

Toll-Like Receptors and Immune Response in Allergic Disease

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Abstract

Allergic reactions are dominated by the preferential development of specific Th2 responses against innocuous antigens in atopic individuals. This can reflect alterations in innate immune mechanisms. Toll-like receptors (TLRs) have evolved as key molecules in innate and adaptive immunity. Their activation by structurally distinct exogenous or endogenous ligands present at the cell microenvironment plays a critical role in antimicrobial defense. The global view is that TLR activation induces antigen-presenting cells to produce cytokines that favor Th1-type immune responses, suggesting that it might prevent the development of deleterious Th2 responses in allergy. On the basis of epidemiological studies and recent data, it has been established that TLRs play a role in the development of Th2 responses. However, more information is needed to fully understand the mechanism of TLR involvement and the implication of immune cells that express TLRs in the Th1/Th2 cytokine profiles. Several TLRs, such as TLR9, TLR7, and TLR8, can be considered as good target candidates. Some TLR ligands, such as CpG DNA, are effective adjuvants, strong inducers of both IL-5 and eosinophilia downregulation. They are also potential links to allergen epitopes that could provide new allergen-specific immunotherapy regimens for the treatment of allergic disorders.

Index Entries

TLR; allergy; Th1; Th2; cytokine; immunotherapy; T cell; B cell; dendritic cell; mast cells.

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Introduction

Allergic reactions are under the control of events that occur sequentially following allergen exposure: recognition by the immune system, IgE production, and interaction of the allergen with effector cells bearing Fcε receptors. Effector cells (eosinophils, mastocytes) release a variety of pro-inflammatory and vasoactive mediators, including histamine, prostaglandins, leukotrienes, and cytokines, that produce allergic inflammatory responses. Cytokines produced by immune and non-immune cells are involved in the polarized Th1/Th2 development of the specific immune response. T cell differentiation is an important parameter for the establishment of effective adaptive immunity. These cells can differentiate into two distinct subsets, Th1 and Th2. Th1 cells secrete mainly IFN-γ and are involved in cellular immunity. Th2 cells, involved in humoral immunity, play a central role in allergic diseases through the secretion of a range of cytokines (particularly IL-4, IL-5, IL-13, etc.) that specifically modulate defined aspects of pathogenesis. The preferential development of specific Th2 responses against innocuous environmental antigens (aeroallergens, food allergens, etc.) in atopic individuals reflects, at least partially, alteration in innate immune mechanisms. Genetic alterations as well as environmental factors are involved in regulating the development and function of Th2 cells. It has been suggested that exposure to specific infection drives the maturing immune system in infancy/childhood toward a Th1 phenotype and away from the Th2 cell phenotype that is associated with atopy. The so-called "hygiene hypothesis" (1), although attractive, is still a matter for debate (2,3). Many studies point to a strong genetic impact in the pathogenesis of IgE-mediated diseases. For example, it has been found that one or more loci on chromosome 5q may control total IgE, as well as asthma and bronchial hyperresponsiveness to nonspecific stimuli (4,5). Genetic variations in

CD14, a key gene of innate immunity, may modulate the effects of bacterial ligands on the development of Th2 responses. Bacterial lipopolysaccharide (LPS), which binds to the CD14/TLR4 complex (a member of the toll-like receptor [TLR] family), or other microbial products can influence the development and severity of asthma.

TLRs are a family of vertebrate recognition receptors, homologous to the *Drosophila* Toll receptor. They have evolved as key molecules in innate and adaptive immunity. They play a crucial role in the recognition of conserved microbial components, alerting the host innate immune system to invading pathogens. It is currently believed that TLR activation participates in protective immunity by supporting the overall development of cell-mediated, Th1-biased T cell responses (6,7).

Many chronic diseases, including allergy, may occur as a result of genetically based or environmentally induced changes in mechanisms regulating natural or acquired immune responses and immune tolerance. Consistent with their function, TLRs are expressed mainly on cell types (macrophages, dendritic cells [DCs], mast cells, etc.) that are involved in the first line of defense against foreign pathogens. Although most studies have focused on the role of TLRs and their signaling pathways in the control of Th1-type immune responses and on the implications for the use of TLR ligands as antimicrobial agents or adjuvants in vaccines, it appears that there are multiple points at which TLR signaling could modulate Th2 responses. In this review we will focus particularly on the possible role of TLRs in Th2-directed responses in allergy. We will also discuss the potential use of TLRs as therapeutic targets in allergic diseases.

