

REVIEW

Strategies of mucosal immunotherapy for allergic diseases

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Incidences of allergic disease have recently increased worldwide. Allergen-specific immunotherapy (SIT) has long been a controversial treatment for allergic diseases. Although beneficial effects on clinically relevant outcomes have been demonstrated in clinical trials by subcutaneous immunotherapy (SCIT), there remains a risk of severe and sometimes fatal anaphylaxis. Mucosal immunotherapy is one advantageous choice because of its non-injection routes of administration and lower side-effect profile. This study reviews recent progress in mucosal immunotherapy for allergic diseases. Administration routes, antigen quality and quantity, and adjuvants used are major considerations in this field. Also, direct uses of unique probiotics, or specific cytokines, have been discussed. Furthermore, some researchers have reported new therapeutic ideas that combine two or more strategies. The most important strategy for development of mucosal therapies for allergic diseases is the improvement of antigen formulation, which includes continuous searching for efficient adjuvants, collecting more information about dominant T-cell epitopes of allergens, and having the proper combination of each. In clinics, when compared to other mucosal routes, sublingual immunotherapy (SLIT) is a preferred choice for therapeutic administration, although local and systemic side effects have been reported. Additionally, not every allergen has the same beneficial effect. Further studies are needed to determine the benefits of mucosal immunotherapy for different allergic diseases after comparison of the different administration routes in children and adults. Data collected from large, well-designed, double-blind, placebo-controlled, and randomized trials, with post-treatment follow-up, can provide robust substantiation of current evidence.

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INTRODUCTION

Allergic diseases are a global health problem and result from a complex interaction between genetic and environmental factors. Type 2 T helper (Th2) immune responses play a critical role in the development of allergic diseases.¹ The cellular response to allergens, occurring in the skin, leads to atopic dermatitis. The disruption of the skin barrier initiates the subsequent atopic development toward allergic airway disease. Allergic airway disease encompasses a variety of symptoms and conditions that affect the mucosal lining of the airways, from the nose (allergic rhinitis) to the lungs (asthma).^{2–4} Atopic dermatitis and allergic rhinitis in children have been reported to be significant risk factors for subsequent development of asthma.^{5–7}

The treatment of allergic diseases is based on allergen avoidance, pharmacological treatment and immunotherapy. In the pharmacological therapy of atopic dermatitis, only symptomatic anti-inflammatory and anti-allergic treatments, local or systemic, exist. However, no prophylactic or long-term treatment regimens are available at present to prevent, attenuate or cure sensitizations in atopic dermatitis patients.⁸ Current available pharmacological agents for the treatment of allergic rhinitis include intranasal corticosteroids, H1 antihistamines, decongestants, cromolyn sodium, leukotriene antagonists and anticholinergics.⁹ Medications to treat asthma can be classified as controllers or relievers.

In controller treatment of asthma, corticosteroids and long-acting β_2 -agonists in fixed-combination inhalers are currently the most effective therapy.^{10,11} However, long-term side effects of corticosteroid inhalation, such as osteoporosis and stunting of growth, need more consideration.¹² Inhalation of corticosteroids does not seem to modify the course of the disease significantly and is not curative because asthma symptoms and inflammation rapidly recur when treatment is discontinued. Also, a small percentage of patients do not respond to the inhaled corticosteroids.^{13,14} Immunotherapy is the only controller treatment currently available with the potential to change the natural history of allergic disease and delay the allergic march observed in many atopic individuals.¹⁵ According to current guidelines for asthma treatment (GINA), the appropriate immunotherapy requires the identification and use of a single well-defined clinically relevant antigen. Antigen-specific immunotherapy (SIT), which often uses the subcutaneous route (subcutaneous immunotherapy; SCIT), is the first choice for induction of hyporesponsiveness to the respective allergens.¹⁶ Specific immunotherapy should be considered only after strict environmental avoidance and pharmacological intervention, including inhaled glucocorticosteroids, have failed to control asthmatic symptoms.¹⁷ SCIT involves the injection of increasing amounts of the allergen under the skin. The long-term time course for these injections may reduce the

