthma, including allergic, nonallergic, and intrinsic and in some patients these forms exist together [12]. Allergic asthma triggered by environmental allergens is mediated by Th2 immune responses. Nonallergic asthma is a less common form of the disease characterized by airway neutrophilia, with or without eosinophils and can also be caused by several factors, such as air pollutants (smoke, diesel particles, and ozone), respiratory viruses, stress, and obesity [13].

Despite allergic asthma, non-Th2 cells (such as Th1, Th9, and Th17) along with other immune cells contribute to non-allergic asthma. Presence of Th2-independent factors, including IFN- γ , IL-17, and neutrophils has been reported in patients with nonallergic asthma [13]. One of the key players in nonallergic asthma is ILC2 cells which through production of IL-5 and IL-13 induce eosinophilia [12]. Most patients with nonallergic asthma have negative skin prick responses to common inhalant allergens, because ILC2 cells produce little IL-4, which does not activate B cells to produce allergen specific IgE. Moreover, this phenotype of asthma poorly responds to common drugs used for corticosteroids, implying it is independent of Th2-associated responses [12, 13].

Atopic asthma is the most common form of asthma and is often characterized by eosinophils in bronchoalveolar lavage (BAL) and sputum [14]. Environmental allergens such as house dust mites (HDM), molds, plant pollen, and animal dander play a key role in breaking pulmonary tolerance and triggering Th2 response. Apart from environmental allergens, other agents such as physical stimuli, drugs, infection particularly viruses, and airborne pollutants can exacerbate Th2-based asthma and trigger other forms of this disease [15].

In asthmatic patients, the integrity of epithelial cells is disrupted, which assists allergens to pass easily through this first line of defense and reach airway submucosa [15]. DCs in the submucosa of airways actively monitor airway lumen where antigens sampled constantly. Most of the experimental evidence suggests that chemokine receptors of DCs are upregulated upon antigen uptake and TLRs stimulation. Chemokine receptors such as CCR7 and CCR8 direct DCs toward mediastinal lymph nodes [15]. Presentation of allergen peptides in the presence of Th2 cytokines evolves to Th2 phenotype differentiation. Allergen-specific Th2 cells along with recruited innate cells secrete major Th2-related cytokines such as IL-4, IL-5, IL-9, and IL-13 [16]. IL-4 and IL-13 followed by CD40-CD40L interactions are essential mediators of IgE class switch recombination of B cells. IL-5 plays a central role in induction of eosinophilia, which is a hallmark of asthma. Eosinophils are abundantly recruited to the lung tissue and BAL fluid of asthmatic patients [17]. IL-5 promotes their maturation and survival, then recruited to the lung through chemokines such as CCL-1 [18]. Eosinophils release inflammatory mediators, including Th2 cytokines (IL-4 and IL-13), and lipid eicosanoids (prostaglandins, leukotrienes, and lipoxins), which are responsible for clinical symptoms of asthma. These products promote pathophysiological hallmarks of asthma via induction of repetitive cycles of tissue damage and chronic inflammation even in the allergen free environment [19].

Severe asthma

Asthma is a complex respiratory disorder ranging from mild to severe form. Traditionally, it was thought that asthma is an allergic (Th2-related or eosinophilic) disease which mostly responds to corticosteroids. Afterwards, on the basis of clinical manifestations, it was

categorized into different subgroups [14]. Thus, instead of a term which previously described a single disease, asthma classification now includes several subgroups that are called phenotypes [14]. Indeed, asthma can also be divided in two general phenotypes including Th2 high and Th2 low. Patients who represent Th2 high asthma, high levels of IL-13 and IL-5 mRNA expression, as well as elevated numbers of eosinophils and mast cells are present in their lungs [20]. In supporting this, Th2 high asthma is well controlled by corticosteroid therapy, suggesting the critical role of Th2 responses in this phenotype. While, in Th2 low asthma, another subsets of Th cells, such as Th1, Th17, and Th9 are involved, explaining why common inhaled corticosteroids are unable to suppress the symptoms of Th2 low asthma [12].

Th2 low asthmas are frequently triggered by agents inducing nonallergic asthma, including viral infections, stress, obesity, and air pollutants. Th2 low asthma has main properties such as low airway and systemic eosinophilia, presence of neutrophils in the BALF, and lack of responsiveness to glucocorticoids and inhibitors of Th2-associated cytokines [12]. It is thought that factors such as oxidative stress, IL-17 and neutrophil products along with intrinsic abnormalities in airway smooth muscle affect the structural elements of the airway leading to airway hyperresponsiveness and airway obstruction [20]. Despite Th2 high phenotype, Th2 low phenotype is considered as a severe form of asthma. This phenotype is normally present in patients who do not show typical eosinophilia and poorly respond to corticosteroids. Although various studies indicate Th17 and Th1 cells play a substantial role in development of severe asthma (SA), the exact mechanisms by which severe asthma (Th2 low) is occurred have not yet been discovered [21].

The neutrophilic asthma is strictly triggered by amplification of Th17/Th1 responses. Th17 cells are a newly described T cell subtype generated in an IL-6, IL-21, and TGF- β -dependent manner. IL-17, as a major proinflammatory cytokine, is a potent chemoattractant for neutrophils. It has been suggested that Th17 cells contribute to noneosinophilic nonallergic asthma, where neutrophilic inflammation is prominent [22]. Double positive Th2/Th17 cells are elevated in BAL fluid of severe asthmatics, implying Th17 cells have a significant role in severe asthma and inhaled corticosteroid-resistant patients [23]. Large amount of neutrophils without eosinophils in sputum of severe asthma highlighted the critical role of IL-17 [24]. In addition, IL-17 mRNA and protein in the lung tissue of severe asthma patients are higher than milder patients or healthy people [25, 26]. Besides human studies, several investigations on mice prove the pivotal role of IL-17 in neutrophilic asthma. Examination of BAL and IL-17 mRNA expression in lungs of mice chronically exposed to OVA showed significant neutrophilia along with elevated level of IL-17 mRNA [27]. Adoptive transfer of *in vitro* polarized Th17 and Th2 cells derived from OVA-specific TCR-transgenic mice supports the central role of Th17 in severe asthma. Adoptive transfer of Th17 cells led to neutrophil influx in BAL and resistance to dexamethasone therapy, whereas transfer of Th2 cells resulted in eosinophilia and sensitivity to dexamethasone treatment [28]. Recently, an interesting study on mice has revealed that macrolides are able to suppress infection-induced, severe, steroid insensitive neutrophilic allergic airway disease (SSIAAD). In this study, *Chlamydia* was used to induce SSIAAD. Administration of clarithromycin followed by dexamethasone could decrease total cells, neutrophils, and eosinophils in BALF of SSIAAD mice. In addition, airway inflammation, T cell responses, airway hyperresponsiveness (AHR), and infection were suppressed. Clarithromycin also arrested Th17 responses and TNF α production. This study suggests that clarithromycin as a macrolide with antimicrobial and immunomodulatory properties could be considered as a novel treatment for steroid-insensitive severe asthma. But, it needs to be determined whether therapeutic effects of clarithromycin on severe asthma are mediated by its antimicrobial or anti-inflammatory features [29].

The critical role of Th1 cells along with Th17 cells in pathogenesis of severe asthma has been documented [30]. Th1 cells followed by their cardinal cytokines, in particular IFN- γ are significantly increased in severe asthma [31]. In comparison with moderate asthmatics and healthy individuals, SA patients possess higher amount of IFN- γ expressing cells in their tissue samples of airways and BALF [32]. In addition, chronic exposure of IFN- $\gamma^{-/-}$ and IFN- γ R $^{-/-}$ mice to OVA did not show airway resistance and leukocytosis in BAL [33].

Studies show that nearly 50% of SA patients have airway eosinophilia [34]. It should be taken into account that neutrophilic asthma and eosinophilic asthma are not mutually exclusive, suggesting there is not an inverse relationship between eosinophil and neutrophil numbers [34]. Although, neutrophils accumulate in the airways of patients with severe asthma, eosinophils may significantly be increased in the airways. In fact, type 2 innate lymphoid cells (ILC2) by production of IL-5 and IL-13 can recruit eosinophils to the lungs independent of Th2 cells. In addition, airway neutrophils through secretion of proteases and lipid mediators, such as matrix metalloproteinase 9 (MMP9), leukotriene B4, elastase, and platelet-activating factor (PAF), accelerate recruitment of eosinophils [12].