TLRs, a Link Between Innate and Adaptive Immunity

Innate recognition of bacteria involves complex binding to receptors that recognize

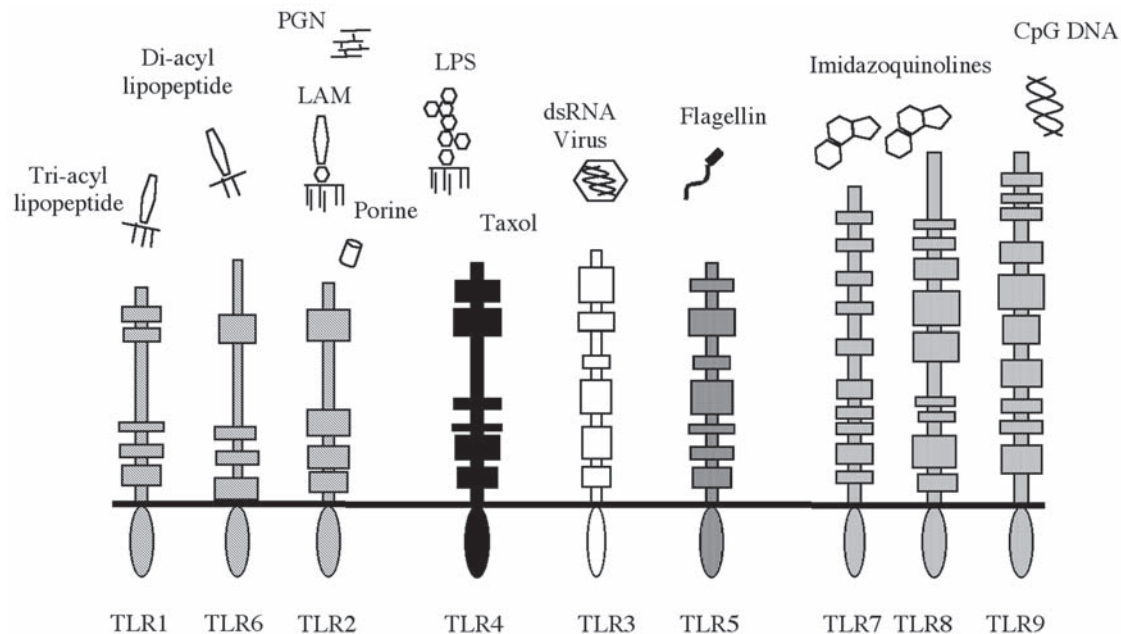


Fig. 1. The different members of the TLR family and their major ligands. The TLRs with similarity and close phylogeny have been placed next to each other and have the same color pattern. PGN: peptidoglycan; LAM: lipoarabinomannan; LPS: lipopolysaccharide; dsRNA: double-strain RNA.

the conserved pathogenic patterns on microbes. The expression and intervention of such pattern recognition receptors depend not only on the cell type but also on the nature of the stimulus, bacterial or other. Integrins, CD55, ion channels, activated clusters containing heat-shock proteins, chemokine receptors, and more specifically CD14, TLRs, and their comolecules (MD2, MD1, MyD88, etc.) have been shown to serve as key molecules in the recognition of invading microorganisms and in the development of the infectious process (8–15).

TLRs (TLR1–TLR10) belong to a large innate receptor repertoire family, the TLR/IL-1/IL-18 receptors, that has evolved to recognize conserved molecular patterns of microbial origin not expressed in the host. Activation of most TLRs leads to receptor interaction with the adapter molecules MyD88 and Toll/IL-1 receptor domain-containing adapter protein (TIRAP, for TLR2 and TLR4). This results in activation of nuclear factor- κ B via a signaling pathway that involves IL-1 receptor-associated

kinase and tumor necrosis factor (TNF)-associated factor-6. Nuclear factor- κ B activation leads to its nuclear translocation and the induction of the expression of genes that are involved in inflammation and antimicrobial reactions (6,16). TLR activation participates in protective immunity by mediating the secretion of antibacterial effector molecules including cytokines, chemokines, and the like, and by supporting the development of a cell-mediated, Th1-biased T cell response.

