

Novel Ways for Immune Intervention in Immunotherapy: Mucosal Allergy Vaccines

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Subcutaneous immunotherapy (SCIT) has been investigated for almost a century [1,2] and is currently used to treat allergies to insect venom, house dust mites (HDMs), grass and tree pollens, and animal epithelia [3–8]. Currently, allergen-specific immunotherapy is considered the only curative treatment for type I allergies because of its capacity to reorient inappropriate humoral and cellular immune responses. Although SCIT is considered a reference therapy, it requires multiple injections and can be associated with severe side effects, including anaphylactic shock. Thus, safer and noninvasive mucosal routes, including nasal, oral, and sublingual routes of administration, are considered an alternative to SCIT.

A rationale for administering immunotherapy through the mucosal route is that the immune system in aerodigestive mucosae is poised to induce immune tolerance rather than immunostimulation when exposed to antigens and allergens from the environment [9,10]. Mucosal tolerance is believed to be a consequence of either anergy (after the depletion of responsive cells) or the induction of suppressive mechanisms mediated by regulatory T lymphocytes [11]. In light of a better understanding of immune mechanisms associated with successful antiallergic treatments, new strategies can be implemented to improve mucosal allergy vaccines. The latter may rely on modified or unmodified recombinant

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Table 1
Comparison between systemic and mucosal routes for allergy vaccination

Route of immunization	Allergens	Comments	References
Subcutaneous immunotherapy (SCIT)	Pollens (ragweed, birch, grasses, <i>Parietaria</i>, olive), dust mites, cat dander, venoms Used as natural biologic extracts, allergoids, recombinant protein (Bet v 1, Phl p 1, 2, 5, 6), purified natural allergen (Amb a1) or peptides (Fel d 1, PLA A2), associated with an adjuvant (eg, CaPO₄, alum, monophosphoryl lipid A [MPL])	Clinical efficacy: SCIT with house dust mites (HDM) or pollen (grass and tree) extracts reduces asthma and rhinoconjunctivitis symptoms and antiallergic drug intake. SCIT decreases allergen-specific skin, nose, and eye sensitivity after natural allergen exposure or challenge. SCIT with hypoallergenic Bet v 1 forms (peptide fragment and trimer) inhibits immediate-type allergic reactions. Patients treated with Fel d 1 peptides experience a decrease in skin reactivity concomitantly with a decrease in Th1/Th2 responses and an induction of circulating IL10–producing regulatory T (Treg) cells. Immunotherapy with peptides from PLA A2 (a major bee venom allergen) induces tolerance after allergen and bee sting challenge in allergic patients. Immune mechanisms: Antibody responses: During SCIT (grass, ragweed and birch pollens), an improvement in symptoms is often associated with an increase in allergen-specific IgG (mostly IgG4) and a reduction of specific IgE antibodies. IgG4 inhibit binding of allergen-IgE complexes to inflammatory cells, thereby preventing histamine release. SCIT with recombinant grass pollen allergens (hypoallergenic Bet v 1 or a mixture of five allergens) improves rhinitis symptoms while up-regulating IgG (1, 2, and 4) antibodies. MPL associated with grass pollen allergens increases the production of specific IgG1 and IgG4 antibodies.	[3–8,24] [118–120] [17,22,24,80,81,121,122]

Cellular responses:

SCIT prevents migration and activation of inflammatory cells (mast cells, eosinophils, basophils, neutrophils) within target organs (skin, nose, lung). After successful SCIT, the level of inflammatory mediators (histamine, kinins, PGD₂, myeloperoxidase) is reduced.

SCIT reorients allergen-specific T-cell responses in atopic patients from a Th2 to Th1 pattern. A correlation is often established between successful SCIT and an increase in the Th1/Th2 cytokine balance. In certain studies, the increase of the Th1/Th2 ratio is not observed at the beginning of the treatment, but only during high-dose maintenance therapy. The shift from Th2 to Th1 responses is sometimes noticeable mostly within the target organ (eg, nasal mucosa, skin).

Successful SCIT with recombinant hypoallergenic Bet v 1 (peptide fragments and trimer) is associated with a reduced production of IL-4, IL-5, and IL-13 by allergen-specific T cells, whereas IFN- γ and IL-12 production is increased. SCIT for wasp venom, HDM, cat, and grass or birch pollen allergies induces allergen-specific regulatory cells (CD4+ CD25+ or Type 1 regulatory cells [Tr1]) in the blood.

Clinical efficacy:

LNIT with HDM or pollen (tree and grass) allergens in natural or allergoid forms reduces symptoms/medication scores in patients with allergic rhinitis. Allergoids are better-tolerated than aqueous extracts when used intranasally but the therapeutic benefit is lower. Macronized powder forms of pollens (eg, *Parietaria*, birch, grass) or mites have been used, leading to improved clinical efficacy and long-lasting protective effects in patients who have rhinitis.

Local nasal immunotherapy (LNIT)

Pollens (ragweed, grasses, *Parietaria*), dust mites
Used as natural biologic extracts, allergoids, macronized powder forms, without any adjuvant

[18,19,25–30]

[19,33–37,123]

[23,119,124–126]

[41–47,50]

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Table 1 (continued)

Route of immunization	Allergens	Comments	References
Local nasal immunotherapy (LNIT)		Immune mechanisms: Antibody responses: A rise in IgA, IgE, and IgG antibodies is observed during LNIT with aqueous or chemically modified (allergoid) pollens (ragweed and grass). No significant immunologic change is induced in LNIT with macronized powder pollen extracts (eg, <i>Parietaria</i>). Cellular responses: Successful LNIT to <i>Parietaria</i> significantly reduces inflammatory infiltration and intercellular adhesion molecule (ICAM-1) expression on nasal epithelial cells after allergen challenge. No change in soluble eosinophil cationic protein is detected. In preclinical models, nasal administration of recombinant Ves v 5 before sensitization with wasp venom reduces Th2 polarization (humoral and cellular responses) in association with an increase in TGF-β and IL-10 mRNA in spleen cells. In another study, immunologic tolerance was achieved after intranasal immunization with ovalbumin (OVA) as a consequence of induction of anergy and stimulation of OVA-specific CD4 + regulatory T cells.	[41–47,50] [48] [51,52]
Oral immunotherapy (OIT)	Grass and birch pollens Used as natural biologic extract in aqueous, freeze-dried, powder forms, tablets, enterosoluble capsules, without any adjuvant	Clinical efficacy: OIT using low or high doses of grass pollen does not improve skin and nasal reactivity to allergens. In contrast, OIT with birch pollen extracts reduces asthma/rhinitis symptoms in adults and children. Oral tolerance induction with myelin basic protein or collagen-derived peptides in	[10,55–57,60–62]

multiple sclerosis or rheumatoid arthritis, respectively, leads to some level of clinical improvement.		
Immune mechanisms:		
Antibody responses:	No changes in specific IgE and IgG have been detected in OIT with grass pollen allergen extracts. OIT with birch pollen extracts decreases specific IgE and up-regulates IgG antibodies.	[55-57,60-62,127]
Cellular responses:	OIT with grass pollen does not change histamine secretion by basophils.	[56]
	Numerous studies in murine models show that CD4+ Treg cells are involved in oral tolerance induction.	
	These Treg cells include Th3 cells synthesizing TGF- β and/or IL-10, Tr1 cells producing IL-10, and CD4+ CD25+ T cells.	[11]
	Clinical efficacy:	
Sublingual immunotherapy (SLIT)	Venoms, pollens (ragweed, birch, grasses, <i>Parietaria</i> , olive), dust mites Used as natural biologic extracts, allergoids without any adjuvant	[69,70,86,128]

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Table 1 (continued)

Route of immunization	Allergens	Comments	References
Sublingual immunotherapy		Immune mechanisms: Antibody responses: SLIT enhances allergen-specific IgG4 production in many studies, whereas IgE is often unaltered. In a few studies, a dose-dependent induction of allergen-specific IgA is observed.	[68,69,71,129]
		Cellular responses: SLIT with grass pollen or HDM down-regulates ICAM-1 expression in epithelial cells. Preseasonal rush SLIT using <i>Parietaria</i> in patients who have asthma reduces recruitment of neutrophils and eosinophils and ICAM-1 expression in the nose after allergen provocation. In children allergic to olive pollen, SLIT reduces rhinitis and skin reactivity in parallel with seric eosinophil cationic protein (ECP) during the peak season. SLIT against grass pollen or HDM decreases nasal secretion of ECP and tryptase in children. In several studies, SLIT had no impact on the Th1/Th2 balance. Children who have asthma treated with <i>Dermatophagoides pteronyssinus</i> extracts exhibited a decrease in seric IL-13 and T-cell proliferation. A pilot study indicated that peripheral blood mononuclear cells from patients treated with HDM-SLIT produce high levels of IL-10.	
			[72,75,82-86]
			[87-89]
			[90]

allergens or derived peptides associated with new vectors and adjuvant molecules to enhance allergen-specific regulatory T-cell responses.

