



REVIEW

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TLR9-Based Immunotherapy for Allergic Disease

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ABSTRACT

A significant amount of data generated over the last few years supports the contention that Toll-like receptor (TLR) 9-based immunotherapy is effective in the prevention and treatment of animal models of allergic disorders. We will review here our experience with two distinct therapeutic strategies: TLR9-based immunomodulation and TLR9-based vaccination. Immunomodulation of allergic inflammation by TLR9 ligand (TLR9-L) is transient. It prevents both the early and late phases of the allergic reaction in experimental models of allergic asthma, rhinitis, and conjunctivitis. It also reverses ongoing allergic inflammation. Indoleamine 2,3-dioxygenase, the rate-limiting enzyme of tryptophan, is induced by TLR9-L and mediates, in part, these anti-inflammatory effects. TLR9-based immunomodulation is independent of allergens and, therefore, has a potential therapeutic advantage in a broad spectrum of allergic patients. On the other hand, TLR9-based vaccination therapy is an allergen-specific mode of immunotherapy, which provides long-term inhibition of allergen-specific hypersensitivities. Current clinical trials with TLR9-based immunotherapy demonstrate high immunogenic and therapeutic efficacy, as well as improved safety when compared with conventional allergen desensitization. Thus, if proven efficient, therapeutic strategies with TLR9-L may revolutionize the current treatment of allergic diseases. © 2006 Elsevier Inc. All rights reserved.

KEYWORDS: TLR9 ligands; Immunostimulatory sequence oligonucleotides (ISS-ODN); CpG-ODN; Allergic asthma; Allergic rhinitis; Allergic conjunctivitis; Indoleamine-2,3 dioxygenase

IMMUNOBIOLOGY OF TOLL-LIKE RECEPTORS

Toll-like receptors (TLRs) are expressed mainly on macrophages and dendritic cells and recognize specific structural components conserved among microorganisms. Activation of TLRs leads to the induction of inflammatory responses and shapes the subsequent development of adaptive immunity.¹ TLR9-Ls are immunostimulatory sequence oligonucleotides (ISS-ODN) containing unmethylated CpG dinucleotides (also known as CpG-ODN).^{2,3} In retrospect, TLR9-Ls were initially discovered in Freund's adjuvant.⁴⁻⁶ Synthetic TLR9-L induces a robust and multifaceted innate immune response in mice analogous to the response elicited with bacterial DNA.⁷⁻⁹ The innate response to TLR9-L is characterized by the production of cytokines such as TNF α , IL-12, IL-18, IFNs (α/β and γ) and IL-6, and the upregulation of co-stimulatory molecules by

antigen-presenting cells (APCs), B cells, and NK cells.^{10,11} Functionally, TLR9-L increases NK cell activity, induces maturation of APCs to elicit Th1 immune responses, and stimulates antigen-independent B-cell proliferation.^{7,12}

THE HYGIENE HYPOTHESIS AND TLR9-BASED IMMUNOTHERAPY

Over the past 3 decades, the prevalence of allergic diseases has increased in industrialized countries.¹³ One proposed explanation is the "hygiene hypothesis," which is based on the assumption that a reduction of microbial burden in early childhood that would induce a strong Th1-biased immunity, redirects the immune response toward a Th2 phenotype and, therefore, predisposes the host to allergic disorders.¹⁴ Because TLR9-L stimulates innate immunity and has a long-lasting Th1 biasing effect,¹⁵ it may mimic an infectious exposure and shift the Th2 deviation towards a more appropriate Th1/Th2 balance. Increasing amounts of experimental data have suggested that TLR9-based immunotherapy is effective in the treatment of animal models of allergic disorders and can even reverse the

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underlying Th2-biased immune-dysregulation.¹⁶ We will discuss here two distinct strategies for TLR9-based therapy: immunomodulation and vaccination (Figure 1), both of which have shown promise in numerous mouse models of allergic disease by our group,¹⁷⁻²⁰ as well as other investigators.^{21,22} Furthermore, phase I/II clinical trials with a TLR9-based vaccination have already been initiated,²³⁻²⁵ and a phase III trial is in progress.