Administration of anti-IL5 monoclonal antibody to SA patients could control symptoms in 50% [35]. Also, inhibition of eosinophilia in SA leads to reduced airway wall thickness and extracellular matrix deposition [36]. Eosinophils along with other recruited cells such as neutrophils, mast cells, basophils and CD8 $^{+}$ T cells mediate asthma exacerbation, persistent symptoms and airway remodeling [37]. Mast cells (MC) strategically reside near the surfaces of airway epithelium and the deeper layers of the mucosa. In asthma condition, they are accumulated in both submucosa and smooth muscles of airways [38]. Cross-linking between IgE and allergens on the surface of MC causes degranulation and release of mediators including histamine, prostaglandins, and leukotrienes which results in exacerbation of symptoms. Moreover, high level of proliferation and recruitment of mast cells during SA supports the persistence of airway inflammation [39, 40]. Unraveling the correlation between cellular inflammation and disease phenotypes of asthma can address specific cellular and molecular markers, optimizing personalized treatment protocols.

Innate immune cells and their TLRs in asthma

Various immune cells contribute in asthma pathogenesis, including airway epithelial cells, mast cells, granulocytes, natural killer (NK) cells and natural killer T (NKT) cells, innate lymphoid cells (ILC), and macrophages [41, 42]. In the following part, we briefly discuss the role of innate immunity in asthma and point out the importance of TLRs in the activation of innate cells.

Epithelial cells and type 2 innate lymphoid cells (ILC2)

Airway epithelial cells have been equipped by a vast array of PRRs, enabling them to detect a wide variety of environmental stimuli. Epithelial cells through release of chemokines (CCL2 and CCL20) recruit immature monocytes or DCs and produce cytokines such as IL-1, granulocyte-macrophage colony-stimulating factor (GM-CSF), and thymic stromal lymphopoietin (TSLP) inducing DC maturation [16].

ILC2 are able to produce Th2-related cytokines, including IL-4, IL-5, and IL-13. They can be activated by microbes involving TLRs through IgE-independent mechanism. ILC2 activated by airway epithelial cells contribute to airways hyper-reactivity and acute allergic immune response [16]. Another pathway promoting airway inflammation and Th2-biased

response is activation of PRRs on epithelial cells. PRRs stimulation results in release of a distinct subset of cytokines known as alarmins; including TSLP, IL-33, and IL-25. All these innate cytokines have pleiotropic effects and drive DCs into a Th2-activating mode [16].

The expression of TLR1-10 mRNA by primary bronchial epithelial cells has been indicated [43]. TLR3 activation in epithelial cells due to viral infection evolves to release of TSLP. TSLP followed by other epithelial cell-derived mediators such as IL-25, IL-33, GM-CSF, IL-1 β , and chemokines increases airway inflammation [44]. Moreover, contamination of most environmental allergens with PRRs agonists, as well as the existence of a structural homology between some allergens and TLRs coreceptors increase the strength of allergenicity causing DC maturation. For example, HDM contains a myeloid differentiation protein (MD)-2 homologue antigen known as Derp2 that induces asthma via TLR4 on airway structural cells [45].

Granulocytes and NK cells

Nearly all TLRs are expressed by mast cells, eosinophils, basophils, and neutrophils. Activation of their TLRs mostly evolves to cytokine and chemokine secretion. For example, it has been demonstrated that stimulation of TLR4 on mast cells promotes asthma development [46], as well as TLRs engagement enhances eosinophils degranulation [46].

Although, basophils and eosinophils express MHC Class II, it is thought that they are unable to induce antigen-specific T cell activation and proliferation [47]. Recently, Suurmond et al. detected TLR1-8 transcripts in human basophils and showed that stimulation of TLRs leads to Th2 cytokine production [48]. A recent investigation on mice displayed that sensitization to *Blomia tropicalis* (house dust mite-derived allergen) by dual activation of TLR2 and TLR4 dominates Th17 cells in lung and promotes airway neutrophilic inflammation. Interestingly, in this study airway inflammation was abrogated in TLR2 and TLR4 knockout mice [49].

A member of NKT cells known as invariant NKT (iNKT) cells has been detected in airway inflammation [50]. In experimental ovalbumin (OVA)-induced asthma model, stimulation of TLR7-bearing iNKT by R848, a TLR7 agonist, through IFN- γ secretion redirected Th2 response toward Th1. Moreover, it was proved that adoptive transfer of R848-treated spleen cells derived from wild-type mice suppresses allergic inflammation, while spleen cells derived from iNKT cell-deficient $J\alpha 18$ (-/-) mice lack this ability [50]. The role of NKT cells in murine experimental asthma has been well established, whereas their relevancy in human asthma due to limited data is obscure and needs to be further investigated [51].

The inflammatory cascades triggered by epithelial cells are directed toward submucosa where the sensitized resident cells and memory cells amplify and maintain inflammation even in the absence of allergens. Better understanding of the exact role of PRRs in propagation and longevity of recruited cells may offer novel therapeutic approach to control airway inflammation particularly in the early stages [15] (Figure 1).

Beside Th2 cells, involvement of other T cell populations such as Th1, Th17, and Th9 cells in asthma has recently been confirmed. Th9 cells are generated under the influence of TGF- β , IL-4 and IL-2. Production of IL-9 by Th9 cells promotes IgE production, mast cell recruitment and survival [54]. Likewise, IL-9 induces goblet cell hyperplasia, mucus production, and eosinophilic inflammation in mice sensitized to *Aspergillus fumigatus* [55]. In OVA-induced asthma model also presence of Th9 cells both in the airway and draining lymph nodes has been reported [56]. In addition, high levels of IL-9 expressing cells in BAL fluid (BALF) from asthmatic patients showed that IL-9 likely play an essential role in human asthma [57].

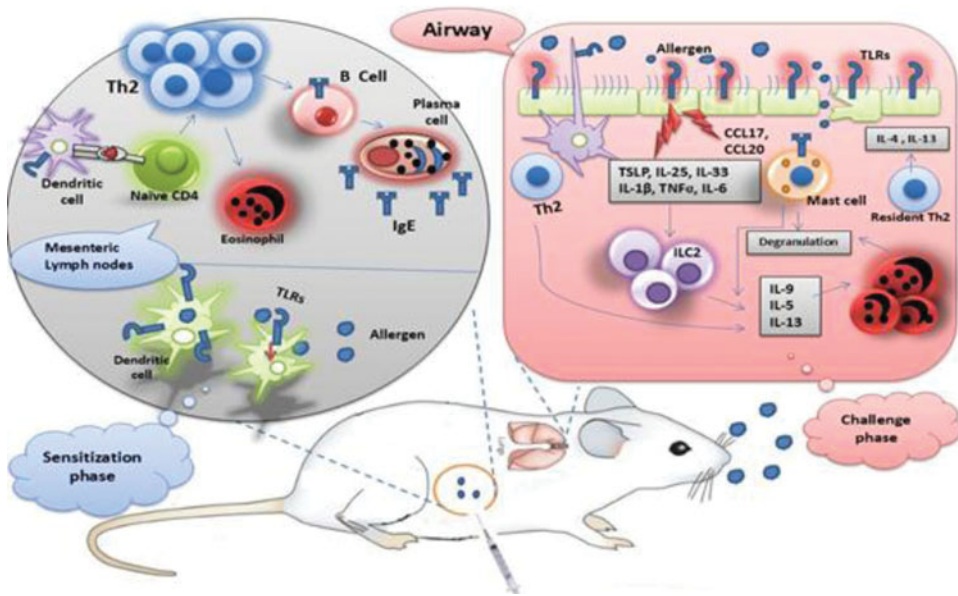


Figure 1. Immunopathology of airway inflammation in mice as a useful model for human asthma. So far, several experimental animal models have been developed to study on airway inflammation. These models depending on the route of administration (intraperitoneal, intranasal, and subcutaneous), type of allergens (OVA, HDM, and fungal allergen), and presence of adjuvant, at least, could be divided into three different categories [52, 53]. Classically, administration of OVA (as antigen) accompanied by aluminium hydroxide (Alum) (as adjuvant) is routinely being used. Unlike the wide use of the OVA/Alum model, some challenges concerning the efficacy of this model, have been discussed. In terms of clinical relevancy of allergens contributing in human asthma, several models have recently been developed. In this context, most investigators believe that intranasal administration of house dust mite extract (HDM) and fungal-derived allergens, not only efficiently represent asthma features, but also are more related to clinical and steroid resistant asthma in human [53]. Here, we briefly illustrated a customary method that is routinely being used for induction of OVA-Alum asthma model.