Bacterial cell wall components, bacterial DNA, and double-stranded RNA are apparently recognized by different TLRs (Fig. 1, 17–19). For example, among other immunostimulatory responses, bacterial lipopolysaccharide (LPS), recognized by CD14 and TLR4, provokes the production of TNF- α , IL-12, and nitric oxide (20, personal data). Peptidoglycan (PGN) induces the same pro-inflammatory responses through TLR2 (21,22). CpG motifs recognized by TLR9 (23) and synthetic nucleic acid-like structures, for example, imidazoquinoline (imiquimod, R-848) are distinguished by

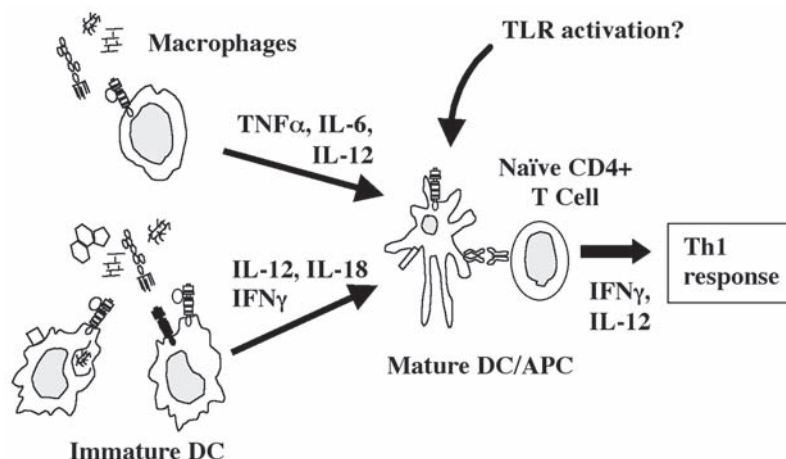


Fig. 2. TLRs in innate immunity and in the maturation of dendritic cells toward a Th1 response. DC, dendritic cell; APC, antigen-presenting cell.

TLR7 and TLR8 (phylogenetically related to TLR9), and they can trigger antigen-presenting cells (APCs) to produce IL-12 (24,25). In addition, TLRs including TLR2, TLR4 and TLR9 can be involved in signaling in response to endogenous proteins (26) associated with cell damage, inflammation, and oxidative stress for example, heat-shock proteins (i.e., HSP60, HSP70, and GP96), extracellular matrix components (hyaluronic acid, fibronectin, and fibrinogen), lung surfactant protein-A, and necrotic cell products.

In mammals, the activation of the innate immune response in peripheral tissues has limited ability to eradicate pathogens. To accomplish complete eradication, a more complete and specific immune response must occur. DCs play an important and essential role in instructing the adaptive immune system and allowing communication between peripheral and lymphoid tissues (27,28). TLR signaling stimulates the maturation of DCs and triggers adaptive immunity by enhancing the expression of MHC molecules, chemokines, and chemokine receptors as well as CD80/CD86 costimulatory molecules on DCs (29,30). CD80 and CD86, which interact with CD28 expressed on CD4⁺ T cells, provide a critical costimulatory signal for T-cell activation.

TLR-mediated activation allows DCs to leave peripheral tissues and migrate to lymph nodes where they can activate T cells (31), which then leads to the secretion of cytokines such as IL-12, IL-23, and IL-27 that preferentially promote Th1 cell type differentiation. Thus, the global view is that TLR activation generally induces APCs to produce cytokines that favor Th1-type adaptive immune responses (Fig. 2). Using deficient mouse models, it has been shown that TLR4 also plays a critical role in the generation of Th2-type cytokines through the modulation of DC costimulatory molecule expression (32) and in low-dose LPS-induced Th2 responses to inhaled antigens (33).

TLRs are also expressed on T and B cells. Thus, TLRs may potentially regulate T-cell immunity directly. Human CD4⁺ T cells express a variety of TLRs, with TLR1, TLR2, TLR3, TLR5, and TLR9 being the predominant forms (34,35). Therefore, it is plausible that TLR signaling pathways may play a direct role in CD4⁺ T-cell activation and differentiation, in addition to their well-characterized effects on DCs and monocytes. However, it remains to be shown whether selective triggering of a particular set of TLRs expressed on T cells leads to differential expression of Th1/Th2 cytokines.