TLR9-BASED IMMUNOMODULATION

TLR9-L Suppresses Experimental Asthma

The inflammatory response in allergic asthma is characterized by cellular infiltration of the airways with eosinophils and T lymphocytes.^{26,27} A large number of therapies are currently available for the treatment of allergic asthma; corticosteroids for the reversal of allergic inflammation, antihistamines and leukotriene inhibitors for the attenuation of pathology associated with mast cells and eosinophils, and adrenergic receptor agonists for the reversal of bronchospasms.^{28,29} However, none of these therapies have been shown to reverse the underlying hypersensitivities to allergens that perpetuate the allergic phenotype. Allergen desensitization can be achieved with traditional allergen (ie, protein)-based vaccination. However, this approach is accompanied by critical disadvantages such as the requirement of repeated injections and the associated risks of life-threatening anaphylaxis.^{30,31}

Immunomodulation by TLR9-L, which is allergen-independent, by systemic administration (eg, intraperitoneal [i.p.] injection) or by mucosal administration (ie, intranasal [i.n.] inoculation) provided almost immediate protection against experimental asthma upon allergen challenge in allergen-sensitized mice.^{17,22,32} Administration of TLR9-L before allergen challenge significantly inhibited both early- and late-phase reaction of asthma, for example, airway hyperresponsiveness (AHR) to inhaled metacholine (MCh),^{17,22,32} eosinophilia in bronchoalveolar lavage fluid (BALF), and IgE levels in the serum³³ (Figure 2). TLR9-L also inhibits IL-4 dependent IgE synthesis by human B cells in vitro.³⁴ Administration of TLR9-L not only inhibited eosinophil infiltration into the airways and into the lung parenchyma but also significantly inhibited blood and bone marrow eosinophilia. The inhibition of asthmatic parameters (eg, AHR) by TLR9-L was associated with a significant inhibition of Th2 cytokine production (eg, IL-5) and induction of allergen-specific IFN- γ production. Antibody neutralization studies have shown that the inhibitory effect of TLR9-L on AHR and on Th2 cytokine secretion was mostly mediated by innate cytokines (IFNs and IL-12) secreted

by TLR9-activated monocytes/macrophages and NK cells. The effects of TLR9-L on reducing the number of tissue eosinophils were both immediate and sustained. TLR9-L administration was effective in inhibiting eosinophilic airway inflammation when administered either systemically (i.p.) or mucosally (i.n.).³⁵

Administration of TLR9-L when given after allergen challenge also promotes the resolution of airway inflammation at a level similar to that seen with dexamethasone (DXM),³⁶ the standard therapy for allergic asthma. Mice that had already developed significant levels of eosinophilic airway inflammation did not develop AHR when treated with either TLR9-L or DXM.³⁶ The combined administration of TLR9-L and DXM was more effective in inhibiting AHR than the administration of either TLR9-L or DXM alone. Both TLR9-L and DXM significantly reduced eosinophil infiltration and the levels of Th2 cytokines (ie, IL-5) in BALF, as well as the number of mucous-producing airway epithelial

cells. However, administration of TLR9-L induced a significant level of IFN- γ in BALF whereas DXM did not.³⁶ Thus, administration of TLR9-L is as effective as DXM, and the administration of both is synergistic in the prevention and reversal of inflammation in experimental asthma.

TLR9-L Inhibits Airway Remodeling

Allergic asthma is characterized by chronic inflammation followed by airway remodeling. This process results in subepithelial fibrosis, an increase in smooth muscle mass and an increase in mucous glands.³⁷ Chronically allergic mice developed sustained eosinophilic airway inflammation and AHR to MCh, as well as other features of airway remodeling. We and other investigators showed that systemic administration of TLR9-L significantly inhibited the development of AHR, eosinophilic inflammation, airway mucous production, and most importantly, airway remodeling.³⁸⁻⁴⁰ In addition, TLR9-L significantly reduced the level of the profibrotic cytokine, transforming growth factor (TGF)- β , in BALF and the lungs.³⁸ These studies demonstrate that the administration of TLR9-L prevents not only the Th2-mediated airway inflammation in response to acute allergen challenge, but also the consequent airway remodeling associated with chronic allergen exposure.

Administration of TLR9-L also can reverse established airway remodeling. Its administration to mice with established airway remodeling significantly reduced the degree of airway collagen deposition.⁴¹ These findings were accompanied with a reduction in the levels of Th2 attracting chemokines, the number of peribronchial Th2 lymphocytes, and the levels of Th2 cytokines that promote peribronchial fibrosis.^{38,41}

CLINICAL SIGNIFICANCE

- Immunomodulation by TLR9-L has been found to prevent, reverse or to treat allergic symptoms in experimental animal models of allergic asthma, rhinitis, and conjunctivitis.
- In animal experiments, TLR9-based vaccination provided long-term higher immunogenic and therapeutic efficacy, as well as improved safety compared with conventional allergen vaccination.
- Based on on-going clinical trials, TLR9-L may revolutionize the treatment of allergic diseases.