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efficacy of SCIT treatment, owing to the side effects¹⁸ from accompanying potent Th2 adjuvants.^{19,20} The possibility of local or systemic adverse effects (such as anaphylaxis) must be considered. The review of SCIT trials²¹ found that immunotherapy could reduce asthma symptoms, the need for medications and the risk of severe asthma attacks after future exposure to the allergen. Immunotherapy was also found to be possibly as effective as inhaled steroids. Overall, there was a significant improvement in asthma symptom scores (standardized mean difference: -0.59 ; 95% confidence interval: -0.83 to -0.35). Furthermore, it would have been necessary to treat three patients (95% confidence interval: 3–5) with immunotherapy to avoid worsening of asthma symptoms in one patient and to treat four patients (95% confidence interval: 3–6) with immunotherapy to avoid increased medication in one patient. Immunotherapy was found to reduce allergen-specific bronchial hyper-reactivity, with some reduction in non-specific bronchial hyper-reactivity as well. However, if 16 patients were treated with immunotherapy, one would be expected to develop a local adverse reaction. Also, if nine patients were treated with immunotherapy, one would be expected to develop a systemic reaction, of any severity. A review by the Mayo Clinic in Rochester confirmed the safety and efficacy of allergen immunotherapy for allergic rhinitis and conjunctivitis, allergic forms of asthma and insect stings based on numerous well-designed scientific studies.²² Additionally, national and international guidelines confirm the clinical efficacy of injection immunotherapy in rhinitis and asthma, as well as the safety, provided that recommendations are followed.²³ Thus far, SIT is not indicated for atopic dermatitis without accompanying allergic rhinitis or asthma, and only with the caution that it might induce exacerbations manifesting in atopic dermatitis or relapses of latent atopic dermatitis. In the study by Werfel *et al.*,²⁴ adult patients with severe forms of atopic dermatitis benefited from SIT with house dust mite (HDM) allergen extract lasting 12 months.

Improved strategies and targets for immunomodulation of allergic diseases should consider the following: (i) fewer side effects; (ii) antigen-specific modulation for long-term effects; and (iii) non-injection routes. Mucosal immunotherapy is an ideal choice based on these considerations. The mucosa-associated lymphoid tissues are the largest mammalian lymphoid organ system. Unique characteristics of the mucosal immune system, including the large production of secretory IgA antibodies and routine maintenance of immune tolerance, contribute to the efficacy of mucosal immunotherapy.²⁵ Akbari *et al.* found that pulmonary dendritic cells (DCs) collected from antigen-exposed mice produced IL-10 and lead to the development of IL-10 secretion by CD4⁺ T regulatory 1-like cells.²⁶ In another study, mucosal DCs derived from mesenteric lymph nodes produced transforming growth factor- β (TGF- β), which induced the development of Th3 cells.²⁷ However, environmental factors, level of antigen exposure and DC subtype each contribute to the results obtained in these studies. The characteristics of mucosal DCs critical for Th-type development still require further study.²⁸ Hufnagl *et al.* indicated that mucosal application of peptides is superior to systemic application for preventing both local and systemic polyallergic Th2 immune responses, which suggests that mucosal tolerance induction is an attractive strategy for the primary and secondary prevention of allergic lung pathology.²⁹

MUCOSAL IMMUNOTHERAPEUTIC STRATEGIES FOR ALLERGIC DISEASES

In animal experiments, the successful application of mucosal immunotherapy for allergic diseases depends on antigen dose or formulation, mucosal adjuvants and Th-type immune manipulation. These

studies have also led to the development of combination therapies. Here, we clarify these strategies and summarize the effect of treatment in the experimental model or clinic in Tables 1 and 2 (combination strategies). Although the outcome of mucosal immunotherapy in human trials still needs to be clarified, non-injection immunotherapy is an attractive therapy for allergic diseases.

Antigen dose and formulation

Mechanisms of mucosal tolerance induced by high- and low-dose antigens are different.^{30,31} High doses of oral or mucosal antigen lead to T cell receptor activation without costimulation and the simultaneous presence of inhibitory ligands leads to anergy³² or deletion.^{16,33} Low-dose tolerance is induced by regulatory cells, such as Th3,^{18,19} T regulatory 1 cells^{34,35} and CD4⁺CD25⁺ regulatory cells.^{36,37} Also, CD8⁺ T cells, *via* the production of TGF- β ,^{38,39} and γ/δ T cells⁴⁰ have been identified as acting as regulatory cells during oral tolerance induction. However, the characterization and function of, and the interactions between, different types of regulatory T cells still require further study.⁴¹

The use of allergen extracts brings forth the possibility of *de novo* sensitization against natural allergen components delivered in allergen extract preparations. Alternatively, using the allergen in recombinant form,^{42–44} or only the major T-cell epitopes,^{12,45} has enhanced treatment efficacy and safety. Additionally, peptide immunotherapy using peptides against multiple immunodominant allergen-specific T-cell epitopes is a safe and promising strategy for allergy control.⁴⁶ Allergens can be further modified through the production of allergoids, which are allergen extracts that have been polymerized into larger aggregates by a chemical reaction. According to the theoretical concept, this chemical modification is hypothesized to result in reduced allergenicity and maintained immunogenicity in mouse models and in clinics.^{47–49}