Immune tolerance after subcutaneous allergen-specific immunotherapy

After the pioneer studies by Noon [1] almost a century ago, allergen-specific SCIT was established based on clinical efficacy as a reference for curative treatment of allergies [3–8]. Venom SCIT is a very effective treatment in patients allergic to *Vespula* or honeybees. SCIT is also efficacious in treating rhinoconjunctivitis and asthma associated with grass and tree pollens, dust mites (eg, *Dermatophagoides pteronyssinus*, *D farinae*, *Lepidoglyphus destructor*), cat dander, and *Cladosporium* or *Alternaria* [3]. SCIT reduces immediate (ie, linked to the release of inflammatory mediators such as histamine or prostaglandin D₂) and late phase (ie, linked to the activation of mast cells and eosinophils) allergen-induced symptoms in target organs (Table 1) [4,12–16].

Successful SCIT is often associated with a decrease in the sera of allergen-specific IgE antibody production concomitant with an up-regulation of IgGs, particularly IgG1 and IgG4s, some of which may exhibit a potential blocking activity (see Table 1) [17]. Such an altered IgE/IgG4 antibody balance contributes to the reduction of IgE-mediated histamine release by basophils and antigen presentation to T cells [18–21]. Researchers have suggested that during SCIT, the affinity of IgE antibodies for allergens is potentially decreased, whereas the affinity of allergen-specific IgG4 antibodies seems enhanced [22]. A limited number of studies have also documented an increase in seric IgA antibodies [23,24]. Successful SCIT also inhibits the recruitment and activation of proinflammatory cells, such as mast cells, basophils, and eosinophils in the skin, nose, eye, and bronchial mucosae, as shown after provocation or natural exposure to allergens (see Table 1) [18,19,25–30].

In the current view of the immune system (Fig. 1), CD4⁺ T lymphocytes play a critical role in controlling the various immune effector mechanisms contributing to allergic inflammation [31]. That allergic patients mount allergen-specific CD4⁺ T-cell responses of the T helper 2 (Th2) type (ie, associated with the secretion of interleukin (IL)-4, IL-5, and IL-13 cytokines [13,32]) is well established. Successful SCIT redirects those allergen-specific Th2 responses toward the Th1 type [33–37], with an increased production of IL-12 and interferon (IFN)- γ within the blood and target organs, such as the nasal mucosa or the skin (see Table 1). More recently, research established that SCIT also stimulates allergen-specific subsets of CD4⁺ regulatory T cells capable of down-regulating Th1 and Th2 responses through the production of IL-10 or transforming growth factor (TGF)- β and cell-to-cell contact (see Table 1) [31].

Taken together, SCIT is widely agreed to be effective in curing allergic rhinoconjunctivitis and preventing progression to asthma. However, repeated injections are needed over several years in the course of SCIT, which makes this treatment cumbersome, particularly for children. In addition, a major drawback

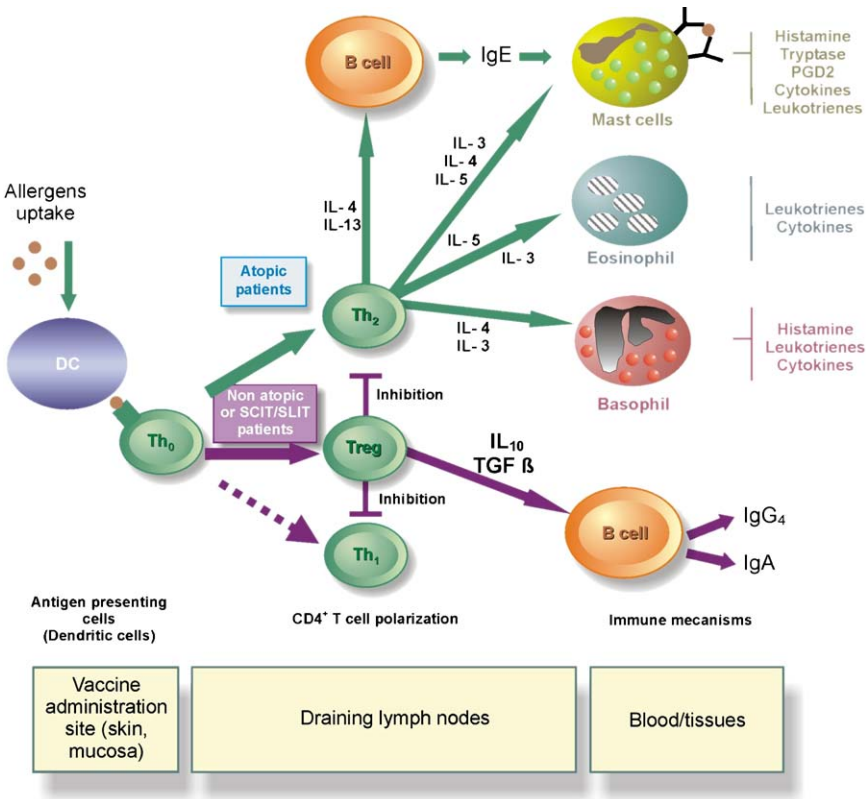


Fig. 1. Cellular and humoral mechanisms of allergen-specific immunotherapy. After allergen capture at the administration site, dendritic cells mature and migrate to draining lymph nodes where they stimulate naïve T cells (Th₀). Depending on the cytokine milieu, Th₀ cells can differentiate into various CD4⁺ T helper cells producing distinct patterns of cytokines. Such CD4⁺ T helper cells stimulated after allergen exposure or desensitization with the vaccine are critical in controlling antibody production and isotype switching, and recruitment and activation of proinflammatory cells (eg, mast cells, eosinophils, basophils). The purpose of allergen-specific immunotherapy is to restore immune tolerance by shifting T-cell responses from Th₂ to Th₁ regulatory T cells.

is the risk for serious systemic reactions such as anaphylaxis, which is infrequent but can be lethal [38]. Therefore, alternative vaccination strategies through the nasal, oral, or sublingual mucosal routes are being investigated.

Tolerance induction by mucosal immunotherapy

Intranasal immunotherapy

Local nasal immunotherapy (LNIT) has been evaluated in clinical trials for almost 3 decades with some encouraging results in terms of safety and efficacy (see Table 1) [39]. For example, LNIT with either aqueous extracts or chemically

modified allergens (allergoids) elicited a marked reduction in symptoms and medication scores in patients who had allergic rhinitis to ragweed and grass pollens [40–42]. In general, chemically modified allergens were better-tolerated than aqueous extracts, although a lower therapeutic benefit was sometimes observed with the former [41,42]. Several studies have shown clinical efficacy of LNIT in patients who have allergic rhinitis to various pollens (eg, *Parietaria*, birch, grass) or mites when using allergens in a macronized powder form [43–47]. Medication and symptoms scores were improved and a long-lasting protective effect was observed, most particularly with regard to nasal symptoms [48]. In contrast, no significant clinical efficacy of LNIT could be shown in the second year after treatment with an allergoid grass vaccine [49]. Adverse reactions during LNIT are infrequent and generally restricted to the upper respiratory tract.