It is assumed that a particular DC subset, DC maturation stage, or the DC/T-cell ratio can affect the cytokine production determined by TLR expression and, as a consequence, the differentiation into Th1 or Th2 cells (36,37). Thus, TLRs (and/or the co-molecules) serve multiple functions: they are active in the early and late stages of the immune response, they play an important role in sensing invading pathogens as well as other exogenous antigens, and they instruct the adaptive immune system via cell-cell interactions.

TLRs in Th2-Directed Responses in Allergic Diseases

The initial immune response that is responsible for the development of allergic diseases such as asthma is thought to be the generation of allergen-specific CD4⁺ Th2 cells. Analysis of blood, bronchoalveolar lavage fluid, and bronchial mucosal biopsies from patients with allergic asthma have shown predominantly activation of Th2-like cells that produce IL-4, IL-13, and IL-5 but not IL-12 or IFN- γ (38,39). Overexpression of IL-13 in the lung and in models of acute asthma in mice plays an important role in induction of mucus hypersecretion, goblet cell hyperplasia, subepithelial fibrosis, eotaxin production, eosinophil accumulation, and the promotion of enhanced airway hyperactivity to cholinergic stimuli. In chronic asthma, IL-13 is also likely to play a key role in regulating airway wall remodeling. The molecular mechanisms that govern Th1 or Th2 polarization are clearly defined. The presence of IL-4 at the time of antigen presentation is the most potent stimulus for Th2 differentiation, whereas IL-12 and IFN- γ favor Th1 development. IL-4 and IL-13, secreted primarily by activated Th2 cells, regulate immunological processes that underpin the pathophysiology of allergic diseases by contributing to the differentiation of B cells and isotype switching to promote IgE secretion (40). When specific IgE produced by allergen-stimulated B cells

encounters mast cells and basophils, it binds to the cell surface via IgE Fc receptors. Subsequently, when the same allergen encounters these antibody-sensitized cells, it crosslinks pairs of IgE antibody and thereby activates the cells, resulting in degranulation with secretion of the mediators of the allergic reaction (Fig. 3).

A substantial body of epidemiological evidence has pointed out that the prevalence of allergy has increased in the last two to three decades, especially in school-age children (41,42). An increase of indoor allergens due to changes in the domestic environment and of outdoor allergens linked to industrial pollution, tobacco smoke, and changes in the overall diet have been implicated in the decrease of Th1-type inflammation and the augmentation of the prevalence of allergic diseases. Some surveys also suggest that there is an inverse correlation between infection and the development of allergy. According to the "hygiene hypothesis" (43), exposure of young children to specific bacterial and viral infections, endotoxin and CpG DNA drives the maturing immune system toward a Th1 phenotype and away from an atopy-associated Th2 phenotype in the environment of the airways. Consequently, contact with an allergen leads to the initiation of a Th1 immune response specific for the allergen, which reduces the likelihood of inducing Th2-dependent IgE antibody production. Early contact with microbes seems to be essential to sculpt the immune response toward a Th1 response. However, animals grown under strict germ-free conditions (in the absence of endogenous microbial flora) also fail to develop Th2-type responses, suggesting that contact with microbes is necessary for the complete maturation of the immune system (44).

Recent research has explored the impact of genetic variation on the regulation and potential role of CD14 in the pathogenesis of IgE-mediated diseases. Most of the data suggest that genetic variations in CD14 can modulate the effect of bacteria on the development of Th2