TLRs and asthma

TLRs are the best known PRRs contributing to immunosurveillance. As mentioned, most immune cells involved in asthma are armed with TLRs, thus the key role of TLRs in allergic diseases and their relevance in asthma pathogenesis is undisputed. A large body of *in vitro* and *in vivo* evidences proves the therapeutic potential of synthetic TLRs ligand for controlling of asthma [58]. TLRs are not only expressed by resident lung cells and variety of immune cells, but also they have been detected in human airway smooth muscle cells and airway nerves [59]. In the following part, the role of TLRs (manipulated by helminths) in the pathogenesis of human and murine model of asthma is discussed (Table 1).

TLR1/2 and TLR2/6

Mice

In the murine models of OVA-induced asthma, activation of TLR1 or TLR2 can either reverse established airway inflammation [60–62] or aggravate allergic asthma [63]. In fact, stimulation of TLR1/2 has shown controversial results in experimental asthma. For example, during chronic fungal asthma in TLR2^{-/-} mice, the temporal changes in airway hyperresponsiveness, decreased Th2-type cytokines and chemokines were reported [64]. Stimulation of TLR2

Table 1. Role of TLRs in experimental murine model of asthma.

TLRs	Activation by	Role in experimental asthma	Effect on Tregs	Ref
TLR1/2	Agonists	Controversial (induce or inhibit)	Expansion of Tregs in OVA-induced asthma model [65]	[60, 63,65,71]
TLR3	Agonists	Controversial (induce or inhibit)	—	[72, 73]
TLR4	LPS (HDM and fungal allergens)	Controversial (induce or inhibit)	—	[86, 87]
TLR5	Flagellin	Induce asthma	—	[166]
TLR2/6	Agonists	Attenuate	—	[67]
TLR7/8	Agonists	Attenuate or inhibit	Enhances the immunosuppressive activity of murine Treg [125]	[50, 72, 161–163]
TLR9	Agonists	Attenuate or inhibit	—	[162–164]

in asthma condition also showed conflicting results. For instance, TLR2 activation inhibits both OVA and mite allergen-induced Th2 response [65], whereas in another study TLR2 stimulation resulted in asthma exacerbation [63]. It seems that TLR2 ligands likely potentiate airway inflammation and confine Th2-biased immune responses in murine model of OVA-induced asthma [63, 66]. Thus, given these controversial results the exact role of TLR2 in asthma remains ambiguous and more investigations need to be done.

Promising results have been obtained concerning the efficacy of TLR2/6 agonists in attenuation of a murine model of chronic allergic airway inflammation. Evidence provided by Moreira et al. suggested that TLR6 activation is critical for regulation of allergic responses in chronic fungal-induced asthma. They showed that stimulation of TLR6 on macrophages and DCs mediates IL-23 production. IL-23 is able to direct naive CD4⁺ T cells toward Th17 population. Indeed, lung levels of IL-23 and IL-17 in TLR6^{−/−} mice were markedly lower than wild-type asthmatic mice. Interestingly, administration of exogenous IL-23 resulted in the restored production of IL-17 and mitigating asthmatic symptoms as compared to the untreated asthmatic TLR6^{−/−} mice [67]. In human, the expression of TLR6 in PBMCs of asthmatic patients is less than healthy cases [68].

Humans

High expression of TLR2 mRNA has been reported in peripheral blood mononuclear cells (PBMCs) of patients with COPD [69]. This is consistent with Chun et al. findings that showed the expression levels of TLR1 and TLR2 are significantly higher in PBMCs of asthmatics than controls [68]. On the contrary, up-regulation of TLR2 has been detected in PBMCs of farmer’s children with decreasing risk of allergy [70]. Some investigators believe that TLR2 heterodimer is responsible for this discrepancy [60], while others believe that stimulation of TLR2 leads to IL-10 secretion and inhibition of Th1 cytokines [66, 71]. Thus, there is not clear evidence so far to pinpoint the exact role of TLR1/2 in asthma and it remains poorly described.

TLR3

A study on mice showed that inhalation of TLR3 agonist has stimulatory effects on OVA-induce asthma. In this study, allergic airway inflammation was exacerbated when mice received OVA accompanied by poly (I:C), TLR3 agonist. On the contrary, in mice treated with OVA along with R848, TLR7/8 ligand, asthma development was suppressed [72]. In addition,

it has been shown that activation of TLR3 in combination with human TSLP promotes Th17 differentiation [44]. As mentioned in severe asthma section, Th17 cells by recruitment of neutrophils play a key role in pathogenesis of inflammatory disorders, especially asthmatics who are steroid resistant [73].

TLR4

Mice

Most natural allergens such as, cockroach antigens, HDM and fungi are contaminated with LPS or peptidoglycans [74]. The presence of LPS in OVA is necessary to induce Th2 responses in a mouse model of allergic asthma. It appears that there is a direct link between stimulation of TLR4 and induction of Th2 responses [75], probably through MyD88-dependent mechanism [76]. A large body of literature shows that administration of low doses of LPS along with allergen promotes airway inflammation [75,76]. By contrast, others have suggested that commercial OVA depleted LPS significantly increases AHR and airway inflammation in comparison to commercial OVA that contains LPS [77].

Several factors appear to impact on TLR4-mediated development of allergen-induced Th2 responses, including gene-environment interactions [78], polymorphism of CD14, [79], the cell type which TLR4 is engaged on, the dose of stimuli, and the timing of exposure [80]. Depending on the time and dose, TLR4 can have either protective or detrimental effect on asthma. In support of the importance of stimulation dose, it was shown that combination of OVA with low-dose LPS exacerbates allergic asthma in mice. Histological findings demonstrated that administration of OVA with low dose LPS increases infiltration of eosinophils and neutrophils, airway mucus secretion and Th2 cytokines production. By contrast, those mice exposed to OVA with high doses of LPS showed Th1 associated response such as airway neutrophilia without mucus secretion and high level of IFN- γ production [81]. In an interesting study, dermal application of TLR4 ligand (LPS) or TLR2 ligand (Pam3Cys) during sensitization with OVA arrested asthma (Th2 responses) in mice. It is proposed that production of IFN- γ due to LPS administration redirects immune response toward Th1, and consequently suppresses asthma [82]. Thus, different effects of TLR4 are thought to be due to signal strength. It means that low LPS administration shifts immune responses toward Th2, whereas high LPS dominates Th1/Th17 responses. Stimulation of TLR2 and TLR4 of murine alveolar macrophages by HDM allergens could potentially shift the immune responses toward Th2 [83]. Regardless of high or low dose of LPS, TLR4 in alveolar macrophages (AM) was upregulated in the lungs of asthmatic mice [81]. Also, many investigations have been done concerning the time of LPS exposure as another factor affecting TLR4-mediated Th2 response. In this regard, the inhibitory effect of LPS exposure on subsequent antigen sensitization in newborn mice was confirmed [84]. In addition, prenatal LPS exposure prevented later allergen sensitization in offspring through inhibition of Th2 immune responses [85], indicating bacterial compounds can modulate immune responses during gestation time and it might be an effective way for prevention of allergic diseases.

The functional involvement of TLR4 in development of allergic asthma has been extensively studied in mice deficient in TLR4 or downstream pathways molecules. Most investigators suggest the expression of TLR4 on epithelial cells is crucial for induction of DC recruitment and intramucosal migratory behavior in response to LPS or HDM. Interestingly, inhalation of TLR4 antagonist at the time of HDM injections could also reduce the features of asthma [86]. On the contrary, there is evidence that proves administration of *Alternaria* extract with OVA can induce airway allergy even in TLR4 KO mice. In this study, after

exposure to allergens, MyD88xTRIF-deficient mice had no detectable BAL or lung eosinophilic, whereas levels of BAL, lung eosinophilia, and IL-13 production in TLR4-deficient were similar to wild-type mice [87].