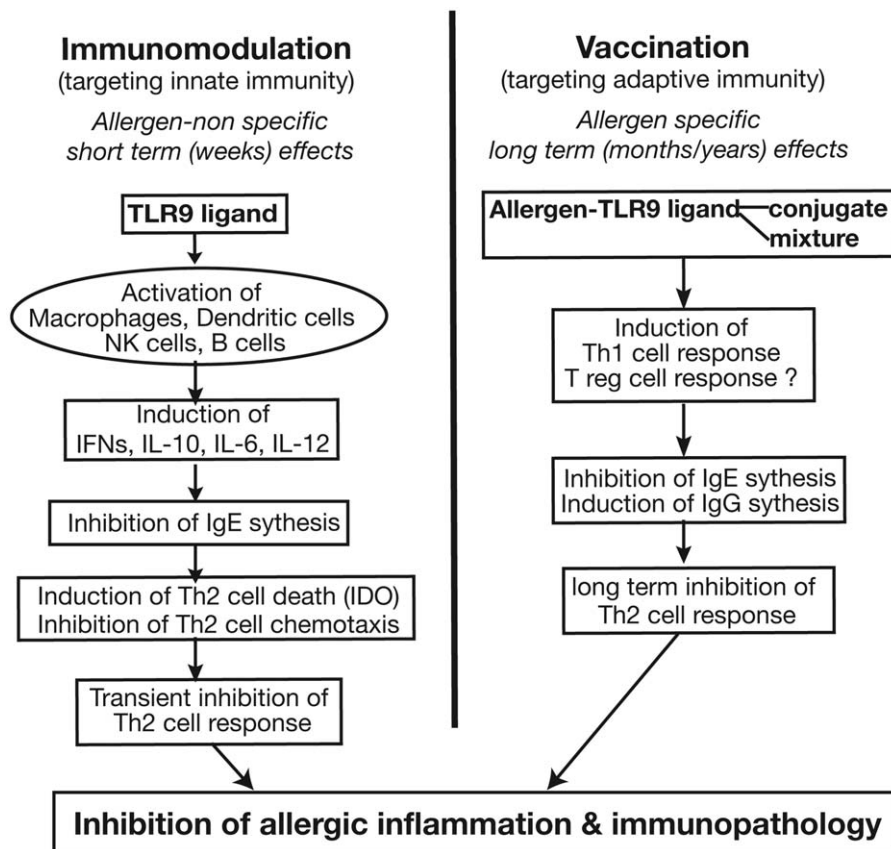


Figure 1 The principles of TLR9-based immunotherapy; immunomodulation and vaccination. Immunomodulation of allergic inflammation by TLR9-L is independent of allergens and its effect is transient (up to 6 weeks). TLR9-based vaccination therapy is an allergen-specific therapy that provides long-term inhibition of allergen-specific hypersensitivities.

The inhibitory effect of TLR9-L in airway remodeling was further demonstrated in an allergic primate model⁴² using rhesus monkeys where allergic asthma was induced with house dust mite allergens. AHR and eosinophil infiltration were reduced 2-fold in monkeys treated with inhaled TLR9-L compared with those that were sham-treated. Airways from TLR9-L-treated monkeys had thinner reticular basement membranes, fewer mucous goblet cells, fewer eosinophils, and fewer mast cells than sham-treated allergic monkeys.⁴² These findings indicate that inhaled TLR9-L is able to attenuate the magnitude of AHR and airway remodeling produced in a nonhuman primate model of allergic asthma that shares similar immunopathological mechanisms to the human disease.

TLR9-L Inhibits Experimental Allergic Rhinitis

Allergen-sensitized mice that received TLR9-L had attenuated immediate and late phase responses to intranasal allergen challenge. Specifically, TLR9-L-treated mice had less histamine and cysteinyl leukotriene release, and less eosinophilic inflammation in their nasal passages. In addition, splenocytes from TLR9-L-treated mice displayed attenuated allergen-specific IL-4, IL-5, and IL-13 but increased IFN- γ secretion.²⁰ Thus, systemic or local TLR9-L delivery attenuates both the immediate and the late phase responses in an experimental allergic rhinitis (AR) model.

TLR9-L Inhibits Experimental Allergic Conjunctivitis

Short ragweed (RW) is the major cause of late summer allergic conjunctivitis (AC) in North America.⁴³ We have investigated the ability of TLR9-L to modulate allergic responses in a RW-induced mouse model of seasonal AC.¹⁹ Systemic or mucosal administration of TLR9-L before or simultaneously with RW challenge inhibited the immediate hypersensitivity and the late-phase responses and the induction of cellular infiltration in the conjunctiva. TLR9-L-administration significantly suppressed the development of RW-specific IgE titers after repeated allergen challenge and improved the clinical symptoms of AC to the same degree observed for DXM.