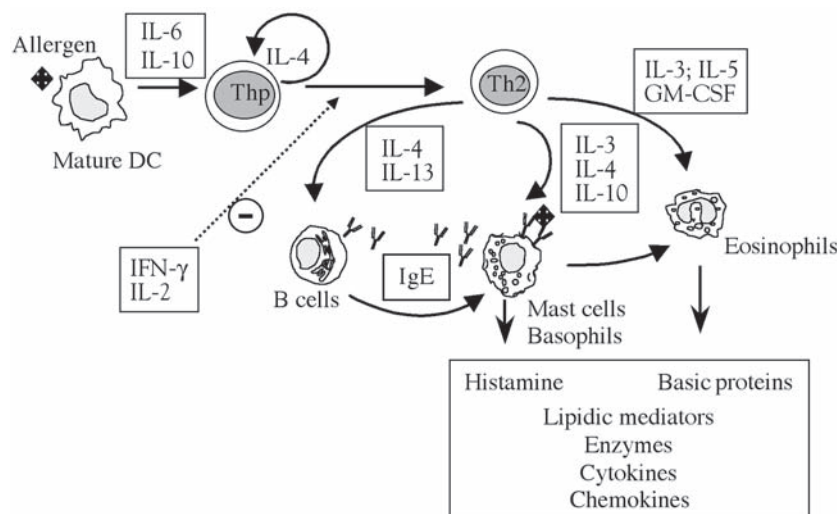


Fig. 3. The Th2 response in allergy.

responses (45–47). In other studies, TLR4 gene polymorphism was used to explain the diverse ability of individuals with asthma or other forms of airway disease caused or exacerbated by endotoxins to respond to environmental stress and LPS (48–51). Exposure to microbial products, including LPS, can influence the severity of asthma; endotoxins in inhaled dusts may be as relevant as allergen levels for the allergic asthmatic (52). However, the mechanism by which LPS influences the pathogenesis of asthma remains undefined. There is some evidence that in TLR4- and MyD88-deficient mice TLR activation directly regulates Th2 responses. Dendritic TLR4-deficient cells express lower level of CD86 and they induce less Th2 cytokine production by antigenically naïve CD4⁺ T cells (32). Moreover, TLR4-deficient mast cells do not produce IL-5, IL-10, or IL-13 (53). Given the role of mast cells and DCs in host defense against microorganisms, it is plausible that signals acting on mast cells also favor the development of Th2 responses in vivo.

Therefore, a pathogenetic role for TLRs and TLR ligands cannot be ruled out, especially once allergic inflammation is established. Depending on the time frame of the interaction with pathogens, the role of TLRs could be dual: in addition to the potentially important role of

TLRs in the induction phase of an allergic phenotype, a TLR-driven immune response might also have an essential role in disease exacerbation.

TLRs and Allergy Immunotherapy

One of the most interesting aspects of TLR biology with regard to allergic disease is the possibility of exploiting the powerful properties of TLR signaling in the modulation of allergic inflammation (Fig. 4). In fact, there is a vast amount of data demonstrating that the Th1-inducing ability of TLRs can be used to prevent the development of deleterious Th2 responses in allergic diseases. This is based on the belief that Th1 and Th2 responses reciprocally regulate their activity in a negative manner. This modulation can occur via the activation of a global set of TLRs and comolecules by whole bacteria (or viruses) or of selective triggering of certain types of TLR and comolecules such as MyD88, TIRAP, etc. (24,54).

TLR activators are extremely potent adjuvants, acting on DCs to drive typical Th1-type responses during antigen presentation. Nevertheless, some complexity is provided by the fact that TLR signaling results in contrasting cytokine output in different cell types. The following gives some examples of potential targets for the treatment of allergy.