Humans

Clinical studies suggest that exposure to house dust endotoxin, a ligand for TLR4, is a significant risk factor for asthma [88]. Whereas, epidemiological studies demonstrated that high levels of endotoxin exposure may protect against allergen sensitization and inversely correlate with atopy rates [89]. The clinical investigations that have been done on human asthmatic patients suggest an increased level of TLR4 on BAL cells from patients with COPD [90]. Study on monocytes, lymphocytes, and DCs from asthmatic patients implied that the expression of TLR4 in these cells is lower than healthy individuals [91]. Overall, there are some studies that corroborate TLR4–ligand interactions may have both pro- and antiasthmatic effects, but it still remains a matter of debate.

Effects of helminths on TLRs

The exact method by which helminths manipulate the innate immune system is not fully understood. There are growing proofs showing that TLRs might be a suitable target for most asymptomatic infections to escape immune response. Some worms such as *Heligmosomoides polygyrus* and *Fasciola hepatica* produce molecules that through manipulation of TLRs induce regulatory DCs [92, 93]. However, it has been reported that some helminth-derived products via TLR-independent manner can inhibit murine experimental asthma and delayed hypersensitivity reaction (DTH) [94]. Limited data have been provided regarding the receptors utilized by helminth-derived immunomodulatory products. Indeed, it should be taken into account that except TLRs, other receptors are also involved during helminth infections. In this regard, many helminth-derived molecules have been recognized that exquisitely engage TLRs and C-type lectins (CTL) to induce either regulatory, Th17 and Th2 responses [95–97].

Helminths and TLRs expression

Schistosoma spp.

An *in vivo* study on mice delineated that early stage of *Schistosoma japonicum* utilizes TLR2 and TLR4 for conducting distinct adaptive immune responses [98], whereas another study showed that crude antigens from this worm induce high expression of TLR2 mRNA rather than TLR4 on murine DCs [99]. In this regard, it has been demonstrated that *Schistosoma* egg antigens (SEA) could not upregulate FoxP3 in TLR2- deficient naive CD4⁺ T cells of mice [100], suggesting SEA contribute to Treg expansion, Th2 modification and IL-10 production through TLR2-dependent pathway [95, 101, 102]. Similarly, Wang et al. showed that administration of SJMHE1 (a peptide from *S. japonicum*) to TLR2^{-/-} mice abolishes Treg proliferation both *in vivo* and *in vitro*. In this study, it was proved that stimulation of TLR2 not only enhances Treg proliferation, but also promotes their suppressive activity. However, it needs to be determined whether SJMHE1 activates TLR2 on Tregs or APCs [103].

Brugia malayi

Patients with *Brugia malayi* infection, a filarial nematode, had lower expression of TLR1, TLR2, TLR4, and TLR9 on B cells and monocytes in comparison to uninfected individuals. An *in vitro* study showed that live filarial can downregulate the expression and function of TLR3

and TLR4 in human DCs [104], whereas Babu et al. showed that *B. malayi* antigens (BmA) significantly induce the expression of TLR2, 4, 7, and 9, also Nod1 and Nod2 mRNA in PBMCs of patients with lymphedema relative to asymptomatic infected patients [105]. Recently, the effect of L3 stage of *B. malayi* was examined on TLR4, TLR3 and TLR2 mRNA expression in human epidermal Langerhans cells (LCs). Preliminary findings represented that L3 is slightly able to increase the expression of TLR2 mRNA. Interestingly, the expression level of TLR4 and TLR3 mRNA in LCs exposed to L3 was not altered [106]. Besides human LCs, the impact of L3 on the expression of TLR1-10 mRNA in human dermal DCs (DDC) was also evaluated. In this study, suppressive effects of L3 on TLRs were quietly confirmed. Levels of TLRs expression in LCs and DDCs were not affected after exposing to L3. Moreover, L3 blocked production of inflammatory cytokines in response to TLR ligands [107]. However, it has been suggested that lack of TLRs signaling does not significantly alter host responses to filarial nematodes. But, it appears that manipulation of TLRs is an appropriate mechanism for immune evasion by filarial parasites [108].

Larval stage of tapeworms

Chronic cysts are installed by larval stage of some parasites known as cestodes. Although, these cysts have massive size and longevity in parenchymal tissues, they intriguingly elicit trivial inflammation [109]. Larval stage of *Taenia solium*, a tapeworm, usually involves brain tissue and makes a disease known as neurocysticercosis. Expression of TLR4 was evaluated in PBMCs of asymptomatic and symptomatic neurocysticercosis (NCC) patients. Expression of this receptor was significantly higher in symptomatic NCC as compared to asymptomatic NCC individuals or healthy controls [109]. *Echinococcus granulosus*, also as a tapeworm in larval stage, forms cystic bodies known as echinococcosis. Shan et al. indicated that TLR2 and TLR4 mRNA expression in PBMCs of patients with chronic cystic echinococcosis (CE) are significantly higher than healthy controls. They found a positive correlation between the expression of these receptors and the serum level of IL-10, suggesting likely manipulation of TLRs expression during chronic CE infection is responsible for cytokine modulation [110].

Intestinal and liver fluke worms

The expression of TLRs can be modulated on intestinal epithelial cells in response to helminth infections. For example ES products of *Hymenolepis diminuta* could increase TLR2 and TLR4 expression in murine intestine [111]. Also, an *in vitro* study showed that the antigens derived from *Gymnophalloides seoi* and *N. brasiliensis* are able to up-regulate TLR2 and TLR4 mRNA expression in human intestinal epithelial cells (HT29 cells) [112]. Interestingly, Ninlawan et al. proved that *Opisthorchis viverrini*, a carcinogenic liver fluke, produces excretory/secretory molecules (OVES) that upregulate cholangiocyte TLR4 mRNA expression. Molecular dissection revealed that OVES activate NF- κ B transcription factor to induce inflammatory cytokines [113]. Re-stimulation of peripheral blood leukocytes (PBL) of opisthorchiasis patients with parasite antigen upregulates TLR2, NF- κ B, and cyclooxygenase 2 (COX-2) genes. Thus, a possible way by which this worm causes liver tumor might be due to induction of oxidant/antioxidant imbalance [114]. Recently, study on another liver fluke, *Clonorchis sinensis*, showed that the crude antigens (CA) of this worm have regulatory effects on DCs. Detailed examinations revealed that the anti-inflammatory effects of CA are extracellular signal-regulated kinase (ERK) signaling-dependent. CA markedly enhanced IL-10 and TGF- β through activation of ERK1/2. Although, ERK was essential for up-regulation of anti-inflammatory cytokines, expression patterns of several activation molecules such as MHC II, CD40, CD80, CD86, TLR2, and TLR4 were not altered following treatment with

CA as compared with the control group [115]. Murine splenocytes also upregulated TLR1 and TLR4 mRNA in response to *Trichinella spiralis* infection, whereas newborn larvae of *Trichinella spiralis* inhibited all TLRs expression, except TLR2, TLR5 and TLR6 in murine splenocytes [116]. Given the migration of these large multicellular eukaryotes through vital host tissues, it remains poorly described how they interact with tissue-resident immune cells.

Helminths and modulation of TLRs signaling

Worms are able to redirect PRRs signaling to intracellular pathways promoting immune subversion and establishment of a persistent infection [117]. The effect of some helminth products on APCs and induction of regulatory DCs is largely dependent upon their ability to signal through TLR4 [96]. ES products can markedly blunt TLR-ligand interaction. For instance, helminth-derived cathelicidin-like peptides hamper TNF- α production from macrophage-exposed to LPS. Likewise, macrophages isolated from animals and humans infected with helminth parasites also exhibited neutralized reaction in response to LPS and IFN- γ [118].

F. hepatica

F. hepatica tegumental coat antigens (FhTeg) have inhibitory effects on DCs of mice through MAPKs pathway. FhTeg by upregulation of suppressor of cytokine signaling 3 (SOCS3), a negative regulator of the TLRs pathway, suppress LPS-induced cytokine secretion. Also, FhTeg arrest LPS-induced NF- κ B and ERK/MAPK pathway activation in TLR4 signaling [119, 120]. Similarly, other ES antigens from the *T. spiralis* and *S. mansoni* egg antigens can decrease LPS-induced phosphorylation of p38 and ERK in macrophages [93, 121]. TRIF-dependent signaling also might be manipulated by some helminths. In this regard, Donnelly *et al.* discovered that cathepsin L1 cysteine protease (FheCL1) from *F. hepatica* by degradation of TLR3 arrests MyD88-independent TRIF-dependent signaling pathway [122–124]. Also, it has been revealed that *T. spiralis* inhibits TLR4 and TRIF-dependent signaling pathway. This worm by modifying TLRs signaling regulates Th1/Th2/Th17-related cytokine production [116].