Takagi *et al.* found that mice orally fed with transgenic rice seeds co-expressing the Cryj I and Cryj II peptide-defining T-cell epitopes before challenge with cedar pollen inhibited the development of serum allergen-specific IgE and IgG antibodies and Th cell proliferative responses. The serum levels of IL-4, IL-5, IL-13 and histamine were significantly decreased, and the development of pollen-induced clinical symptoms was inhibited in this mouse model. These results indicate the potential of transgenic rice seeds in the production and mucosal delivery of allergen-specific T-cell epitope peptides for the induction of oral tolerance to pollen allergens.⁵⁰

Adjuvants for mucosal immunotherapy

Cholera toxin B (CTB). CTB is produced by *Vibrio cholera*. Despite being a transmucosal carrier-delivery system for induction of peripheral immunological tolerance, CTB has also been used as a non-toxic mucosal immunomodulatory adjuvant through its binding ability to the asialo-GM-1 receptor on B cells, T cells and DCs.⁵¹ Smits *et al.* found that intratracheal administration of CTB can suppress allergic inflammation through the induction of airway luminal IgA secretions in a TGF- β -dependent manner, which is necessary for its preventive and curative effect.⁵²

CpG oligodeoxynucleotides (CpG ODNs). CpG ODNs contain unmethylated CpG motifs, which confer the immunostimulatory properties of bacterial DNA through the ability to induce immune responses.^{53,54} CpG ODNs can enhance Th1 immune responses,^{55,56} suppress Th2 responses^{57,58} and induce regulatory T cells.^{59,60} These findings suggest that CpG ODNs can be a therapeutic approach for the

Table 1 Summary of mucosal therapy for allergic diseases

Strategy	Animal model	Human studies
Antigen dose and formulation	Recombinant form/i.n.; ⁴³ major T epitopes/i.h., ⁴⁵ i.n. ⁴⁶ and oral; ⁴⁸ monomeric allergoid/oral (intragastric administration) ⁴⁷	Monomeric allergoid (Lais, Lofarma S.p.A., Milan) for mite-sensitized patients with rhinitis and intermittent asthma/SLIT ^{48,49}
Adjuvants for mucosal immunotherapy	Cholera toxin B/i.t. ⁵² CpG ODNs/i.t. ⁵³ and i.n. ⁶¹ Chitin/oral ⁷⁰ and i.n.; ⁷¹ Chitosan/i.n. ⁷⁷	No recent study for allergic diseases CpG ODNs for atopic asthma (RDPC*)/i.h. ⁶⁴ Phase I/IIa study on chitin microparticles for allergic rhinitis subjects (ClinicalTrials.gov, identifier: NCT00443495)
Probiotics	<i>Lactobacillus</i> spp., <i>Bifidobacterium</i> spp./oral ^{81,82}	Clinical trial results are still diverse for each study/oral ⁸⁷

Abbreviations: i.h., inhalation; i.n., intranasal; i.t., intratracheal; ODN, oligodeoxynucleotide; RDPC*, randomized, double-blind, placebo-controlled clinical trials; SLIT, sublingual immunotherapy.

treatment of Th2-mediated allergic asthma. The immunomodulatory effects of CpG ODNs on the development of HDM *Dermatophagoides farinae* (Der f)-induced airway inflammation and remodeling in mice have been reported.⁵³ Simultaneous intratracheal instillation of CpG ODNs with Der f at the first allergen exposure showed significant inhibition of inflammation in a dose-dependent manner of CpG. For intranasal therapy, Ramaprakash *et al.* found that intranasal CpG therapy attenuated experimental fungal asthma in both a TLR9-dependent and an independent manner.⁶¹ A clinical study also showed CpG ODNs to have promising experimental and clinical results in allergic rhinitis.^{62,63} However, a subsequent study of CpG ODNs (delivered by nebulization) showed fewer benefits in asthma.⁵⁶ Although CpG ODNs could increase the expression of IFN- γ and IFN- γ -inducible genes, they did not sufficiently inhibit allergen-induced responses in asthmatic subjects.^{56,64} However, the ability of CpG ODNs to promote Th1 responses has already led to the design of phase I clinical trials with allergy patients.^{65,66}

Chitin/chitosan. Chitin is a key structural component of helminths, arthropods and fungi.^{67,68} The immune response to chitin is still considered controversial.^{69–73} Oral⁷⁰ and intratracheal⁷¹ administration of chitin has been shown to downmodulate allergic airway inflammation in murine models. Controversially, Reese *et al.*⁷³ found that intranasal administration of chitin resulted in eosinophil and basophil accumulation in helminth-infected mice. The sensitizing role for chitin may, through alternatively activated macrophages, mediate eosinophil recruitment *via* leukotriene B4 production. Several factors, such as the administration route or particle size, may account for the Th1 vs. Th2 response to chitin. There are still many controversial and unsolved issues in this field to be discussed.⁷⁴

Chitosan is formed naturally through the action of chitin deacetylases or by the deacetylation of chitin oligosaccharides.⁷⁵ It is a natural

biodegradable mucoadhesive polysaccharide derived from crustacean shells. This slowly degrading polymer has been shown to increase transcellular and paracellular transport of macromolecules across intestinal epithelial monolayers.^{75,76} Chen *et al.* found that soluble chitosan delivered intranasally with water during allergen sensitization⁷⁷ could attenuate airway inflammation in the Der f-induced murine allergy model. Furthermore, a phase I/IIa study on chitin microparticles delivered by the nasal route to subjects suffering from allergic rhinitis has entered clinical trials.