Mechanism of Immunomodulation by TLR9-L: Induction of Indoleamine 2,3-Dioxygenase

Initially, we and other investigators speculated that the immunomodulatory activities induced by TLR9-L are mediated by redirecting an allergen-specific Th2 response toward a Th1 response.^{17,19} Recently, we found that indoleamine 2,3-dioxygenase (IDO), the rate-limiting tryptophan (trp) catabolizing enzyme, is induced in the lungs by TLR9-L and suppresses Th2-driven allergic asthma.⁴⁴ IDO is induced by TLR9-L, as well as some other TLR-Ls,^{45,46} mainly via the production of

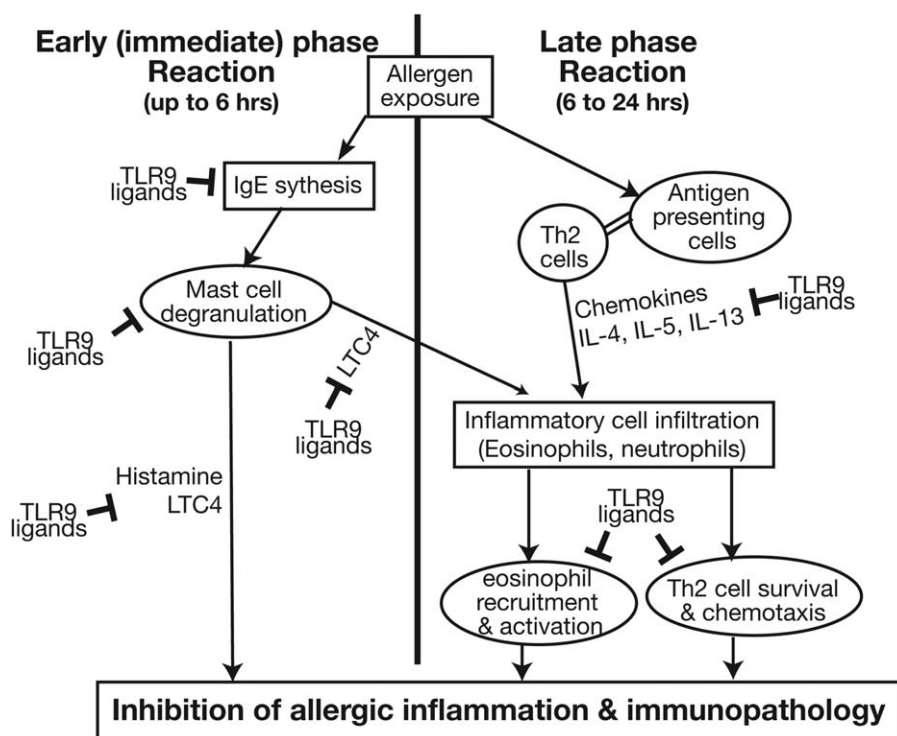


Figure 2 Immunomodulation by TLR-9-L affects the early and late phases of the allergic reaction. The early phase reaction occurs within minutes after allergen exposure. TLR9-L inhibits IgE synthesis, histamine release, and the induction of leukotriene C4 (LTC4) in the lung. The late phase reaction, which is orchestrated by many types of cells, for example, Th2 cells, eosinophils, neutrophils and mast cells, begins 3-4 hours after allergen exposure, and resolves in 12-24 hours. TLR9-L inhibits the infiltration and survival of inflammatory cells (Th2 cells, eosinophils, and neutrophils) in the late phase reaction. Allergic responses in experimental asthma include bronchoconstriction, submucosal edema, or mucous secretion. $\neg$ indicates the sites of inhibition by the administration of TLR9-L.

IFNs.⁴⁷ IDO expression is enhanced by IL-10 but suppressed by IL-4 and IL-13⁴⁸ and is expressed in various cell types including fibroblasts, macrophages, dendritic cells and epithelial cells.^{49,50} TLR9-L-induced IFN- γ amplifies IDO expression and enzymatic activity in the bronchial epithelial cells. The inhibition of IDO activity by a specific inhibitor, 1-methyl-DL-tryptophan (M-trp), reversed the immunomodulatory effects of TLR9-L in experimental asthma as was demonstrated by an increase in AHR, eosinophilic infiltration, and in the levels of IL-5 and IL-13 in the BALF. We observed that TLR9-L administration, via IDO induction, reduces the survival of allergen-specific Th2 cells and, consequently, Th2 cytokine production using an adoptive transfer model of Th2 cells into SCID mice.⁴⁴ These data indicate that TLR9-induced IDO mediates its immunomodulatory effects by inducing the death of effector Th2 cells and not via the induction of a Th1 or a regulatory T cell response.