No clear immunologic changes correlating with vaccine efficacy have been noted in LNIT clinical trials [41–47,50]. Immune mechanisms underlying LNIT-induced tolerance have been investigated in mice. In such preclinical models, T-cell anergy, a decrease in Th2 responses, and an induction of TGF- β – and IL-10–producing regulatory T cells have been proposed to be potential mechanisms for tolerance induction through the nasal route [51,52]. Biodistribution studies of radiolabeled *Parietaria judaica* allergen (Par j 1) have been conducted in humans after intranasal administration. Such studies showed that most of the allergen is swallowed and absorbed through the gastrointestinal tract in volunteers who had allergies and those who did not, leading to a plasmatic peak 1 hour after administration [53,54]. A fraction of the allergen seems to persist up for 24 hours in the nasal cavity in healthy volunteers but not in patients who are allergic. The rapid local clearance of the allergen observed in volunteers who were allergic likely involves IgE-mediated capture mechanisms.

Altogether, LNIT represents a safe and efficient treatment against grass pollen rhinitis. However, it requires particular technical skills, and controlling the exact dose administered is not trivial. Thus, these limitations have hampered its broad use in allergen-specific immunotherapy.

Oral immunotherapy

Oral immunotherapy (OIT) was investigated in the early 1980s in allergic patients [55–57]. Several studies were conducted in adults and children using an orally administrated grass pollen extract, either as an aqueous solution, a powder, or enterosoluble tablets [55,56]. Although such oral grass pollen vaccines were well tolerated, no major impact was observed on symptom and medication scores or on skin or nasal reactivity (see Table 1). In addition, no significant alteration of seric allergen-specific IgG or IgE levels was found in patients undergoing OIT compared with those of patients undergoing therapy with placebo [55–57]. In parallel studies, immunotherapy using high doses of allergen formulated as enterosoluble grass pollen capsules also lacked any therapeutic effect [58,59]. In contrast, oral immunotherapy with high doses of

encapsulated birch pollen extracts showed some clinical efficacy in adults and children who were allergic [58,59]. When compared with the placebo group, patients undergoing birch pollen OIT showed an increase in seric-specific IgG antibody levels, associated with a reduction in seasonal IgE levels [60–62]. Encouraging clinical efficacy data were also obtained for OIT in patients allergic to either *Dermatophagoides* sp, *Artemisia*, or ragweed pollen [63–65].

OIT has also been extensively evaluated as a potential treatment for non-allergic inflammatory or autoimmune diseases [9,10]. Because tolerance breakdown of the intestinal immune system is known to cause inflammatory disorders, such as celiac or Crohn's disease, OIT is being considered in these pathologies [66]. Also, oral tolerance has shown some efficacy in preclinical models of autoimmune encephalomyelitis, arthritis, type I diabetes, thyroiditis, uveitis, atherosclerosis, and cerebrovascular stroke [9]. Using peptide vaccines, OIT was evaluated in patients who had rheumatoid arthritis, diabetes, or multiple sclerosis [10,11], with only a limited clinical benefit observed [10,11].

Regarding immunologic mechanisms involved during OIT, high doses of antigen administered orally are believed to promote T-cell depletion or anergy [11]. Alternatively, low-dose antigen tolerance is likely mediated through active suppressive mechanisms involving regulatory T cells. In all circumstances, increasing evidence suggests that dendritic cells play a crucial role in the regulation of mucosal immunity and tolerance as a consequence of their capacity to direct the fate of T cells. The level of costimulatory molecules and the cytokine milieu influence the degree of antigen-presenting cell (APC) maturation and activation, leading to systemic or suppressive T-cell responses [67]. Several studies focusing on CD4⁺ regulatory T cells have shown that such cells are critical in the induction of oral tolerance [11]. Regulatory T cells induced during OIT are highly heterogeneous and include Th3 cells (producing TGF- β or IL-10), Tr1 cells (producing IL-10), and CD4⁺ CD25⁺ cells, which mediate their regulatory function through cytokine secretion or cell contact-dependent mechanisms involving the cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) or programmed death-1 (PD-1) molecules [11].

Although oral tolerance induction has been successful in several animal models, its clinical efficacy has not been firmly established in humans. Thus, a purely oral route of administration is not recommended as a routine approach in allergic immunotherapy.

Sublingual immunotherapy

More than a decade ago, SLIT was investigated in patients with allergies [68–75]. During sublingual immunization, the allergen extract is held under the tongue for at least 1 to 2 minutes to allow capture by oral Langerhans-like dendritic cells (LLDCs) before being swallowed. Wilson and colleagues [70] conducted a meta-analysis encompassing 22 randomized clinical studies evaluating SLIT in 979 patients who had allergic rhinitis to HDMS, pollens (from grass, *Parietaria*, olive, ragweed, *Cupressus*), or cat dander. They concluded that

SLIT significantly reduces symptoms and medication requirements [70]. For example, high-dose SLIT has beneficial effects on nasal symptoms during the peak pollen season in patients who have severe seasonal allergic rhinitis, even if 2 years of treatment are required [76]. The safety and efficacy profile of SLIT has been confirmed in children, including those younger than 5 years [77]. In adults and children who had rhinitis, long-term clinical benefits in the form of prevention of new sensitizations or asthma were observed after SLIT [70]. A comparison of SCIT and SLIT regimens performed in a clinical trial in adult patients allergic to birch pollen showed no statistical difference in terms of efficacy between the two routes when high-dose SLIT regimens were used [78].

Biologic mechanisms underlying SLIT are being clarified [79]. As shown for the subcutaneous route, the IgE/IgG4 ratio is decreased in several clinically effective SLIT studies. SLIT most particularly increases allergen-specific IgG4 levels compared with placebo, as shown with house dust mites (HDM) and *Parietaria*/grass pollen vaccines [68,69,71]. Allergen-specific IgG (and possibly IgA) antibodies induced during SLIT are believed to compete with IgEs for binding to the allergen, thus preventing basophil or mastocyte degranulation (see Table 1) [24,80,81]. Within target organs (eg, eye or nose), SLIT reduces the recruitment of inflammatory cells induced by allergen challenge [75,82–85]. SLIT with grass pollen or HDM extracts also decreases the release of inflammatory mediators such as tryptase or eosinophil cationic protein in sputum or nasal secretions [82,84]. The expression of the *intercellular adhesion molecule 1* (ICAM-1), is also down-regulated on epithelial cells after SLIT [83,85]. Similarly, rush SLIT to *Parietaria* reduces the recruitment of neutrophils and eosinophils to the nasal mucosa, and the expression of the ICAM-1 molecule [86]. The impact of SLIT on allergen-specific T-cell polarization is still poorly documented. Although several SLIT studies have shown no impact on T-cell responses [87,88], successful sublingual immunotherapy in patients allergic to HDMs reduces the production of the Th2 cytokine IL-13 and the proliferation of peripheral blood mononuclear cells [89,90]. In a recent preliminary report, SLIT was found to stimulate IL-10-producing T cells in the peripheral blood of patients allergic HDMs, although no functional test was used to confirm that those cells were bona fide regulatory T cells [91].