Probiotics

Using unique strains of probiotics can improve immunomodulatory effects of mucosal therapy.⁷⁸ Probiotics are dietary supplements that contain beneficial bacteria such as *Lactobacillus GG* (LGG) and are effective in preventing early atopy in children through the modulation of intestinal microbiota.^{79,80} In animal models of asthma, orally administered probiotics can strain-dependently decrease allergen-specific IgE production and modulate systemic cytokine production.⁷⁸ Certain probiotics (LGG or *Bifidobacterium lactis* and *Lactobacillus reuteri*) have been shown to decrease airway hyper-responsiveness and inflammation by inducing regulatory mechanisms.^{81,82} However, definitive conclusions are lacking because of the variety of experimental protocols used. Before using probiotics for asthma prevention, further studies using molecular methods to test for microbiota⁸³ and large-scale analyses are required.

In clinics, the implementation of probiotics for primary prevention early in infancy is increasingly being discussed as the optimal time point for intervention. A recent meta-analysis of several clinical trials suggests that pre- and post-natal probiotic interventions are effective in preventing the development of pediatric dermatitis,⁸⁴ although the effects on allergy development are less clear. Additionally, a double-blind, placebo-controlled study was conducted to examine the

Table 2 Combination effect of mucosal therapy for allergic diseases

Combination effect	Animal model
Antigen–adjuvant	HDM–CTB/i.n., ⁸⁸ OVA–CTB/oral, ⁹¹ i.n. ⁹² or rBet–CTB/i.n. ⁹² Thiolated ODN with maleimide-activated OVA/i.t. ⁹⁵ Der f-CS nanovaccine/i.n.; ⁹⁸ chitosan-pDer p2 ¹⁰⁰ or pDer p1 nanoparticles/oral ¹⁰¹ Mucoadhesive chitosan-formulated OVA/sublingual ⁹⁹
Antigen–probiotics	Co-administration/oral ¹⁰² and i.n. ¹⁰⁴ Recombinant Der p1 111–139 ¹⁰⁵ and Bet v 1 ¹⁰⁷ producing probiotics/i.n.
Induction of Th1, Tr or anti-Th2 immune response	IL-13 peptide-based virus-like particle vaccine/i.n. ¹⁰⁸ Chitosan/IFN- γ pDNA nanoparticles (CIN)/i.n. ¹⁰⁹ Coadministration of live lactococci producing IL-12 and BLG/i.n. ¹¹⁰

Abbreviations: BLG, bovine β -lactoglobulin; CS, chitosan; CIN, chitosan/IFN- γ pDNA nanoparticle; CTB, cholera toxin B; HDM, house dust mite; Der p, *Dermatophagoides pteronyssinus*; i.h., inhalation; i.n., intranasal; i.t., intratracheal; ODN, oligodeoxynucleotide; OVA, ovalbumin.

effectiveness of LGG and *L. gasseri* TMC0356 in alleviating Japanese cedar pollinosis, a seasonal allergic rhinitis caused by Japanese cedar pollen. Fermented milk prepared with these two bacteria, or placebo yoghurt, was administered to 40 subjects with a clinical history of Japanese cedar pollinosis for 10 weeks.⁸⁵ The allergic rhinitis alleviating effects of LGG and *L. gasseri* (TMC0356) might be due at least partly to their specific downregulation of the human Th2 immune response. In the clinical trials, randomized, placebo-controlled, double-blind studies of *Lactobacillus plantarum* No. 14 administration to female students with seasonal allergic diseases found *L. plantarum* No. 14 to strongly induce the gene expression of Th1-type cytokines. This study highlights the clinical effects of *L. plantarum* No. 14 on seasonal allergic diseases,⁸⁶ but a Cochrane systematic review concluded that, when the results for the different probiotic strains used in clinical trials are pooled, probiotics are not effective for the treatment of atopic dermatitis.⁸⁷ Also, synbiotics (90% short-chain galacto-oligosaccharides, 10% long-chain fructo-oligosaccharides: Immunofortis and *Bifidobacteriu breve* M-16V) had no effect on bronchial inflammation and the late asthmatic response but did significantly reduce systemic production of Th2-cytokines after allergen challenge and improved peak expiratory flow for patients with asthma and HDM allergy.⁸⁸