ES products (ESP) from *F. hepatica* are able to prevent DC activation and cytokine production (IL-12 p70, TNF and IL-6) in response to TLRs ligands (TLR4, TLR3, and TLR9) [125]. When ESP-treated macrophages were exposed to TLR4 and TLR2 agonists, nitric oxide (NO) and IFN- γ production were down-regulated, whereas the production of arginase-1 (Arg-1) was upregulated. Flynn *et al.* suggested that FhESP may influence on macrophages in two ways. First, modifying TLR2 or TLR4 expression inducing their differentiation to alternative activated macrophage (AAM) and secondly engaging of these receptors can limit their accessibility for additional signaling [126].

Schistosoma spp.

The impact of *S. mansoni* antigens on TLRs signaling in DCs and macrophages has been widely studied. LPS-mediated TLRs signaling in human monocytes can be modulated by Sm16 protein from *Schistosoma* [127]. Recently, Correale *et al.* indicated that SEA through TLR2-dependent ERK signaling, induce DCs to upregulate SOCS3, IL-10R, retinoic acid receptor and several genes contributing in retinoic acid synthesis. Further experiments uncovered that SEA-treated DCs via aforementioned genes, not only suppress pro-inflammatory cytokines, but also induce Foxp3 Tregs [128]. Besides TLR2 stimulation, *S. mansoni* expresses molecules stimulating TLR3 and TLR4. dsRNA from schistosomal eggs has RNase A-resistant and RNase III-sensitive structures, enabling it to trigger TLR3 activation [95, 129]. Another

study showed that a carbohydrate derived from *S. mansoni*, Lacto-n-fucopentaose III (LNFPIII), is able to drive *in vitro* differentiation of naive DCs to a DC2 phenotype via TLR4-dependent mechanism. In supporting this, the response of DCs obtained from TLR4^{-/-} mice to LNFPIII was quietly abolished, implying LNFPIII acts through TLR4 [96]. LNFPIII strongly induces phosphorylation of ERK, while phosphorylation of other MAPK molecules such as JNK and p38 was not significant [96]. *S. mansoni* adult worm produces glycolipids that act cooperatively with TLR ligands to modulate the host immune responses. These molecules employ dendritic cell-specific intracellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) for activation of TLR4 in human DCs as a novel mechanism. This study revealed an unusual function of DC-SIGN, as an essential coreceptor for TLR4 that is required for induction of Th1 responses [130].

An intriguing subject is that some *schistosoma spp.* through TLRs manipulation redirect interaction between macrophages and CD4⁺ T cells toward anergy. In this regard, Smith et al. demonstrated that *S. mansoni*, can selectively upregulate PD-L1 on murine macrophages. Expression of PD-L1 results in production of anti-inflammatory cytokines including IL-4, IL-13, IL-10-, and TGF- β [131]. It has only recently become apparent that *S. japonicum*, particularly SEA, through TLR2-dependent signaling increases the expression of PD-1 and PD-L2 on murine BMDCs and CD4⁺ T cells, respectively. Thus, it seems that suppressive effects of *S. japonicum* antigens due to induction of coinhibitory molecules is likely TLR-2 dependent [132]. Moreover, the expression of other genes involved in immune suppression such as gene related to anergy in murine lymphocytes (GRAIL) is elevated during chronic murine schistosomiasis [133].

Beside adult worm, schistosome eggs also produce antigens (SEA) that exquisitely modify the TLRs signaling [93]. *S. mansoni* eggs secrete a glycoprotein known as omega-1 inhibiting TLR-induced DC activation. Moreover, omega-1 has been tested on double deficient MyD88/TRIF mice-derived DCs. Intriguingly, it could drive DCs to induce Th2 cell differentiation in MyD88/TRIF-independent manner [134, 135].

Filarial nematodes

Cystatins are cysteine protease inhibitors produced by most filarial nematodes and recently identified in *Ascaris lumbricoides* (Al-CPI). It is well established that these molecules have strong immunomodulatory effects [136]. The suppressive functions of *Brugia malayi*-derived cystatin (Bm-CPI-2) on antigen processing had previously been proved. Moreover, the modulatory effects of a recombinant AvCystatin (rAvCystatin), from other filarial nematode known as *Acanthocheilonema viteae*, in suppression of murine experimental asthma were recently confirmed [137]. In this regard, Klotz *et al.* provided evidence concerning the detailed signaling triggered by these molecules in murine macrophages. They showed that AvCystatin targets intracellular signaling events to manipulate macrophage. In fact, AvCystatin just like an immunomodulatory protein masterfully modifies host macrophages, as activation of the MAP kinases ERK and p38 induces IL-10 and IL-12/23p40 production. Molecular dissection uncovered that dual specificity phosphatases (DUSPs), as negative regulators of MAPK activation and IL-10 expression, are exploited by *A. viteae* to modulate downstream signals [138].

ES-62 from *A. viteae* is one of the best characterized helminth products. It is able to interact with TLR4 in a manner different from LPS. Melendez et al. examined the effect of ES-62 on mast cells degranulation. They provided solid evidences, implying the internalized ES-62-TLR4 complex could degrade critical molecules in Fc ϵ R signaling. Indeed, they showed ES-62 by interfering in downstream cascades during IgE binding inhibits mast cells degranulation [139]. Similarly, when Th17 cells were exposed to ES-62, production

of IL-17 was hampered due to MyD88 inhibition [140]. Recently, Al-Riyami et al. could design a synthetic small molecule (11a) which is an analog of ES-62. They evaluated the anti-inflammatory effects of 11a on collagen-induced arthritis (CIA) in mice. Interestingly, 11a not only was able to downregulate the expression of MyD88 in TLRs signaling, but also it could decrease proinflammatory cytokines by induction of uncoupling in downstream molecules. Moreover, it is supposed that ES-62 is able to interact with TLR4 on murine $\gamma\delta$ T cells [141] which this interaction can lead to induction of IL-22 producer T cells. Interestingly, administration of anti-IL-22 antibodies to CIA mice abolished the protective effects of ES-62, indicating ES-62 mediates protection against joint pathology via an IL-22-dependent mechanism. However, much remains to be learned whether ES-62 protects against murine collagen-induced arthritis (CIA) via targeting $\gamma\delta$ T cells [142].

Trichuris suis

Recently, a gene expression analysis by microarray was performed to examine the effects of *T. suis* soluble products (SPs) on the human (GM-CSF-differentiated) macrophages [143]. The results showed that SPs arrest the expression of wide range of LPS-induced proinflammatory genes and proteins in the macrophages. In fact, it was revealed SPs induce their anti-inflammatory effects through TLR4-dependent manner. The expression of many LPS-induced inflammatory mediators such as IL-12B, CCL1, and CXCL9, were suppressed by *T. suis* SPs [143]. Similarly, stimulation of *T. suis* excretory/secretory (Ts ES)-treated DCs by CpG and LPS not only could not increase expression of CD86 and MHC class II, but also inhibited IL-6 and IL-12/23p40 production. The suppressive functions of Ts ES were also evaluated in murine OVA-induced asthma model. The results of this study showed that larval Ts E/S can forestall murine asthma in an IL-10-dependent manner [144].

Other worms

Previously, Terrazas et al. showed that the ES products of *Taenia crassiceps* (TcES) suppress maturation of DCs in response to TLR4 and TLR9 agonist, as well as induce tolerogenic DCs [145]. Recently, they found that TcES trigger a new intracellular signaling that is able to block the TLRs pathway in DCs. Furthermore, detailed studies revealed that TcES redirect immune response to Th2 via macrophage galactose C-type lectin (MGL), mannose receptor (MR), and TLR2-dependent manner. TcES phosphorylate c-RAF, which is a MAP₃K acting on downstream pathways of the Ras family, to suppress TLR4 signaling. More precisely, c-RAF by inhibition of its downstream molecules such as p38 and NF- κ B prevents TLR-mediated DC maturation, as blocking cRAF in DCs abrogated the ability of TcES to induce Th2 polarization [145].