Biodistribution studies in humans showed that no direct plasmatic absorption of allergens occurs through the sublingual mucosa [53,92]. Radiolabeled Par j 1 administered sublingually was detected in the plasma only after ingestion, with a peak between 1 and 2 hours after administration, implying absorption through the gut mucosa. Radiolabeled allergens persisted for up to 20 hours at the mucosal surface after SLIT, suggesting that allergen capture by Langerhans cells can occur during this time frame. The sublingual mucosa contains many LLDCs expressing constitutively high-affinity receptors for IgE (FcεRI), which are believed to play a crucial role in tolerance induction during desensitization [93]. Cross-linking of IgE receptors on LLDCs induces TGF-β and IL-10 cytokine production and the synthesis of indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolizing enzyme regulating T-cell proliferation [94,95]. After allergen capture in the sub-

Table 2
Vector systems and adjuvants for tolerance induction with mucosal allergy vaccines

	Comments	References
Immunomodulators		
Whole bacteria		
<i>Lactococcus lactis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus casei</i>	In mice, mucosal administration of commensal bacteria adapted to the intestine such as <i>Lactococcus lactis</i> or <i>Lactobacillus plantarum</i> together with recombinant Bet v 1 induces IgG2a antibodies and IFN- γ production in mice sensitized to the allergen. A mutant of <i>Lactobacillus plantarum</i> altered in the teichoic acid biosynthesis pathway exhibits a superior protective capacity in a murine colitis model, as a consequence of enhanced production of IL-10. <i>Lactobacillus casei</i> reduces CD8+ T-cell-mediated skin inflammation controlled by specific CD4+ Treg cells.	[100–103]
Heat-killed <i>Mycobacterium vaccae</i>	Heat-killed <i>Mycobacterium vaccae</i> induces Treg cells secreting IL-10 and TGF- β , conferring protection against airway inflammation in mice. Some efficacy in children who have atopic dermatitis has also been observed after intradermal administration.	[104,105]
Natural/synthetic bacterial products		
Toxins and derivatives (CT, LT, CTB, LTB)	Genetically detoxified CT and LT (or B subunits) without ADP ribosyl transferase activity retain a strong mucosal adjuvanticity (ie, induction of seric and mucosal neutralizing IgA). Mice vaccinated with influenza virus hemagglutinin concomitantly with LTB or genetically detoxified LT develop strong and protective antigen-specific IgG and IgA responses. The antigen/allergen can be mixed, fused, or chemically conjugated with the toxin moiety.	[106,130,131]
Lipid A analogs (OM-174, monophosphoryl lipid A (MPL), RC 529)	Lipid A derivatives are powerful Th1 adjuvants when administered through a systemic route. OM-174 promotes DC migration to lymphoid organs and their maturation. Mucosal administration (nasal, oral) of antigen of interest with MPL or RC 529 induces seric and local IgA and Th1 responses (IgG2a and CTL). Grass pollen allergens combined with MPL induce a strong production of specific IgG1 and IgG4 antibodies through the subcutaneous route. MPL is being tested in humans as an adjuvant for the sublingual route.	[132–134]
CpG	Synthetic oligonucleotides containing CpG motifs (CpG ODN) are potent Th1 adjuvants. Mucosal delivery of CpG ODN together with proteins promotes systemic (IgG2a and CTLs) and mucosal (IgA and CTLs) responses. Intradermal immunization with Amb a 1 fused to CpG oligonucleotides prevents allergen-induced hyperresponsiveness in mice. A conjugate Amb a1-cpG vaccine is being tested in ragweed allergic humans through the subcutaneous route.	[135,136]

Synthetic muramyldipeptides (MDP)	MDP-Lys (L18) induces mucosal immune responses protecting against a lethal viral challenge (using Sendai virus or rotavirus).	[137]
Bacterial subunits	The purified protein derivative (PPD) from <i>Mycobacterium tuberculosis</i> can induce IL-10-producing T cells in humans. The filamentous hemagglutinin (FHA) from <i>Bordetella pertussis</i> can induce Tr1 cells <i>in vivo</i> in mice.	[138,139]
Cytokines		
IL-1, IL-12, IL-15, IL-18, GM-CSF, IFN- γ / β	Combinations of cytokines (mainly IL-1 + IL-12, IL-18, and GM-CSF) with an HIV-env peptide induce mucosal protection, with specific CTLs and IgG1 and IgA antibodies. IFN- γ / β is a strong mucosal adjuvant against influenza virus. DNA vaccines expressing cytokine genes also induce efficient mucosal immune responses. Coimmunization with plasmid-expressing influenza hemagglutinin with the IL-15 gene generates protective cytotoxic T-lymphocyte responses. In murine models, imidazoquinolines (ligands for TLR7), dihydroxyvitamin D ₃ plus glucocorticoids, calcineurin inhibitors (cyclosporin A, FK 506), rapamycin, and mycophenolate mofetil can enhance regulatory T-cell responses.	[107,108] [140,141] [142]
Small synthetic molecules		
Vectors/delivery systems		
Particulate formulations		
Liposomes, virosomes	Lipid-based vehicles such as liposomes can incorporate functional viral envelope proteins (leading to Virosomes). Mucosal administration of such particulate formulations increases IgG and neutralizing IgA antibodies. Nasal administration of plasmid DNA complexes formulated with cationic lipids induces specific secretory IgA antibodies in the vaginal and rectal mucosae and seric IgG and IgA antibodies. Intranasal immunization with the antigen formulated in liposomes together with immunopotentiators (eg, LPS, MPL, LTB) induces strong systemic and mucosal responses (with IgG and IGA in the lung and nose). These complexes are spherical structures (30–100 nm diameter) comprising the saponin-adjuvant Quil A, cholesterol, and phospholipids mixed with the antigen of interest. ISCOMs exhibit a strong systemic and mucosal adjuvant activity. ISCOMs particles target the endosomal and cytosolic compartments, thus leading to class I and class II presentation of antigens for T cell stimulation. Nasal administration of influenza virus subunits formulated as ISCOMs induce protective immune responses.	[111,143–145] [109,146]
ISCOMs (structured complex of saponins and lipids)	PLGA can be used to present the antigen within nano- or microparticles to target antigen-presenting cells.	[147]
Poly (D,L-lactide-co-glycolic) acid (PLGA)		

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Table 2 (continued)

Comments	References
Virus-like particles (VLPs) From human papilloma viruses, Norwalk virus, porcine parvovirus	[112,113,148–151]
DC-targeting vectors Bacterial molecules (OmpA, CyaA, STxB)	[114–117]
Anti-DEC205 antibodies	[152]

Abbreviations: CT, cholera toxin; CTB, B subunits of CT; CTL, cytotoxic T lymphocyte; CyaA, adenylate cyclase; DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM-1, intercellular adhesion molecule 1; ISCOMs, immunostimulating complexes; LT, heat-labile enterotoxin; LTB, B subunits of LT; OmpA, outer membrane protein A; STxB, B subunit of Shiga toxin.

lingual mucosa, LLDCs rapidly migrate to proximal-draining lymph nodes of the cervical/submaxillary chain [96]. The latter represents a TGF- β -rich environment that likely favors the activation of B cells expressing “blocking IgG” antibodies (IgG2b in mice) and the induction of suppressive T cells [97].

Because of its well-established safety profile, with more than 500 million doses administered to humans, SLIT is currently considered a valid alternative to SCIT [78].

Novel mucosal vector systems and adjuvants

In SCIT, mineral adjuvant molecules (eg, calcium phosphate, aluminum hydroxide) are used as part of allergy vaccines [73]. No such adjuvants are currently used during mucosal immunotherapy, implying that the dose of allergens should be increased. For example, it is well-documented that up to 50 to 100 fold more allergen is needed in SLIT to reach a level of efficacy similar to SCIT [73]. Specialized Langerhans cells in mucosae are prone to elicit regulatory T cells, favoring the development of allergen-specific IgG or IgA antibodies instead of IgE responses through IL-10 and TGF- β cytokine production. Capitalizing on the better understanding of immune mechanisms associated with immune tolerance, more efficacious mucosal vaccines can be rationally designed, particularly adjuvants/formulations that better target mucosal Langerhans cells, to improve stimulation of allergen-specific regulatory T cells. Schematically, antigen presentation platforms that can be used to improve mucosal tolerance include (1) immunopotentiators (ie, synthetic or biologic adjuvant molecules modulating immune responses by providing cosignals to immune cells) and (2) vector systems (targeting the antigen/allergen to APCs within the mucosa).

Immunopotentiators

Using appropriate immunopotentiators together with antigens/allergens may be necessary to redirect T-lymphocyte polarization from Th2 to regulatory T-cell responses. Several adjuvants are currently available from the vaccine industry that could lead to more efficient mucosal and systemic immune responses after administration at mucosal surfaces. Some are explored as novel approaches to potentiate antigen-specific Th1 or regulatory T-cell responses. For example, the Th1 adjuvant monophosphoryl lipid A has been used successfully through the subcutaneous route as part of grass pollen vaccines [98,99] and is currently being tested in humans through the sublingual route. In preclinical models, living or heat-killed bacteria (eg, *Lactobacillus plantarum*, *Lactococcus lactis*, *Mycobacterium vaccae*) showed some benefits as adjuvants in murine models of asthma (Table 2) [100–105]. Other potential mucosal adjuvants include bacterial toxins, such as cholera toxin and the closely related *Escherichia coli* heat-labile enterotoxin or their nontoxic derivatives (genetically detoxified or B subunits).