The other randomized, double-blind, placebo-controlled, allergy-prevention trial used a combination of LGG, *L. rhamnosus* LC705, *B. breve* Bb99 and *Propionibacterium freudenreichii* ssp. *Shermanii* prenatally and during the 6 months after birth. Probiotics might also enhance IgA responses in the gut and regulate inflammatory cytokines, both of which are immunomodulatory effects that could prevent progression of atopy and potential development of disease.⁸⁹ To date, the evidence suggesting that probiotics can be used to treat or prevent allergic diseases in children remains controversial. Data from the recent randomized, double-blinded, placebo-controlled clinical trials using probiotics for the treatment of allergic diseases in children have been collected but are insufficient to strongly recommend probiotics as a standard treatment or preventative measure for pediatric allergic disease. Additional studies with standardized designs, bacterial strains, dosages and durations should be performed for different allergic diseases of children.⁹⁰

Combination effect

An important issue in mucosal immunotherapy is how to improve efficacy. Combining the proper adjuvant with the specific allergens will contribute to more efficient antigen-SIT. We have focused on examples of the combination strategy for the treatment of allergic diseases in mouse models, which are summarized in Table 2. The efficacy of the combination strategy in human studies is still unclear.

CTB–Ag. Many studies have shown that the mucosal administration of relevant antigens (Ag) together with, and preferably linked to, the non-toxic B subunit of cholera toxin (CTB) by either oral⁹¹ or intranasal⁹² administration represents a highly effective way to maximize oral tolerance induction for immunotherapeutic purposes and is superior to the administration of Ag alone. Sun *et al.*⁵¹ found that using *N*-suc-cinimidyl [3-(2-pyridyl)dithio]-propionate as a conjugator to link different antigens can achieve immunotolerance through a single oral administration of low-dose antigen. In a study of the HDM allergen, Lee and Mo found that immune tolerance could be induced through intranasal application of a HDM and CTB conjugate in the murine allergic rhinitis model and that the effect can last for 4 weeks.⁹² Interestingly, Wiedermann *et al.* found that the tolerogenic or immunogenic properties of CTB strongly depend on the nature of

the coupled allergen.⁹³ The clinical trials for mucosal respiratory or gastrointestinal allergies have yet to be performed.⁹⁴

CpG ODN-conjugated Ag (CpG ODN–Ag). Shiota *et al.*⁹⁵ found antigen-conjugated CpG ODN (mixing thiolated CpG ODN with maleimide-activated ovalbumin (OVA)) to be a novel antigen-specific immunomodulator that could regulate murine airway eosinophilia and Th2 cells. Interestingly, the CpG ODN–Ag conjugate was 100-fold more effective than the unconjugated mixture at inducing Th1 differentiation *in vitro* in an IL-12-dependent manner. Mucosal or intratracheal administration of CpG ODN with allergens or CpG ODN–Ag has also been applied to the different animal models of allergic disease.⁹⁶ A variety of clinical trials are currently ongoing to determine the efficacy of CpG ODNs as a therapeutic tool for atopic diseases. In the review by Gupta and Agrawal, therapeutic applications of CpG ODNs in allergy and asthma are discussed. CpG ODNs may be used alone or as an adjuvant for immunotherapy to treat these disorders.⁹⁷

Chitosan–Ag. Liu *et al.*⁹⁸ tested immunotherapeutic efficacy of intranasal administration of Der f entrapped in chitosan microparticles in sensitized mice. Mice treated with the intranasal Der f–chitosan nano-vaccine prior to challenge displayed alleviated airway hyper-reactivity, lung inflammation and mucus production, and had fewer eosinophilic cells in the bronchoalveolar lavage fluid (BALF). The IL-4 cytokine levels in BALF and from splenocytes were reduced, but IgA and IFN- γ in serum were increased. Liu *et al.* also observed that IL-10 levels were increased among splenocytes and in BALF, which contributed to the increase in regulatory T cells in the spleen. These results illustrate how intranasal administration of the Der f–chitosan nano-vaccine plays a role in immunological protection against murine allergic asthma by inducing regulatory T cells and Th1-type reactions.