H. polygyrus induces intestinal mucosal T cells to produce TGF- β through a TLR4-dependent mechanism, whereas in TLR4^{-/-} mice this ability was abrogated. In fact, it seems that the migration of parasite across the intestinal wall disrupts the epithelial barrier, and consequently facilitates engagement of commensal bacterial LPS to TLR4 which results in activation of IL-10/TGF- β producing regulatory T cells [146]. This is consistent with another study indicating that *H. polygyrus* can induce a population of lamina propria CD8⁺ T cells in mice intestine which are able to inhibit the proliferation of other T cells [147]. Likewise, other products of helminths are found to activate DCs that preferentially induce Treg cells. Some of them also influence intracellular pathways such as modulation of MyD88-dependent signaling [126].

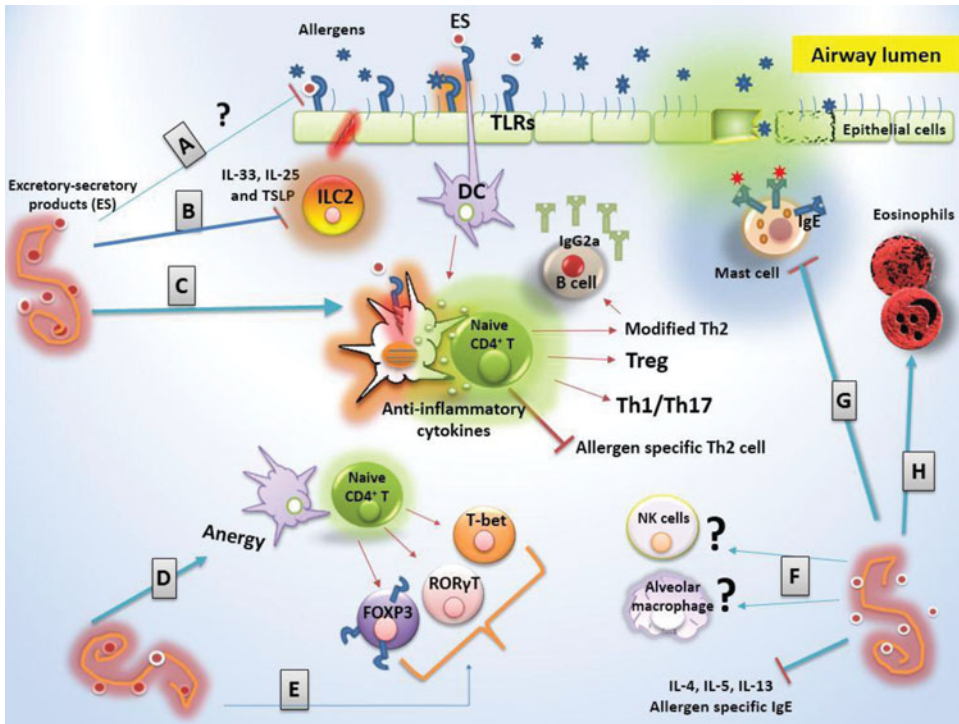


Figure 2. Proposed mechanisms exploited by helminths to alleviate allergic responses in airway. (A) Some helminths produce molecules that efficiently occupy TLR4 and make it unavailable for LPS and other TLR4 ligands. (B) ILC2 and epithelial cell-derived alarmins exacerbate inflammation. It has been confirmed that intranasal delivery of ES inhibits release of IL-33 from ILC2. (C) Some worms (such as *H. polygyrus*) produce molecules that downregulate the expression and function of TLRs of APCs. In addition, helminth-derived products by upregulation of anti-inflammatory cytokines (such as IL-10 and TGF β) facilitate the proliferation of Treg cells. (D) Treatment of APCs with ES results in up-regulation of some co-inhibitory molecules such as PD-L1. *S. mansoni* through TLR2 stimulation redirects macrophages and CD4 $^{+}$ T interaction toward anergy. In addition, the expression of gene related to anergy in murine lymphocytes (GRAIL) was elevated during schistosomiasis. (E) Worms modify expression and functions of TLRs on Tregs and other differentiated T cells, which may enhance their proliferation. For example, SEA induces Treg expansion through TLR2-dependent manner. (F) Up to now, the effect of helminths on NK, NKT cells, and alveolar macrophage in asthma condition has not been determined. Given the important role of these cells in experimental asthma, their response to helminth products needs to be investigated. (G) Inhibition of mast cell degranulation is an interesting mechanism by which helminths suppress airway inflammation. ES-62 from *A. viteae* by constituting an intracellular complex with mast cell TLR4 interferes with Fc ϵ R signaling and hampers mast cells degranulation. (H) It is well established that some of the helminth-derived molecules can induce apoptosis in eosinophils and other immune cells. In addition, ES products can also inhibit eosinophil recruitment.

Helminths and TLR-based therapeutic opportunities: A forward look analysis

In fact, the significant efficacy of helminth products on inhibition of aberrant responses in animal models has motivated attempts to consider them as potential therapeutics in human diseases (Table 2). Many studies are currently ongoing to purify and identify the effector components of such products.

Worms are able to exploit various mechanisms to evade and hijack immune responses. These mechanisms include expansion of regulatory cells (Breg, Treg, and regulatory DC) [148], induction of apoptosis, manipulation of PRRs, and suppression of innate immune cells, especially APCs (Figure 2).

Table 2. Helminth-derived products affecting TLRs and downstream signaling in experimental model.

TLRs	Product	Helminth/location in definitive host	Cytokine profiles	Immunological outcome	Disease model Showing protective effect	Mode of action (signaling)	Ref
TLR4	LNFPIII	<i>Schistosoma mansoni</i> /blood	Low (IFN γ , IL-12) High(IL-4, IL-10)	Th2, AAM, Tolerogenic DCs	Psoriasis, Type1 diabet, EAE (amelioration)	NF- κ B $\uparrow$	[96, 97]
	ES-62	vessels(human and mice) <i>Acanthocheilonema viteae</i> /lymphatic tissue (mice)	Low(IL-12, IL-17)	Th2	Murine OVA-Induced asthma, EAE, Arthritis (amelioration)	ERK $\uparrow$, P38 or JNK MAP $\downarrow$, Transient NF- κ B, Block mast cell degranulation	[139–142]
	FhHDM-1	<i>Fasciola hepatica</i> /Liver (various mammals)	Low pro-inflammatory cytokine	Inhibition of classical macrophage activation	Sepsis (amelioration)	Binding to LPS and inhibits signaling	[170]
	SM16	<i>Schistosoma mansoni</i> /blood	—	Alter LPS activation in monocytes	—	Inhibits degradation of the IRAK1 signaling	[127]
	Fag	vessels(human and mice) <i>Setaria Digitata</i> /abdominal cavities(grazing animals)	Low (IL-1 β , IL-6, TNF α) High (IL-10)	AAM	Endotoxemia (amelioration)	Inhibits LPS mediated classical activation	[171]
	BpA	<i>Brugia pahangi</i> /lymphatic vessels (cat, gerbil, ...)	—	Induce apoptosis in human monocytes	—	Activation of caspase3	[181]
	HDM	<i>Schistosoma mansoni</i> and <i>Fasciola hepatica</i>	Low (TNF- α , IFN γ)	$\downarrow$ activation of macrophages	—	Alter the response of B cells ($\uparrow$ IgG1)	[118]
	Unknown	<i>H. polygyrus</i> /small intestine (rodents)	High (TGF- β) Low (TNF α , IL-12)	Regulatory Th3-type T cells	Colitis	$\uparrow$ TGF- β via TLR4-dependent manner	[146]
	Unknown	<i>H. polygyrus</i>	High (IL-10)	Suppressed both Th1 and Th2/inducing regulatory T cells	—	Suppression of DC response to TRLs	[8]