Mechanism of Immunomodulation by TLR9-L: Inhibition of the Th2 Phenotype Spread

Epidemiological evidence indicates that the key events contributing to the development of allergic asthma occur in early childhood.⁵¹⁻⁵³ An accelerated generation of Th2 memory responses against various aeroallergens also occurs during these early years.^{54,55} We hypothesized that a preexisting Th2/asthmatic response can promote Th2 responses to the same or to

newly encountered allergens (ie, Th2 phenotype spread). TLR9-L administration inhibited the production of chemokines involved in the homing of naive CD4⁺ T and Th2 cells to the bronchial lymph nodes, which resulted in the abrogation of the future development of a Th2 phenotype spread. This suggests that TLR9-L may be effective in reducing the spread of allergen reactivity in atopic children.^{54,55}

TLR9-BASED VACCINATION FOR ALLERGIC DISEASE

The principal goal of a vaccination strategy against allergic disease is to elicit a protective immune response to a specific antigen/allergen. In the past, researchers have modified antigens to make them less allergenic and more immunogenic in order to prevent adverse reactions upon allergen injection, that is, allergen desensitization.⁵⁶ We have developed two strategies for TLR9-based vaccination: the injection of a TLR9-L mixed with allergens and the injection of allergen-TLR9-L (ISS)-conjugates (AIC). Vaccination with allergens mixed or conjugated with TLR9-L has been shown to be effective in reversing Th2-biased immune profiles and is accompanied by a Th1-biased immune deviation.^{21,57}

Physical linking of TLR9-L to allergens substantially improved the immunogenicity while it reduced the allergenicity and the anaphylactogenicity of the related allergen as com-

pared with allergen mixed with TLR9-L.^{18,58,59} In mice, the injection of TLR9-L conjugated to the major short ragweed allergen, Amba1 (the allergen associated with hay fever), induced IFN- γ -producing CD4⁺ T cells and a higher titer of Amba1-specific IgG2a as compared with Amba1 mixed with TLR9-L. Furthermore, AIC treatment in mice sensitized to Amba1 converted a preexisting Th2 response to a Th1 response (ie, increased IgG2a and IFN- γ production and decreased IgE and IL-5 production).⁶⁰ AIC was less allergenic than naïve protein because the bound ODNs sterically prevent its recognition by specific IgE.^{57,59,60} Furthermore, AIC preparations had enhanced allergen uptake^{61,62} and presentation. Indeed, recent data from a clinical trial with TLR9-L conjugated to Amba1 (TOLAMBA; Dynavax, Berkeley, Calif) demonstrated significant symptom relief and immunomodulation of the allergic response in patients with allergic rhinitis.^{23,25,63} AIC led to a shift from Th2 immunity toward Th1 immunity and appeared to be safe.⁶⁴ In addition, AIC exhibited significant fewer local reactions on quantitative intradermal skin titration compared with standardized aqueous ragweed.²⁴ Taken together, AIC demonstrates higher immunogenic and therapeutic efficacy and improved safety compared with the conventional allergen desensitization (vaccination) approach.

CONCLUSIONS

Accumulated experimental data in animal models and emerging data from clinical trials support TLR9-based immunotherapy as effective in the prevention and treatment of allergic disorders. TLR9-based immunotherapy contains 2 distinct strategies: TLR9-based immunomodulation and vaccination (Figure 1). Immunomodulation by TLR9-L prevents or treats allergic symptoms in experimental models of allergic asthma, rhinitis, and conjunctivitis. IDO induced by TLR9-L is one of the mechanisms by which TLR9-L inhibits experimental asthma. TLR9-L intervenes in the sequential development of allergic immunopathology (eg, Th2 phenotype spread, airway remodeling), and TLR9-based vaccination provides long-term, higher immunogenic and therapeutic efficacy, as well as improved safety compared with conventional allergen vaccination. Because the beneficial effects of TLR9-based immunotherapy, vaccination and immunomodulation are different from each other, that is, allergen specific vs allergen nonspecific, or long term vs short term, we propose that these two therapeutic strategies could be used to complement each other for the treatment of allergic disease, once their efficacy has been established through clinical trials.

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