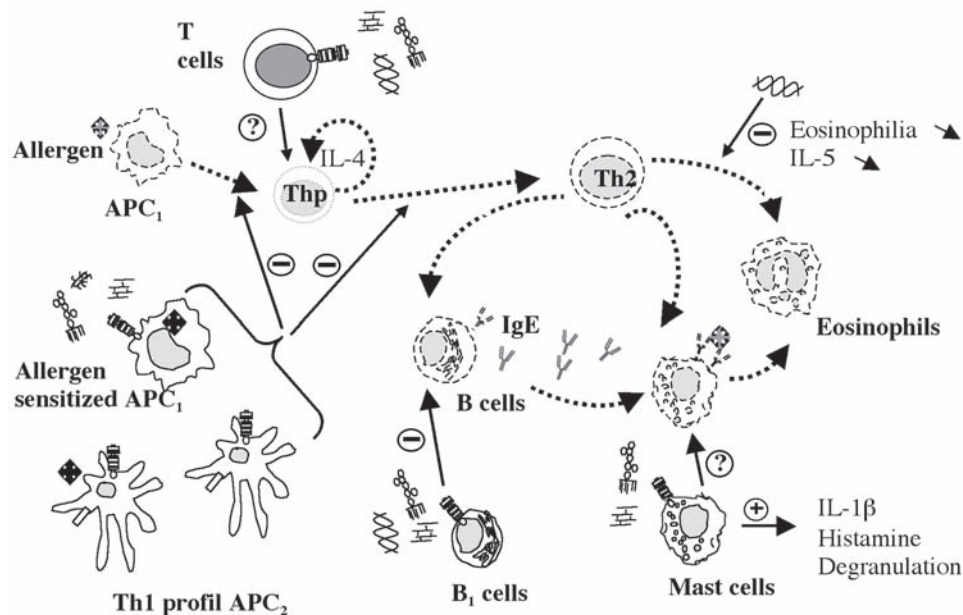


Fig. 4. Potential targets for a TLR-modulated response in allergy.

TLR; PE : peptidoglycan; LPS ; CpG DNA ; allergen .

Downregulation of the Th2 response by TLR activation is designated by $\ominus$.

Upregulation of the Th2 profile is shown as $\oplus$ and still-unknown effects are designated by $\otimes$.

CD14, TLR4, TLR2, and MyD88 can be considered as targets for immunotherapy in patients with allergic disease. This approach is supported by the fact that in MyD88-deficient mice the lack of TLR signaling leads to a Th2 response, with IgE production by default and no induction of a Th1 response (55). Nevertheless, there is not a black or white case for an effect of TLR ligands at the cellular level.

Mycobacteria and their glycolipids (LAM, etc.), which are known to be highly immunostimulatory, can—through recognition, uptake, and presentation by macrophages and DCs—favor TLR-dependent stimulation of the Th1 response by enhancing IL-12 and IFN- γ production (56). It also has been shown that certain mycobacterial strains can reduce the production of specific IgE, eosinophilia, and allergen-induced bronchial inflammation while increasing the production of IFN- γ , a potent suppressor of Th2 activity (57).

Using DCs, it has been suggested that the level of LPS exposure can determine the type of inflammatory response generated and there-

fore can provide a potential mechanistic approach to the therapy of allergy (33). The LPS threshold for induction of a response can be deduced by consideration of the threshold at which low levels of LPS signal through the CD14 pathway and high level of LPS signal through the CD14-independent pathway (20), both pathways involving TLR4 on macrophages (S.C. Gangloff, unpublished data). Thus, distinct levels of signaling intensity lead to differential effects on the adaptive immune response.

Mast cells represent another potential source of multifunctional cytokines that may participate in the recruitment and activation of other cells in the inflammatory microenvironment of an allergic response. Microligands such as peptidoglycan (PGN) from *Staphylococcus aureus* or LPS from *Escherichia coli* can activate mast cells directly in a respective TLR2- or TLR4-dependent manner, leading to a selective production of IL-1 β (with LPS), degranulation and Ca²⁺ mobilization (with PGN) (58) or to the release of histamine (with PGN) (59). Therefore, inhi-

bition of the TLR4 and/or TLR2 pathways in mast cells may downregulate the contribution of these cells to allergic responses.

In an allergic situation, even though TLR4 can be involved in the Th2 profile under certain very specific conditions, the presentation of allergen by APCs simultaneously stimulated by bacterial ligands would activate innate immune pathways and, by enhancing CD14 expression and responsiveness to LPS, would lead to increased expression of IL-12 and IL-18, decreased Th2 differentiation, and suppression of IgE responses.

TLR9 is a critical receptor for the recognition of bacterial DNA and for CpG DNA, as first shown in transfected mice. Macrophages, DCs, and B cells are the main cell types that respond directly to CpG DNA. Macrophages produce mainly Th1 cytokines (IL-12, IL-18, TNF- α , and IFN- α/β) (60,61); DCs produce predominantly IL-6, IL-12, and TNF- α , and they overexpress costimulatory molecules (MHC class II, CD54, CD86, and CD40); while B cells produce essentially IL-6 and IL-12. The actions of CpG motifs and their potential roles and therapeutic abilities were recently reviewed in detail (62,63). Basically, bacterial DNA and oligodeoxynucleotides carrying the CpG motif can produce immunostimulatory activity on human and mouse T cells and APCs. They induce costimulatory molecule upregulation and a Th1-like cytokine production pattern that is dominated by IL-12 and IFN- γ , with little secretion of Th2 cytokines. The therapeutic activity of CpG DNA in a model of asthma was initially thought to be working through induction of Th1-like cytokines (IL-12, IFN- γ). However, it has been shown more recently that CpG DNA could prevent allergic disease in IL-12- and IFN- γ -deficient mice (64), and also inhibit an established Th2 response (65). Furthermore, CpG DNA has been used to treat experimentally induced asthma in mice (66).