These bacterial toxins can be coadministered as a mix, conjugated, or fused with soluble antigens (see Table 2) [106]. A combination of cytokines mimicking signals induced by bacterial toxins can be used instead [107,108]. Triggering Toll-like receptors by lipid A or synthetic muramyl dipeptide analogs is also effective in inducing systemic and mucosal responses (see Table 2). Immunosuppressive drugs (eg, glucocorticoids with vitamin D₃) also represent potential regulatory T-cell adjuvants for allergy immunotherapy because they are powerful inducers of IL-10 through immune cells (see Table 2).

Mucosal vector systems

Selected formulations can be used to present allergens in a particulate form, thereby targeting antigen-presenting cells (eg, Langerhans cells) with phagocytic activity. They may be used in association with immunopotentiators to further increase immune responses (see Table 2). These formulations include liposomes, virosomes, or immunostimulating complexes [109]. Ideal mucosal vectors should protect the allergens in the vaccine (mostly proteins or glycoproteins) from degradation by local proteases while targeting mucosal inductive sites or antigen-presenting cells.

Several vector systems to express antigen/allergen at mucosal surfaces have been tested. Using naked plasmid DNA or DNA absorbed on microparticles, markedly enhanced systemic and mucosal immune responses have been obtained [110,111]. Nonreplicating virus-like particles (VLPs) generated by the spontaneous assembly of viral capsid proteins also represent an attractive alternative for expressing either the whole antigen/allergen, or selected T- or B-cell epitopes within a particulate vector system [112,113]. As an advantage for mucosal application, VLPs are often derived from viruses for which mucosae represent a natural route of transmission (see Table 2). Genetically detoxified or nontoxic bacterial products, such as the adenylate cyclase from *Bordetella pertussis*, the outer membrane protein A (OmpA) from *Klebsiella pneumoniae*, or the Shiga toxin B subunit, can be conjugated to antigens/allergens to target antigen-presenting cells (see Table 2) [114–116]. Delivering epitopes of interest through these vectors generates specific protective cytotoxic T lymphocytes and systemic antibody responses [114–116]. Nasal administration of OmpA induces mucosal immune responses [117]. Collectively, several vector systems are available to target antigens/allergens to mucosal Langerhans cells. Although such vectors were initially designed and tested to elicit effector immune responses of the Th1 and Th2 types, none of these potential mucosal vectors has been established as a means to enhance antigen-specific regulatory T cells.

Summary

Building on the success of SCIT in curing allergic disease, improved allergy vaccines that rely on mucosal routes of administration are needed. To varying

degrees, desensitization through the oral, nasal, and sublingual routes has shown some efficacy. The development of allergy vaccines has almost exclusively been based on natural or modified allergen extracts. A second generation will consist of vaccines based on recombinant proteins. In this regard, a major interest exists in developing hypoallergenic forms of recombinant allergens capable of inducing IgGs and stimulating T cells while exhibiting reduced IgE-binding capacity. Although this approach is definitively promising for vaccines administered parenterally, a more appropriate strategy for mucosal immunization, particularly for sublingual immunotherapy, is perhaps to rely on recombinant allergens presented in the most native form to allow IgE-mediated targeting and capture of allergens by mucosal Langerhans cells [93]. A better understanding of immune mechanisms involved in the induction and maintenance of antigen-specific immune tolerance provides opportunities for the design and development of improved mucosal vaccines. An emergent working hypothesis is that allergy vaccines should induce allergen-specific regulatory T lymphocytes capable of producing suppressive cytokines (eg, IL-10, TGF- β). Developing a new generation of safer and more efficient allergy vaccines administered through non-parenteral routes implies the need for adjuvants or vector systems specifically selected to enhance tolerance at mucosal surfaces. Identifying allergen presentation systems suitable for mucosal application will help develop more efficient allergy vaccines while simplifying immunization schemes.

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References

- [1] Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911;2:1572–3.
- [2] Freeman J. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. *Lancet* 1911;2:814–7.
- [3] Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;102:558–62.
- [4] Larche M. Specific immunotherapy. *Br Med Bull* 2000;56:1019–36.
- [5] Gehlhar K, Schlaak M, Becker W, et al. Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy* 1999;29:497–506.
- [6] Michils A, Mairesse M, Ledent C, et al. Modified antigenic reactivity of anti-phospholipase A2 IgG antibodies in patients allergic to bee venom: conversion with immunotherapy and relation to subclass expression. *J Allergy Clin Immunol* 1998;102:118–26.
- [7] Pichler CE, Helbling A, Pichler WJ. Three years of specific immunotherapy with house-dust-mite extracts in patients with rhinitis and asthma: significant improvement of allergen-specific parameters and of nonspecific bronchial hyperreactivity. *Allergy* 2001;56:301–6.
- [8] Moller C, Dreborg S, Ferdousi HA, et al. Pollen immunotherapy reduces the development of

- asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002;109:251–6.
- [9] Mowat AM, Parker LA, Beacock-Sharp H, et al. Oral tolerance: overview and historical perspectives. *Ann N Y Acad Sci* 2004;1029:1–8.
- [10] Mayer L, Shao L. Therapeutic potential of oral tolerance. *Nat Rev Immunol* 2004;4:407–19.
- [11] Faria AM, Weiner HL. Oral tolerance. *Immunol Rev* 2005;206:232–59.
- [12] Akdis CA, Blaser K. Mechanisms of allergen-specific immunotherapy. *Allergy* 2000;55:522–30.
- [13] Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998;102:157–64.
- [14] Till SJ, Francis JN, Nouri-Aria K, et al. Mechanisms of immunotherapy. *J Allergy Clin Immunol* 2004;113:1025–34 [quiz 35].
- [15] Ebner C. Systemic immune response to specific immunotherapy. *Clin Exp Allergy* 1998;28:781–3.
- [16] Garcia NM, Lynch NR, Di Prisco MC, et al. Nonspecific changes in immunotherapy with house dust extract. *J Investig Allergol Clin Immunol* 1995;5:18–24.
- [17] Wachholz PA, Durham SR. Induction of 'blocking' IgG antibodies during immunotherapy. *Clin Exp Allergy* 2003;33:1171–4.
- [18] Iliopoulos O, Proud D, Adkinson Jr NF, et al. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991;87:855–66.
- [19] Durham SR, Ying S, Varney VA, et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4 + T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996;97:1356–65.
- [20] van Neerven RJ, Wikborg T, Lund G, et al. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4 + T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999;163:2944–52.
- [21] van Neerven RJ, Arvidsson M, Ipsen H, et al. A double-blind, placebo-controlled birch allergy vaccination study: inhibition of CD23-mediated serum-immunoglobulin E-facilitated allergen presentation. *Clin Exp Allergy* 2004;34:420–8.
- [22] Pierson-Mullany LK, Jackola D, Blumenthal M, et al. Altered allergen binding capacities of Amb a 1-specific IgE and IgG4 from ragweed-sensitive patients receiving immunotherapy. *Ann Allergy Asthma Immunol* 2000;84:241–3.
- [23] Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;33:1205–14.
- [24] Niederberger V, Horak F, Vrtala S, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004;101(Suppl 2):14677–82.
- [25] Hakansson L, Heinrich C, Rak S, et al. Priming of eosinophil adhesion in patients with birch pollen allergy during pollen season: effect of immunotherapy. *J Allergy Clin Immunol* 1997;99:551–62.
- [26] Durham SR, Varney VA, Gaga M, et al. Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin Exp Allergy* 1999;29:1490–6.
- [27] Monteseirin J, Bonilla I, Camacho J, et al. Elevated secretion of myeloperoxidase by neutrophils from asthmatic patients: the effect of immunotherapy. *J Allergy Clin Immunol* 2001;107:623–6.
- [28] Wilson DR, Irani AM, Walker SM, et al. Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium. *Clin Exp Allergy* 2001;31:1705–13.
- [29] Furin MJ, Norman PS, Creticos PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J Allergy Clin Immunol* 1991;88:27–32.
- [30] Wilson DR, Nouri-Aria KT, Walker SM, et al. Grass pollen immunotherapy: symptomatic

- improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J Allergy Clin Immunol* 2001;107:971–6.
- [31] Robinson DS, Larche M, Durham SR. Tregs and allergic disease. *J Clin Invest* 2004;114:1389–97.
- [32] El Biaze M, Boniface S, Koscher V, et al. T cell activation, from atopy to asthma: more a paradox than a paradigm. *Allergy* 2003;58:844–53.
- [33] Mavroleon G. Restoration of cytokine imbalance by immunotherapy. *Clin Exp Allergy* 1998;28:917–20.
- [34] Hamid QA, Schotman E, Jacobson MR, et al. Increases in IL-12 messenger RNA + cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997;99:254–60.
- [35] Benjaponpitak S, Oro A, Maguire P, et al. The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. *J Allergy Clin Immunol* 1999;103:468–75.
- [36] Ebner C, Siemann U, Bohle B, et al. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997;27:1007–15.
- [37] Secrist H, Chelen CJ, Wen Y, et al. Allergen immunotherapy decreases interleukin 4 production in CD4 + T cells from allergic individuals. *J Exp Med* 1993;178:2123–30.
- [38] Medicines CoSo. Desensitising vaccines: an allergist's view. *BMJ* 1986;293:1169–70.
- [39] Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. *J Allergy Clin Immunol* 2003;111:437–48 [quiz 49].
- [40] Deuschl H, Johansson GO. Hyposensitization of patients with allergic rhinitis by intranasal administration of chemically modified grass pollen allergen. A pilot study. *Acta Allergol* 1977;32:248–62.
- [41] Nickelsen JA, Goldstein S, Mueller U, et al. Local intranasal immunotherapy for ragweed allergic rhinitis. I. Clinical response. *J Allergy Clin Immunol* 1981;68:33–40.
- [42] Georgitis JW, Reisman RE, Clayton WF, et al. Local intranasal immunotherapy for grass-allergic rhinitis. *J Allergy Clin Immunol* 1983;71:71–6.
- [43] Andri L, Senna GE, Betteli C, et al. Local nasal immunotherapy in allergic rhinitis to *Parietaria*. A double-blind controlled study. *Allergy* 1992;47:318–23.
- [44] Andri L, Senna G, Betteli C, et al. Local nasal immunotherapy for *Dermatophagoides*-induced rhinitis: efficacy of a powder extract. *J Allergy Clin Immunol* 1993;91:987–96.
- [45] D'Amato G, Lobefalo G, Liccardi G, et al. A double-blind, placebo-controlled trial of local nasal immunotherapy in allergic rhinitis to *Parietaria* pollen. *Clin Exp Allergy* 1995;25:141–8.
- [46] Andri L, Senna G. Local nasal immunotherapy. *Allergy* 1995;50:190.
- [47] Andri L, Senna G, Betteli C, et al. Local nasal immunotherapy with extract in powder form is effective and safe in grass pollen rhinitis: a double-blind study. *J Allergy Clin Immunol* 1996;97:34–41.
- [48] Passalacqua G, Albano M, Riccio AM, et al. Local nasal immunotherapy: experimental evidences and general considerations. *Allergy* 1997;52:10–6.
- [49] Nickelsen JA, Georgitis JW, Mueller UR, et al. Local nasal immunotherapy for ragweed-allergic rhinitis. III. A second year of treatment. *Clin Allergy* 1983;13:509–19.
- [50] Nickelsen JA, Goldstein S, Mueller U, et al. Local intranasal immunotherapy for ragweed allergic rhinitis. II. Immunologic response. *J Allergy Clin Immunol* 1981;68:41–5.
- [51] Tsitoura DC, DeKruyff RH, Lamb JR, et al. Intranasal exposure to protein antigen induces immunological tolerance mediated by functionally disabled CD4 + T cells. *J Immunol* 1999;163:2592–600.
- [52] Winkler B, Bolwig C, Seppala U, et al. Allergen-specific immunosuppression by mucosal treatment with recombinant Ves v 5, a major allergen of *Vesputula vulgaris* venom, in a murine model of wasp venom allergy. *Immunology* 2003;110:376–85.
- [53] Bagnasco M, Mariani G, Passalacqua G, et al. Absorption and distribution kinetics of the

- major *Parietaria judaica* allergen (Par j 1) administered by noninjectable routes in healthy human beings. *J Allergy Clin Immunol* 1997;100:122–9.
- [54] Passalacqua G, Altrineti V, Mariani G, et al. Pharmacokinetics of radiolabelled Par j 1 administered intranasally to allergic and healthy subjects. *Clin Exp Allergy* 2005;35:880–3.
- [55] Rebiën W, Puttonen E, Maasch HJ, et al. Clinical and immunological response to oral and subcutaneous immunotherapy with grass pollen extracts. A prospective study. *Eur J Pediatr* 1982;138:341–4.
- [56] Taudorf E, Weeke B. Orally administered grass pollen. *Allergy* 1983;38:561–4.
- [57] Cooper PJ, Darbyshire J, Nunn AJ, et al. A controlled trial of oral hyposensitization in pollen asthma and rhinitis in children. *Clin Allergy* 1984;14:541–50.
- [58] Mosbech H, Dreborg S, Madsen F, et al. High dose grass pollen tablets used for hyposensitization in hay fever patients. A one-year double blind placebo-controlled study. *Allergy* 1987;42:451–5.
- [59] Urbanek R, Burgelin KH, Kahle S, et al. Oral immunotherapy with grass pollen in enterosoluble capsules. A prospective study of the clinical and immunological response. *Eur J Pediatr* 1990;149:545–50.
- [60] Moller C, Dreborg S, Lanner A, et al. Oral immunotherapy of children with rhinoconjunctivitis due to birch pollen allergy. A double blind study. *Allergy* 1986;41:271–9.
- [61] Taudorf E, Laursen LC, Lanner A, et al. Oral immunotherapy in birch pollen hay fever. *J Allergy Clin Immunol* 1987;80:153–61.
- [62] Taudorf E, Laursen L, Lanner A, et al. Specific IgE, IgG, and IgA antibody response to oral immunotherapy in birch pollinosis. *J Allergy Clin Immunol* 1989;83:589–94.
- [63] Giovane AL, Bardare M, Passalacqua G, et al. A three-year double-blind placebo-controlled study with specific oral immunotherapy to *Dermatophagoides*: evidence of safety and efficacy in paediatric patients. *Clin Exp Allergy* 1994;24:53–9.
- [64] Leng X, Fu YX, Ye ST, et al. A double-blind trial of oral immunotherapy for *Artemisia* pollen asthma with evaluation of bronchial response to the pollen allergen and serum-specific IgE antibody. *Ann Allergy* 1990;64:27–31.
- [65] Litwin A, Flanagan M, Entis G, et al. Oral immunotherapy with short ragweed extract in a novel encapsulated preparation: a double-blind study. *J Allergy Clin Immunol* 1997;100:30–8.
- [66] Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol* 2002;2:647–55.
- [67] MacPherson G, Milling S, Yrlid U, et al. Uptake of antigens from the intestine by dendritic cells. *Ann N Y Acad Sci* 2004;1029:75–82.
- [68] Bousquet J, Scheinmann P, Guinépain MT, et al. Sublingual-swallow immunotherapy (SLIT) in patients with asthma due to house-dust mites: a double-blind, placebo-controlled study. *Allergy* 1999;54:249–60.
- [69] La Rosa M, Ranno C, Andre C, et al. Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized *Parietaria judaica* extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999;104:425–32.
- [70] Wilson DR, Torres LI, Durham SR. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev* 2003;2:CD002893.
- [71] Clavel R, Bousquet J, Andre C. Clinical efficacy of sublingual-swallow immunotherapy: a double-blind, placebo-controlled trial of a standardized five-grass-pollen extract in rhinitis. *Allergy* 1998;53:493–8.
- [72] Vourdas D, Syrigou E, Potamianou P, et al. Double-blind, placebo-controlled evaluation of sublingual immunotherapy with standardized olive pollen extract in pediatric patients with allergic rhinoconjunctivitis and mild asthma due to olive pollen sensitization. *Allergy* 1998;53:662–72.
- [73] Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 2001;108:S147–334.
- [74] Pajno GB, Morabito L, Barberio G, et al. Clinical and immunologic effects of long-term sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, placebo-controlled study. *Allergy* 2000;55:842–9.