Interestingly, Saint-Lu *et al.*⁹⁹ tested two types of chitosan microparticles, differing in size and surface charge, for the *in vitro* capacity to improve antigen uptake and presentation by murine bone marrow-derived dendritic cells or purified oral antigen-presenting cells (CD11b⁺ CD11c[–] cells in buccal floor and lingual tissues). Also, OVA-sensitized BALB/c mice were treated sublingually with soluble or chitosan-formulated OVA twice a week for 2 months. Saint *et al.* found that only a mucoadhesive, especially one that is positively charged, and a micro particulate form of chitosan enhances OVA uptake, processing and presentation by murine bone marrow-derived dendritic cells, and oral antigen-presenting cells. Sublingual administration of such chitosan-formulated OVA particles enhances tolerance induction in mice with established asthma. Mucoadhesive chitosan microparticles represent a promising formulation for use in sublingual allergy vaccines. In other studies, chitosan nanoparticles, containing plasmid DNA encoding the HDM allergen *Dermatophagoides pteronyssinus* 2 (Der p2)¹⁰⁰ or Der p1,¹⁰¹ induced IFN- γ in serum and prevented subsequent sensitization of Th2 cell-regulated allergen-specific IgE responses following oral vaccination in mice. The data on Der p2 also indicate that oral administration of chitosan–Der p2 DNA nanoparticles results in the expression of Der p2 by epithelial cells in both the stomach and small intestine. Levels of IFN- γ from chitosan–DNA nanoparticle-treated mice were higher than those in the phosphate-buffered saline-treated group, the group receiving chitosan nanoparticles without the Der p2 plasmid, and those given the naked Der p2 plasmid.¹⁰⁰

Co-administration with Ag and probiotics/recombinant probiotics. Recent experimental studies have shown a reduction in IgG1 or IgE when the

specific lactic acid bacteria (LAB) strain *Lactobacillus casei* strain Shirota was orally administered¹⁰² or injected¹⁰³ together with the particular allergen. In the murine model of birch pollen allergy, Repa *et al.* demonstrated that intranasal co-application of *Lactococcus lactis* and *L. plantarum* strains with the recombinant Bet v 1 protein, before and after sensitization with the allergen, resulted in a shift from Th2 to Th1 responses characterized by a marked reduction in the IgE/IgG2a ratio and increased IFN- γ production.¹⁰⁴ Successful immunomodulation was further demonstrated by the suppression of allergen-induced basophil degranulation. These results indicate that combined mucosal application of LAB with a specific allergen could be another prophylactic and therapeutic approach to allergy treatment. Furthermore, recombinant *L. plantarum* expressing the HDM antigen Der p1 also could reduce Th2 cytokines in sensitized mice.¹⁰⁵ Similarly, therapeutic effects from administration of recombinant *L. lactis* and *L. plantarum* strains expressing Bet v 1 have also been reported. Intranasal or intragastric pretreatment with the Bet v 1-producing LAB (*L. lactis* and *L. plantarum*) strains led to significantly reduced allergen-specific IgE and increased IgG2a levels, indicating a shift to non-allergic Th1 responses.^{106,107} However, in sensitized mice, mucosal application of these recombinant strains did not sufficiently reduce allergic immune responses, which indicates that LAB-inducing immunosuppressive cytokines, such as TGF- β or IL-10, rather than Th1-like cytokines, may be more beneficial in therapeutic settings.

Mucosal induction of Th1, regulatory T or anti-Th2 immune response

Ma *et al.*¹⁰⁸ showed that intranasal vaccination with the IL-13 peptide-based virus-like particle vaccine could induce more effective suppression than subcutaneous immunization, characterized by OVA-driven Th2 patterns of antibody responses, airway IL-13 and eosinophil accumulation. Another example is the study that used chitosan/IFN- γ pDNA nanoparticles to generate *in situ* production of IFN- γ and *in vivo* effects.¹⁰⁹ Mucosal chitosan/IFN- γ pDNA nanoparticle therapy was found to have both prophylactic and therapeutic effects in the OVA animal model by reducing allergen-induced airway inflammation and airway hyper-responsiveness. This effect was dependent on signal transducer and activator of transcription 4 (STAT4) signaling. Importantly, chitosan alone could not efficiently alleviate airway inflammation in this study. Similarly, intranasal co-administration of live lactococci producing IL-12 and bovine β -lactoglobulin, a major cows' milk allergen, could also improve the efficiency of tolerance induction by intranasal administration of bovine β -lactoglobulin.¹¹⁰ Interestingly, IL-10-inducing adjuvants such as 1 α , 25-dihydroxyvitamin D3 plus dexamethasone and *L. plantarum*¹¹¹ can both significantly enhance sublingual immunotherapy (SLIT) efficacy in a murine asthma model.

ROUTES OF MUCOSAL THERAPY

Many routes for mucosal immunotherapy have been proposed and investigated, including oral (straight swallow), nasal or trachea, and sublingual. Here, we have summarized each route and discussed the efficiency of the immunotherapy. The dose, side effects and technical limitations for clinical use are taken into consideration.