Certain chemical compounds can stimulate TLR7 and TLR8, receptors for which no natu-

ral ligands have as yet been identified. These agonists, like imidazoquinoline derivatives that exhibit immunomodulatory activity, might have a beneficial effect by redirecting the dominant Th2-type response in asthma to a more protective Th1 response. The Resiquimod (R-848) antitumor, antiviral effects have been associated with increased production of IL-12, IFN- α , IL-18, TNF- α , IL-10, and IL-15 by CD14⁺ cells, as well as with an increase in the number of IFN- γ -producing natural killer cells (67). More generally, ligands to TLR7 exert differential biological effects in a DC subset-specific manner, plasmacytoid DCs producing IFN- α and myeloid DCs producing IL-12 (68). Therefore TLR9, TLR7, and TLR8 should be considered as potential useful candidates for the treatment of allergic diseases, especially since their ligands in addition to direct interaction have been demonstrated to be adjuvants, similar to CpG DNA, which is now being tested in clinical trials in patients with allergy and asthma.

Another new strategy for the treatment for allergy is to prevent the development of sensitization, to which end potential human vaccines are being developed. They will probably aim to induce strong Th1 cytokine responses, leading to the induction of allergen-specific tolerance as well as to the downregulation of eosinophilic inflammation (69,70). The most promising approach to this objective includes the induction of systemic or local immune responses through the use of bacterial vaccines, substances that can activate several TLRs, or the use of selective TLR-specific ligands such as the CpG oligonucleotides.

In a mite-induced allergy model, injection of mite allergen *Dermatophagoides pteronyssinus* group 2 (Der p2) together with BCG can cause a Th1-type immune response that hinders Der p2-induced allergic sensitization and the development of airway inflammation. Also, direct conjugation of CpG to an allergen—for example, Amb a 1—might be an effective new method for inducing prophylactic and thera-

peutic protection against atopic disorders (71,72). An Amb a 1-ISS conjugate has enhanced Th1-biased immunogenicity and reduced allergenicity. It may therefore offer a more effective and safer approach for allergen immunotherapy than currently available methods. Such compounds might be used as adjuvants in novel allergen-specific immunotherapeutic regimens for the treatment of allergic disorders.

Conclusions

TLRs are crucial elements not only in the early phase of antigen recognition but also in adaptive immunity throughout the entire course of the host defense response. Their involvement in multiple immunomodulatory activities defines TLRs as general adjuvant receptors. By directing the function of APC, TLR-dependent activation plays a critical role in the induction of Th1-type immune responses, and this is well-established as important for host antimicrobial defense. In addition, TLR4 and MyD88 may be relevant to the development of Th2-type immune responses that are characteristic of atopy and allergic disease development. TLR-dependent activation of T cells and mast cells, which also exhibit TLRs, could participate in the Th2 response profile in allergic patients. However, the mechanism by which selectivity of the downstream response is achieved is yet to be elucidated. A clearer understanding of this mechanism will allow us to better appreciate how the host adaptive immune system communicates with the environment and will provide potential ways by which TLRs and their signaling pathways can be used as targets for therapeutic intervention. There is also substantial interest in the development of TLR agonists and antagonists that can produce pharmacological effects on the immune response to obtain more effective vaccines and novel therapeutic approaches in the treatment of atopic, autoimmune, malignant, and infectious diseases. In allergy, efforts to

improve the safety of immunotherapy by means of chemical modification of allergens have not been particularly successful because the modifications studied so far greatly reduce their antigenicity. We anticipate that TLR ligands, now known to be effective adjuvants, will be regarded as potential linkers to allergen epitopes, with the aim of developing novel allergen-specific immunotherapy regimens for the treatment of allergic disorders.

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