- [75] Marogna M, Spadolini I, Massolo A, et al. Clinical, functional, and immunologic effects of sublingual immunotherapy in birch pollinosis: a 3-year randomized controlled study. *J Allergy Clin Immunol* 2005;115:1184–8.
- [76] Smith H, White P, Annila I, et al. Randomized controlled trial of high-dose sublingual immunotherapy to treat seasonal allergic rhinitis. *J Allergy Clin Immunol* 2004;114:831–7.
- [77] Baena-Cagnani CE, Passalacqua G, Baena-Cagnani RC, et al. Sublingual immunotherapy in pediatric patients: beyond clinical efficacy. *Curr Opin Allergy Clin Immunol* 2005;5:173–7.
- [78] Khinchi MS, Poulsen LK, Carat F, et al. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 2004;59:45–53.
- [79] Moingeon P, Batard T, Fadel R, et al. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006;61:151–65.
- [80] Garcia BE, Sanz ML, Gato JJ, et al. IgG4 blocking effect on the release of antigen-specific histamine. *J Investig Allergol Clin Immunol* 1993;3:26–33.
- [81] Mothes N, Heinzkill M, Drachenberg KJ, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy* 2003;33:1198–208.
- [82] Marcucci F, Sensi L, Frati F, et al. Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications. *Allergy* 2001; 56:1091–5.
- [83] Passalacqua G, Albano M, Fregonese L, et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite-induced rhinoconjunctivitis. *Lancet* 1998;351: 629–32.
- [84] Marcucci F, Sensi L, Frati F, et al. Effects on inflammation parameters of a double-blind, placebo controlled one-year course of SLIT in children monosensitized to mites. *Allergy* 2003; 58:657–62.
- [85] Silvestri M, Spallarossa D, Battistini E, et al. Changes in inflammatory and clinical parameters and in bronchial hyperreactivity asthmatic children sensitized to house dust mites following sublingual immunotherapy. *J Investig Allergol Clin Immunol* 2002;12:52–9.
- [86] Passalacqua G, Albano M, Riccio A, et al. Clinical and immunologic effects of a rush sublingual immunotherapy to *Parietaria* species: A double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 1999;104:964–8.
- [87] Rolinck-Werninghaus C, Kopp M, et al. Lack of detectable alterations in immune responses during sublingual immunotherapy in children with seasonal allergic rhinoconjunctivitis to grass pollen. *Int Arch Allergy Immunol* 2005;136:134–41.
- [88] Lima MT, Wilson D, Pitkin L, et al. Grass pollen sublingual immunotherapy for seasonal rhinoconjunctivitis: a randomized controlled trial. *Clin Exp Allergy* 2002;32:507–14.
- [89] Ippoliti F, De Santis W, Volterrani A, et al. Immunomodulation during sublingual therapy in allergic children. *Pediatr Allergy Immunol* 2003;14:216–21.
- [90] Fenoglio D, Puppo F, Cirillo I, et al. Sublingual specific immunotherapy reduces PBMC proliferations. *Allerg Immunol (Paris)* 2005;37:147–51.
- [91] Ciprandi G, Fenoglio D, Cirillo I, et al. Induction of interleukin 10 by sublingual immunotherapy for house dust mites: a preliminary report. *Ann Allergy Asthma Immunol* 2005;95: 38–44.
- [92] Bagnasco M, Passalacqua G, Villa G, et al. Pharmacokinetics of an allergen and a monomeric allergoid for oromucosal immunotherapy in allergic volunteers. *Clin Exp Allergy* 2001; 31:54–60.
- [93] Allam JP, Novak N, Fuchs C, et al. Characterization of dendritic cells from human oral mucosa: a new Langerhans' cell type with high constitutive FcεpsilonRI expression. *J Allergy Clin Immunol* 2003;112:141–8.
- [94] Novak N, Bieber T, Katoh N. Engagement of Fc epsilon RI on human monocytes induces the production of IL-10 and prevents their differentiation in dendritic cells. *J Immunol* 2001; 167:797–804.

- [95] von Bubnoff D, Bezold G, Matz H, et al. Quantification of indoleamine 2,3-dioxygenase gene induction in atopic and non-atopic monocytes after ligation of the high-affinity receptor for IgE, Fc(epsilon)RI and interferon-gamma stimulation. *Clin Exp Immunol* 2003;132:247–53.
- [96] van Wilsem EJ, Breve J, Savelkoul H, et al. Oral tolerance is determined at the level of draining lymph nodes. *Immunobiology* 1995;194:403–14.
- [97] van Helvoort JM, Samsom JN, Chantry D, et al. Preferential expression of IgG2b in nose draining cervical lymph nodes and its putative role in mucosal tolerance induction. *Allergy* 2004;59:1211–8.
- [98] Drachenberg KJ, Wheeler AW, Stuebner P, et al. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 2001;56:498–505.
- [99] Drachenberg KJ, Heinzkill M, Urban E, et al. Efficacy and tolerability of short-term specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A (MPL) for children and adolescents. *Allergol Immunopathol (Madr)* 2003;31:270–7.
- [100] Repa A, Grangette C, Daniel C, et al. Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy. *Vaccine* 2003;22:87–95.
- [101] Grangette C, Muller-Alouf H, Goudercourt D, et al. Mucosal immune responses and protection against tetanus toxin after intranasal immunization with recombinant *Lactobacillus plantarum*. *Infect Immun* 2001;69:1547–53.
- [102] Grangette C, Nutten S, Palumbo E, et al. Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. *Proc Natl Acad Sci USA* 2005;102:10321–6.
- [103] Chapat L, Chemin K, Dubois B, et al. *Lactobacillus casei* reduces CD8 + T cell-mediated skin inflammation. *Eur J Immunol* 2004;34:2520–8.
- [104] Zuany-Amorim C, Manlius C, Trifilieff A, et al. Long-term protective and antigen-specific effect of heat-killed *Mycobacterium vaccae* in a murine model of allergic pulmonary inflammation. *J Immunol* 2002;169:1492–9.
- [105] Arkwright PD, David TJ. Effect of *Mycobacterium vaccae* on atopic dermatitis in children of different ages. *Br J Dermatol* 2003;149:1029–34.
- [106] Pizza M, Giuliani MM, Fontana MR, et al. Mucosal vaccines: non toxic derivatives of LT and CT as mucosal adjuvants. *Vaccine* 2001;19:2534–41.
- [107] Bradney CP, Sempowski GD, Liao HX, et al. Cytokines as adjuvants for the induction of anti-human immunodeficiency virus peptide immunoglobulin G (IgG) and IgA antibodies in serum and mucosal secretions after nasal immunization. *J Virol* 2002;76:517–24.
- [108] Staats HF, Bradney CP, Gwinn WM, et al. Cytokine requirements for induction of systemic and mucosal CTL after nasal immunization. *J Immunol* 2001;167:5386–94.
- [109] Sanders MT, Brown LE, Deliyannis G, et al. ISCOM-based vaccines: the second decade. *Immunol Cell Biol* 2005;83:119–28.
- [110] Hobson P, Barnfield C, Barnes A, et al. Mucosal immunization with DNA vaccines. *Methods* 2003;31:217–24.
- [111] Klavinskis LS, Barnfield C, Gao L, et al. Intranasal immunization with plasmid DNA-lipid complexes elicits mucosal immunity in the female genital and rectal tracts. *J Immunol* 1999;162:254–62.
- [112] Boisgerault F, Moron G, Leclerc C. Virus-like particles: a new family of delivery systems. *Expert Rev Vaccines* 2002;1:101–9.
- [113] Niikura M, Takamura S, Kim G, et al. Chimeric recombinant hepatitis E virus-like particles as an oral vaccine vehicle presenting foreign epitopes. *Virology* 2002;293:273–80.
- [114] El Azami El Idrissi M, Ladant D, et al. The adenylate cyclase of *Bordetella pertussis*: a vector to target antigen presenting cells. *Toxicon* 2002;40:1661–5.
- [115] Jeannin P, Renno T, Goetsch L, et al. OmpA targets dendritic cells, induces their maturation and delivers antigen into the MHC class I presentation pathway. *Nat Immunol* 2000;1:502–9.
- [116] Haicheur N, Benchetrit F, Amessou M, et al. The B subunit of Shiga toxin coupled to full-