Oral

Oral delivery is attractive because of the ease of administration. This type of administration has direct access to the gastrointestinal tract, which has an abundant mucosal immune system. Oral administration offers improved convenience and leads to compliance with patients,

thereby reducing overall healthcare costs.¹¹² Many animal studies have reported that immune therapy for allergic diseases could be given by oral administration of OVA or purified allergens.^{113–115} In addition to the adjustment of antigen dose and the frequency of dosing¹¹⁶ for the mucosal adjuvants, including CTB, CpG ODNs and chitosan as previously mentioned, oral tolerance is enhanced by IL-4,¹⁹ IL-10,^{19,116,117} anti-IL-12,¹¹⁸ TGF- α ,¹¹⁹ Flt-3 ligand¹²⁰ and anti-CD40 ligand.¹²¹ Antigen absorption following oral administration is less dangerous in regards to the airways or skin, which suggests that the mouth is likely to be a tolerogenic site. Interestingly, in the study of Allam *et al.*, the administration of the Phlp5 allergen (a major grass pollen allergen) was found to induce oral mucosal Langerhans cells to bind to Phlp5 in a dose- and time-dependent manner and to lead to an increased production of the tolerogenic cytokines IL-10 and TGF- β and an enhanced migratory capacity, but decelerated maturation, of oral Langerhans cells.¹²² However, in clinical trials, oral immunotherapy requires doses thousands of times higher than those for conventional SCIT owing to gastrointestinal degradation after ingestion.¹²³ Moreover, gastrointestinal side effects seemed to increase with increasing oral administration doses of pollen birch extract in the birch pollinosis study.¹²⁴ For these reasons, it is no longer considered a feasible option for immunotherapy in clinics.¹²⁵

Nasal or tracheal administration

Nasal or tracheal administration is a promising route for immunotherapy because doses are lower than for the oral route. Through the intratracheal administration route, Haneda *et al.* found that TGF- β secreted by T cells plays an important role in the down-modulation of immune responses to high doses of antigens, which might otherwise induce deleterious inflammation in the airway mucosal tissues.¹²⁶ Honey *et al.* addressed the mechanisms underlying peripheral T-cell tolerance following intranasal or inhalation administration of a high dose of immunogenic peptide (p1 111–139) derived from the HDM allergen Der p1^{127,128} and found this treatment to involve a downregulation of the Th cell response. Data from clinical trials for intranasal immunotherapy have been reported,^{129,130} but the administration of this therapy required great skill and sometimes the immunotherapy itself was found to provoke allergic responses in patients.¹³¹ The clinical trial results for tracheal administration have suggested that clinical efficacy is unproven and that the risk/benefit ratio is unfavorable.^{132,133}

SLIT

The mechanisms behind SLIT include the production of blocking IgG4 antibodies,^{134,135} the presence of high numbers of tolerogenic DC subsets, the induction of regulatory T cells,^{136,137} and the programming of the immune system toward a regulatory state of unresponsiveness to specific allergens. SLIT may also increase IL-10, which has a clear role in suppressing allergic immune responses.¹³⁸ The study of O'Hehir *et al.* found that TGF- β mediates the immunological suppression seen early in clinically effective sublingual HDM immunotherapy and the increase in regulatory T cells with suppressor function.¹³⁹ In a mouse model of rhinitis,¹⁴⁰ SLIT can reduce allergic symptoms. Brimnes *et al.* established a mouse model using a clinically relevant allergen to produce hallmarks of allergic rhinitis.¹⁴¹ Using this model, SLIT was demonstrated to reduce allergic symptoms in a time and dose-dependent manner.

Comparisons of skin biopsies from the subcutaneous injection sites¹⁴² and the oral mucosa¹⁴³ of SLIT-treated allergic subjects confirmed the negligible presence of inflammatory cells.¹⁴⁴ In addition, the high doses administered with oral immunotherapy resulted in

significant local reactions, including gastrointestinal bleeding, which were possibly able to interfere with antigen absorption and thus with immunization. However, it was evident that the sublingual mucosa could tolerate higher allergen levels than the mucosa in the nose or skin.¹⁴⁵

Clinically, use of the sublingual route is supported by numerous controlled trials showing its efficacy in asthma and rhinitis in adults and children.^{143,146} Additionally, no severe adverse events occurred during the trial, and the most common adverse events were mild asthma attack and local rash. Cao *et al.* evaluated the safety and efficacy of SLIT with Der f drops in Der f allergic asthma and/or rhinitis patients.¹⁴⁷ After 25 weeks of treatment, the SLIT and placebo groups did not show significant difference in the production of Der f-specific IgE antibody, while specific IgG4 increased significantly in SLIT patients after 25 weeks of treatment compared to those in a control group. The peak expiratory flow rates and rhinitis symptoms in the SLIT group improved, and the medical score of asthma significantly decreased. Furthermore, no severe adverse events occurred in the trial, and the most common adverse events were mild asthma and local rash. However, SLIT is not always a safe alternative to subcutaneous therapy.¹⁴⁸ The most frequent side effect of oral sublingual therapy is itching after antigen intake. Shortness of breath, wheezing and severe asthma attacks have also been reported,¹⁴⁹ yet SLIT is now accepted by the World Health Organization as a valid alternative to subcutaneous therapy in children.⁶³ The magnitude of clinical efficacy is reported to range between 20% and 50%, owing to the reduction of symptoms and medical scores, and is greater than the effects of placebo therapy.⁴³ Optimal therapeutic doses are still unknown, but range from three to five times or as high as 375 times the doses used in subcutaneous immunotherapy. The review written by Larenas-Linnemann discusses the shortcomings of SLIT in terms of efficacy, dosing, timing of treatment and patient selection, which all need to be taken into serious consideration.¹⁵⁰