TLR2	PS lipids	<i>Schistosoma mansoni</i>	Low (IL-12, TNF- α)	Th2	—	↓ p38	[95, 102]
	Lyso-PS lipids	<i>Schistosoma mansoni</i>	Low (IL-12)	Treg (Tr1)	—	ND	[95]
	PS lipids	<i>Ascaris lumbricoides</i> /small intestine (Human)	Low (IL-12, TNF- α)	Th2	—	↓ p38	[102]
	rAS-MIF	<i>Anisakis simplex</i> /intestine (marine mammals, human)	High (IL-10) TNF- α , IFN- γ and Th2 cytokines	Treg	Murine OVA-Induced asthma	↑ Treg via TLR2-dependent	[186]
	pseudocoelomic fluid (PCF)	<i>Ascaris suum</i> /Intestine (pigs and human)	IL-10 independent	Inhibiting APC	Mouse model of allergic inflammation	Through either TLR2 or TLR4	[187]
	SEA	<i>Schistosoma mansoni</i>	Low (IL-4, IL-5, IFN- γ) High (IL-10) Low (IL-12, TNF- α , IFN- γ , IL-17)	Treg	MS	TLR2-dependent ERK signaling and retinoic acid	[128]
	TcES	<i>Taenia crassiceps</i> /intestine (wild canine and rat)	High (IL-4, IL-13) Low (IL-12, TNF- α)	Th2	—	TLR2-dependent induction of cRAF signaling pathway	[145]
	SJMHE1	<i>Schistosoma japonicum</i>	High (IL-10, TGF- β)	Tregs	DTH	TLR2-dependent induction of Tregs	[103]
	dsDNA	<i>Schistosoma mansoni</i>	High (IL-12, TNF- α , IFN-I)	Th1	—	Activate STAT1	[129]
	cathepsin cysteine proteases	<i>Schistosoma mansoni</i> , <i>Fasciola hepatica</i>	Inhibit (IL-6, IL-12 and TNF- α)	Skewing and inhibition of Th1 and Th17	Endotoxemic shock	Dampen TRIF signaling by degradation of TLR3	[123, 124]
TLR3							

Abbreviations: EAE, experimental autoimmune encephalitis; AAM, alternative activated macrophage; MS, multiple sclerosis; DTH, delayed-type hypersensitivity; ND, not defined.

Regulatory cells

Tregs via development of the protective tolerance against inhaled antigens, play a cardinal role in controlling inflammation in the respiratory system. Numerous studies in human subjects and animal models of allergic airways disease have proved their essential role during allergic disease [149]. Regulatory effects of Tregs are mostly ascribed to production of anti-inflammatory cytokines such as IL-10 and TGF- β . Tregs broadly affect both innate and adaptive immune cells involved in asthma development, including suppression of DCs and effector Th1, Th2, and Th17 cells. Also, mast cells, basophils, and eosinophils were found to be suppressed by Tregs during asthma exacerbation [150]. Asthmatic patients have fewer and less functional Tregs, as well as there is a breakdown in their regulatory mechanisms, promoting aberrant responses to harmless antigens [151].

Just like Tregs, regulatory B cells (Bregs) can efficiently control allergic responses, both in human and mice [152]. Various mechanisms have been suggested for immunoregulatory functions of Bregs in asthma diseases, including inhibition of DC maturation, production of anti-inflammatory cytokines, induction of Tregs and expression of inhibitory molecules [152–154]. The most important mechanism by which Bregs suppress asthma is production of IL-10 and TGF- β . IL-10-producing B10 cells are the most important Bregs inhibiting asthma development [152, 154]. In addition, it has been shown that B cells through upregulation of FasL are able to attenuate cockroach antigen-induced murine asthma [155].

Thus, development of methods amplifying functional Tregs and Bregs in predisposed or asthmatic patients can be considered as a potential approach for prevention and treatment of asthma.

Regulatory T cells (Tregs)

Many investigations have indicated that helminths and their products can suppress allergic airway disease mostly by recruitment and expansion of Tregs. Moreover, some helminths not only induce Tregs expansion, but also by activating their TLRs promote suppressive functions of Tregs. For example, SEA induce Treg expansion through TLR2-dependent manner [95, 101, 102]. Also, TLR2-dependent ERK signaling in SEA-treated DCs suppresses pro-inflammatory cytokines along with the induction of Foxp3 Tregs [128]. SJMHE1, from *S. japonicum*, via TLR2 stimulation both enhances Treg proliferation and promotes their suppressive activity [103]. With regard to striking role of Tregs in progression of immunosuppressive effects of helminths, more investigations need to be done how helminths through modifying TLRs of Tregs enhance their longevity and suppressive activity.

Regulatory B cells (Bregs)

Their relevancy in helminth infections has been proved in several studies [156–158]. In addition to Tregs, Bregs also contribute to suppression of antihelminth immune responses. Recently, a detailed study on protective effects of ES-62 against collagen-induced arthritis (CIA) in mice revealed that regulatory B cells through IL-10 production suppress pathogenic Th1/Th17 responses. Also, ES-62 could significantly downregulate some surface molecules such as TLR4, CD80, and CD86 on B cell, suggesting ES-62 by setting a balance between effector B cells and regulatory B cells suppresses inflammation [156]. There is solid evidence that suggests *S. mansoni* also through stimulation of Bregs surprisingly avoids allergic airway inflammation in murine models. In this regard, adoptive transfer of spleen B cells from mice with schistosomiasis to OVA-sensitized mice could diminish allergic airway inflammation. Interestingly, it was uncovered that these adoptive B cells via a TGF β -independent manner

recruit CD4⁺CD25⁺ Foxp3⁺ Tregs to the lungs in order to overcome allergic responses [157]. In addition, recently, an *in vitro* study on B cells derived from patients who naturally had *S. haematobium* infection indicated that coculture of these B cells with stimulated CD4⁺ T cells not only results in inhibition of IFN- γ , IL-4, and IL-17 production, but also increase differentiation of Tregs [158]. Up to now, multiple mechanisms of immune modulation by helminth-induced regulatory B cells have been recognized, at least, may include induction of anti-inflammatory antibodies (e.g., IgG4), APCs impairment, restraining of effector T cells, and recruitment of Treg cells [159,160]. Altogether, given the steadily growing body of knowledge concerning the involvement of Bregs during helminth infections and their therapeutic opportunity in inflammatory responses, it is less clear yet whether TLRs expressed on Bregs are markedly manipulated during helminth infections.

TLR-based adjuvants, agonists, and antagonists in asthma therapy: A potential role for helminth-derived products

Many investigations have proved the immunomodulatory properties of TLRs in allergic asthma. Skewing Th2 response toward Th1 through TLRs stimulation has been proposed as an approach to treat patients with asthma. In this regard, the suppressive effects of TLR7 [161–163] and TLR9 agonist (CpG ODN) on asthma have extensively been studied [164, 165]. However, TLR5 agonist (flagellin) was found to promote and exacerbate experimental asthma in mice [166], as well as the exact role of other TLRs such as TLR1/2, 3, and 4 is controversial. Thus, it seems that inhibition of TLRs stimulating asthma or activation of TLRs suppressing asthma would be an approach to treatment.

Up to now, several TLR inhibitors have been developed for the treatment of inflammatory diseases. TLRs antagonists interrupt extracellular and intracellular events during TLR-ligand interaction. They either block TLRs binding to ligand or inhibit downstream signaling pathway. It is believed that TLRs antagonists might lead to impairment of local immunity, also in heterogeneous disease such as asthma it requires more studies. So far, there are no data concerning the efficacy of TLRs antagonists for asthma treatment [167]. TLRs-based adjuvants are being considered as efficacious agents to optimize immune responses in different situation. Administration of TLRs-based adjuvant along with antigens has increased the efficacy of vaccines [168, 169]. Recently, it was shown that adding TLRs agonists, including poly (I:C) (a TLR3 agonist), LPS (a TLR4 agonist), and polyU (a TLR7 agonist), to a vaccine designed against severe acute respiratory syndrome-related coronavirus (SARS-CoV) significantly reduced airway eosinophil infiltration and increased vaccine efficacy [160]. These data reflect the critical role of TLRs on the differentiation, function, and life span of cells that play a key role in initiation and amplification of asthma.

It has only recently been apparent that some helminth-derived products can physically interfere with TLRs-ligand interaction. Up to now, several studies have reported that helminth-derived peptides can bind LPS and prevent it from binding to TLR4/MD2/CD14 complex on APCs [118]. In this regard, Robinson et al. indicated that parasitic helminths secrete molecules that modulate innate cell response to classical TLRs ligands such as LPS. For instance, the helminth-derived molecule secreted by *F. hepatica* (FhHDM-1) through binding directly to LPS, blocks interaction with lipopolysaccharide binding protein (LBP), thus effectively reduces TLRs signaling cascade on the macrophage [170]. A recent study has also provided convincing evidence that proves, chitohexaose residues present in somatic extracts of *Setaria digitata* (FAG) and *Brugia pahangi* effectively compete with LPS for binding to TLR4.