- size antigenic protein elicits humoral and cell-mediated immune responses associated with a Th1-dominant polarization. *Int Immunol* 2003;15:1161–71.
- [117] Goetsch L, Gonzalez A, Plotnicky-Gilquin H, et al. Targeting of nasal mucosa-associated antigen-presenting cells in vivo with an outer membrane protein A derived from *Klebsiella pneumoniae*. *Infect Immun* 2001;69:6434–44.
- [118] Muller U, Akdis CA, Fricker M, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;101:747–54.
- [119] Verhoef A, Alexander C, Kay AB, et al. T cell epitope immunotherapy induces a CD4 + T cell population with regulatory activity. *PLoS Med* 2005;2:e78.
- [120] Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 2005;11:S69–76.
- [121] Jakobsen CG, Bodtger U, Poulsen LK, et al. Vaccination for birch pollen allergy: comparison of the affinities of specific immunoglobulins E, G1 and G4 measured by surface plasmon resonance. *Clin Exp Allergy* 2005;35:193–8.
- [122] Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005;116:608–13.
- [123] Gafvelin G, Thunberg S, Kronqvist M, et al. Cytokine and antibody responses in birch-pollen-allergic patients treated with genetically modified derivatives of the major birch pollen allergen Bet v 1. *Int Arch Allergy Immunol* 2005;138:59–66.
- [124] Francis JN, Till SJ, Durham SR. Induction of IL-10 + CD4 + CD25 + T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003;111:1255–61.
- [125] Nasser SM, Ying S, Meng Q, et al. Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur J Immunol* 2001;31:3704–13.
- [126] Gardner LM, Thien FC, Douglass JA, et al. Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4 + CD25 + interleukin-10 + T cells expressing peripheral tissue trafficking markers. *Clin Exp Allergy* 2004;34:1209–19.
- [127] Bjorksten B, Moller C, Broberger U, et al. Clinical and immunological effects of oral immunotherapy with a standardized birch pollen extract. *Allergy* 1986;41:290–5.
- [128] Passalacqua G, Villa G, Altrinetti V, et al. Sublingual swallow or spit? *Allergy* 2001;56:578.
- [129] Bahceciler NN, Arikian C, Taylor A, et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. *Int Arch Allergy Immunol* 2005;136:287–94.
- [130] Haan L, Verweij WR, Holtrop M, et al. Nasal or intramuscular immunization of mice with influenza subunit antigen and the B subunit of *Escherichia coli* heat-labile toxin induces IgA- or IgG-mediated protective mucosal immunity. *Vaccine* 2001;19:2898–907.
- [131] Hagiwar Y, Tsuji T, Iwasaki T, et al. Effectiveness and safety of mutant *Escherichia coli* heat-labile enterotoxin (LT H44A) as an adjuvant for nasal influenza vaccine. *Vaccine* 2001;19:2071–9.
- [132] Pajak B, Garze V, Davies G, et al. The adjuvant OM-174 induces both the migration and maturation of murine dendritic cells in vivo. *Vaccine* 2003;21:836–42.
- [133] Baldrige JR, Yorgensen Y, Ward JR, et al. Monophosphoryl lipid A enhances mucosal and systemic immunity to vaccine antigens following intranasal administration. *Vaccine* 2000;18:2416–25.
- [134] Mason KW, Zhu D, Scheuer CA, et al. Reduction of nasal colonization of nontypeable *Haemophilus influenzae* following intranasal immunization with rLP4/rLP6/UspA2 proteins combined with aqueous formulation of RC529. *Vaccine* 2004;22:3449–56.
- [135] Gallichan WS, Woolstencroft RN, Guarasci T, et al. Intranasal immunization with CpG oligodeoxynucleotides as an adjuvant dramatically increases IgA and protection against herpes simplex virus-2 in the genital tract. *J Immunol* 2001;166:3451–7.
- [136] Santeliz JV, Van Nest G, Traquina P, et al. Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol* 2002;109:455–62.
- [137] Fukushima A, Yoo YC, Yoshimatsu K, et al. Effect of MDP-Lys(L18) as a mucosal immuno-

- adjuvant on protection of mucosal infections by Sendai virus and rotavirus. *Vaccine* 1996;14:485–91.
- [138] Boussiotis VA, Tsai EY, Yunis EJ, et al. IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. *J Clin Invest* 2000;105:1317–25.
- [139] McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. *J Exp Med* 2002;195:221–31.
- [140] Bracci L, Canini I, Puzelli S, et al. Type I IFN is a powerful mucosal adjuvant for a selective intranasal vaccination against influenza virus in mice and affects antigen capture at mucosal level. *Vaccine* 2005;23:2994–3004.
- [141] Kutzler MA, Robinson TM, Chattergoon MA, et al. Communization with an optimized IL-15 plasmid results in enhanced function and longevity of CD8 T cells that are partially independent of CD4 T cell help. *J Immunol* 2005;175:112–23.
- [142] van Overvelt L, Moingeon P. T cell adjuvants and novel strategies for their identification. In: Schjins V, O'Hagan D, editors. *Immunopotentiators in Modern Vaccines*. Surrey (UK): Academic Press; 2005.
- [143] Durrer P, Gluck U, Spyr C, et al. Mucosal antibody response induced with a nasal virosome-based influenza vaccine. *Vaccine* 2003;21:4328–34.
- [144] Felnerova D, Viret JF, Gluck R, et al. Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. *Curr Opin Biotechnol* 2004;15:518–29.
- [145] de Jonge MI, Hamstra HJ, Jiskoot W, et al. Intranasal immunisation of mice with liposomes containing recombinant meningococcal OpaB and OpaJ proteins. *Vaccine* 2004;22:4021–8.
- [146] Hu KF, Lovgren-Bengtsson K, Morein B. Immunostimulating complexes (ISCOMs) for nasal vaccination. *Adv Drug Deliv Rev* 2001;51:149–59.
- [147] Waeckerle-Men Y, Groettrup M. PLGA microspheres for improved antigen delivery to dendritic cells as cellular vaccines. *Adv Drug Deliv Rev* 2005;57:475–82.
- [148] Ball JM, Hardy ME, Atmar RL, et al. Oral immunization with recombinant Norwalk virus-like particles induces a systemic and mucosal immune response in mice. *J Virol* 1998;72:1345–53.
- [149] Ball JM, Graham DY, Opekun AR, et al. Recombinant Norwalk virus-like particles given orally to volunteers: phase I study. *Gastroenterology* 1999;117:40–8.
- [150] Takamura S, Niikura M, Li TC, et al. DNA vaccine-encapsulated virus-like particles derived from an orally transmissible virus stimulate mucosal and systemic immune responses by oral administration. *Gene Ther* 2004;11:628–35.
- [151] Shi W, Liu J, Huang Y, et al. Papillomavirus pseudovirus: a novel vaccine to induce mucosal and systemic cytotoxic T-lymphocyte responses. *J Virol* 2001;75:10139–48.
- [152] Bonifaz LC, Bonnyay DP, Charalambous A, et al. In vivo targeting of antigens to maturing dendritic cells via the DEC-205 receptor improves T cell vaccination. *J Exp Med* 2004;199:815–24.