Evidence for beneficial effects from SLIT has been confirmed in children with allergic rhinitis^{151–153} or asthma¹⁵⁴ caused by pollen exposure. SLIT was also found to prevent the progression from allergic rhinitis to asthma.¹⁵⁵ However, for HDM-induced asthma, therapeutic effects for patients cannot be determined without data from randomized, large population-based, high-quality studies.^{146,156–161}

As discussed in a systematic review of SLIT for allergic rhinitis,¹⁶² the therapeutic manipulation of SLIT should be interpreted with caution. The use of different allergens, optimal doses, duration of treatment and the application in children or adults should be further examined for SLIT.¹⁵⁵ Interestingly, in the study of Marogna *et al.*, the clinical effects of a monomeric allergoid were assessed across three different maintenance doses in mite-sensitized patients with rhinitis and intermittent asthma. In this clinical trial, SLIT with monomeric allergoids produced clinically significant results across a wide range of doses. The absence of significant side effects, even at high doses, was probably due to the low level of allergenicity.¹⁶³

For immunotherapy of atopic dermatitis, SCIT is not indicated for use because of the likelihood that it could induce exacerbations of manifest atopic dermatitis or relapses of latent atopic dermatitis. In children treated either with SLIT or placebo, Pajno *et al.* reported a benefit from SLIT exclusively in children with atopic dermatitis sensitized against HDMs and in those with mild-to-moderate variants of atopic dermatitis, adjudged by the SCORAD index (SCORing Atopic Dermatitis).¹⁶⁴ However, two

patients in the SLIT group were excluded from the study owing to intense generalized flush reactions occurring within 1 h after sublingual allergen administration.

CONCLUSION

The putative value of SIT is not only in its use as a causal therapy for already manifest sensitizations, but also in its use as a preventive measure to avoid the development of further sensitizations and to counteract the atopic march early in life. This is of particular importance in light of recent developments that provide clear evidence for a genetically determined skin barrier dysfunction that predisposes a subgroup of patients with atopic dermatitis to the manifestation of numerous sensitizations and concomitant asthma.¹⁶⁵ Although SIT is an effective treatment for many allergic diseases, certain drawbacks, such as the long duration of treatment and the risk of anaphylactic reactions, need to be taken into account. Many gene-based strategies for immunotherapy aimed at reversing or preventing abnormal immune regulation and restoring Th1-predominated responses or regulatory T cell function have been developed,^{166–170} but therapeutic efficacy depends highly on delivery efficiency and target selection.¹⁷¹ Mucosal immunotherapy is a better strategy for treating allergic disease because of its non-injection routes and low side-effect profile. SLIT is a better choice for prophylactic and therapeutic approaches to allergy treatment, but the outcomes for allergic disease from the use of different sensitizing allergens will still need further definition. Also, a challenge remains in evaluating whether results from experimental animal studies will hold true in humans.

Continuous improvements have been made in allergen preparation, such as the introduction of highly purified allergoids¹⁷² and recombinant allergens,¹⁷³ the targeting of dominant T-cell epitopes of allergen,^{12,45} and the refinement of treatment schedules,¹⁷⁴ as well as in the concomitant use of adjuvants.¹⁷⁵ Additional types of mucosal adjuvant candidates have been explored, including living parasites,¹⁷⁶ IL-10, TGF- β -inducing compounds^{111,177} and natural compounds derived from plants and herbs.^{178,179}

The collection of data from large, well-designed, double-blind, placebo-controlled, randomized trials with post-treatment follow-up, will provide robust substantiation of current evidence. SCIT has demonstrated long-term clinical effects and the potential to preventing the development of asthma in children with allergic rhinoconjunctivitis for up to 7 years after treatment termination.¹⁸⁰ The role of SCIT in adult asthma treatment is still limited. Mucosal immunotherapy studies in adults and children with allergic diseases that use different types of allergens and different routes of administration and evaluate the side effects from each route will improve our knowledge on this issue.¹⁸¹

We anticipate that continued growth in the understanding of immunotherapeutic strategies for allergic diseases will offer therapies with lower doses, greater safety and more effective application in the future.

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