Treatment of murine macrophages and human monocytes with these molecules could inhibit LPS-induced inflammatory mediators [171]. However, study on antagonistic activity of these biologic products is in preliminary stages and more detailed studies are required to opine on the antagonistic properties of these antigens. The exact mechanism through which helminth products inhibit TLRs-ligand interaction remains unknown. It also necessities to employ sensitive assays for evaluating their affinity to receptors. Prospectively, these studies point out that helminth products could be considered for further assessment in future investigations as potential antagonist of TLRs.

Helminth products through TLRs manipulation could present a novel generation of natural TLRs-based adjuvants for developments of immunoregulatory vaccines. Thus, the possibility of development of a Treg-based, Breg-based, and tolerogenic DC-based vaccines should be investigated [172]. In addition, it needs to be further elucidated whether helminths can up-regulate asthma protective TLRs and downregulate deleterious TLRs.

Innate immunity

Group 2 innate lymphoid cells (ILC2)

Group 2 innate lymphoid cells (ILC2) so-called nuocytes, produce large amount of Th2 cytokines such as IL-5, IL-13, and alarmins. Halim et al. found that ILC2 by production of large amount of Th2 cytokines promote Th2 responses, and increase the migration of activated DCs from the lungs to the mediastinal lymph nodes [173]. The effect of excretory-secretory products on ILC-derived cytokines, especially IL-33 has recently been revealed. This study indicated that *H. polygyrus* soluble products (HES) hamper airway inflammation induced by *Alternaria*/OVA allergens. Detailed study and molecular dissection pinpointed that intranasal administration of HES suppresses early IL-33 release due to *Alternaria*/OVA exposure. Interestingly, suppressive effects of HES were abrogated after administration of recombinant IL-33 [87]. But, it needs to be determined whether ES can arrest IL-33, IL-25, and TSLP release from airway epithelial cells.

Granulocytes, alternative activated macrophages (AAMs), and mast cells

There is convincing evidence implying the inhibitory effects of helminths on eosinophils, basophils, and neutrophils. Recent findings have proved that worm products exploit various mechanisms to suppress granulocytes in experimental asthma. Because of provoking effects of eosinophils, basophils and neutrophils on asthma exacerbation, developing a broad spectrum of anti-inflammatory molecules from helminths could be an invaluable approach [52].

Mast cells are propagated and recruited to submucosa of airways during asthma. These tissue-dwelling cells disgorge their toxic granules due to surface IgE-allergen engagement. Some ES products inhibit mast cell degranulation by interesting mechanisms. For instance, ES-62 from *A. viteae* by involving TLR4, forms an intracellular complex to interfere with FcεR signaling and eventually suppresses mast cells degranulation [139]. In support of this function, a recent study has also documented that *Clonorchis sinensis*-derived proteins known as venom allergen-like proteins (CsVAL), inhibit activation of down-stream molecules contributing in degranulation of IgE-armed mast cells [174]. Thus, avoiding mast cells degranulation by helminth products will confer a novel biological treatment, especially in order to mitigate airway inflammation and asthma. Recently Rzepecka et al. succeeded to design two ES-62-based analogue molecules, termed 11a and 12b, which represented an outstanding ability in suppression of OVA-induced asthma. Importantly, these synthetic products not only

could inhibit eosinophils and mast cells activation, but also prevented neutrophil recruitment to the lungs, paving the way for devising new treatments against severe asthma [175].

Dendritic cells

The expression of wide array of PRRs in DCs strictly equips them for monitoring inhaled aerosols. Activation of these receptors triggers strong signals leading to maturation and migration of DCs. An epidemiological study on school children in Ghana indicated that there is an inverse relationship between *Schistosoma haematobium* infection and their atopic reactivity to allergens. Detailed investigations on their PBMCs revealed that this worm through down-regulation of TLR2 and SOCS3 modifies Th2 responses [176]. SOCS3 is involved in pathogenesis of asthma and its expression significantly increased in experimental asthma. Knocking down of SOCS3 by intranasal administration of short interfering RNA (siRNA) could attenuate asthmatic responses in a chronic asthma mouse model [177]. There is evidence that proves helminth antigens can directly manipulate SOCS3 expression [119, 120, 128]. Helminths intriguingly interfere with TLRs signaling to induce tolerogenic DCs with production of anti-inflammatory cytokines [178]. Altogether, targeting extra- and intracellular molecules that regulate the differentiation, maturation, and migration of DCs with mechanisms discussed in previous sections, could be of potential therapeutic application in allergic asthma.

Induction of apoptosis

Apoptosis as an effective approach to attenuate host immune responses is widely performed by helminths. For instance, some nematodes induce apoptosis of host cells such as murine CD4⁺ T cells, human lung epithelial cells, DCs [178], and NK cells [179]. *Fasciola hepatica*-derived molecules are able to induce apoptosis in eosinophils [180]. Apart from eosinophils, monocytes and APCs are also targeted by helminths to induce programmed cell death. The role of TLR4 in induction of apoptosis by *Brugia pahangi* antigens (BpA) has recently been apparent. The results of an *in vitro* study showed that BpA trigger apoptosis in human monocytes through TLR4-dependent manner. Also, it could markedly induce apoptosis in TLR4 overexpressed transfected cells and bone marrow-derived macrophages (BMDMs) of wild-type (C3H/OuJ) mice as compared with TLR4-deficient cells and TLR4-defective (C3H/HeJ) mice, respectively [181].

Conclusion

It is well established that TLRs efficiently contribute in many Th1 or Th2-associated responses. Although all routes by which helminths modify host inflammatory responses have not exactly been recognized, innate immunity and in particular TLRs, are masterfully manipulated by helminth-derived bioactive molecules. Unfortunately, at present due to lack of deep information concerning the molecular structure and chemical compositions of these products, many promising results from *in vitro* and *in vivo* studies have not been translated into clinical practice yet [182]. In addition, to minimize side effects and exclude deleterious ingredients, using the identified component rather than the whole crude antigens is a substantial step prior to clinical studies [182, 183]. To obtain immunomodulatory molecules from helminths as therapeutics for allergy/asthma disease, more preclinical studies remain to be carried out [182]. Definitely, to characterize helminth-derived crude antigens, high-throughput functional assays and omics-based techniques (such as transcriptomics, proteomics, and

glycomics) are very useful. These techniques will pave the way to explore in detail further promising candidates and yield successful treatments that can be adapted for the clinics [184]. In addition, these methods by providing detailed information on the helminth-derived antigens not only would be very promising for identifying of stage-specific antigens, but also will help the investigators to focus on helminths that their antigens are more closely related to human hookworm [185].

Serious obstacles concerning the therapeutic applications of live helminths in human such as ethical and standardization issues, have redirected most recent investigations to focus on identifying helminth-derived immunoregulatory molecules. Alternatively, developing analogue molecules which structurally and functionally have the most similarity to these natural products would basically eliminate some obstacles concerning the using live helminths and obtaining ES from helminths. In this context, designing ES-62 phosphorylcholine-based small molecule analogues (SMAs) has recently displayed promising results against both the murine collagen induced arthritis (CIA) model [141], and OVA-induced experimental asthma [169]. Better understanding of the molecular mechanism employed by helminths may shed light on novel therapeutic approach, especially for corticosteroid-resistant inflammatory disorders like severe asthma [31]. It is necessary to do more studies on how TLRs actually mediate protective effects against helminths. It also needs to be exactly defined how intestine resident helminths by targeting TLRs can significantly alleviate host airway inflammation in OVA-induced asthma model [186, 187]. Currently, a large body of studies is being carried out on molecules that specifically target TLRs, as therapeutic opportunity, for harnessing inflammation [188]. Particular insight into these antigens not only provides a basis for the development of new therapies for diseases such as asthma, but also offers the potential to develop immune deviation therapies, causing a shift from an adaptive Th2 to Th1-dominated immune response and induction of regulatory T cells. Furthermore, helminth products that influence on TLRs signaling pathways can be considered as potential adjuvants for immunostimulatory and immunoregulatory vaccines [189]. Although, no clinical study has been done concerning the efficacy of helminth products on asthma treatment, promising results achieved from murine experimental asthma are encouraging scientists to launch clinical studies.

Given the emerging data on the effect of helminth antigens on TLRs, many investigations have been done to characterize helminth-derived molecules that are tractable to the pharmaceutical industry, such as ES-62. Full characterization of crude antigens helps us to obtain the potential therapeutic components and unravel the underlying mechanisms mediated by such products. Prospectively, the data that are available at present suggest that, in the near future, it is possible that helminth antigens will offer new therapeutic strategies and druggable targets for fighting allergic diseases.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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