

Adjuvants and Vector Systems for Allergy Vaccines

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KEYWORDS

• Adjuvants • Allergy vaccine • Immunopotentialiation
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Allergen-specific immunotherapy is currently the only curative treatment of type I allergies, with a unique capacity to reorient inappropriate humoral and cellular immune responses from a Th2 to a mixed Th1/T regulatory cell (Treg) pattern.¹ Following the pioneer studies by Noon and colleagues^{2,3} a century ago, allergen-specific subcutaneous immunotherapy (SCIT) was established as an effective treatment of respiratory and venom allergies.^{4,5} Although SCIT has been performed with soluble allergens in the United States, in most circumstances preparations combining allergen extracts with adjuvants such as aluminum hydroxide or calcium phosphate are being used in Europe.^{6,7} This article reviews other adjuvants that are being investigated with the aim of reducing the number of injections needed for desensitization.

More recently, noninvasive mucosal routes of administration have been explored as an alternative to SCIT, most particularly sublingual immunotherapy (SLIT).⁸ SLIT was first investigated more than 15 years ago in patients with respiratory allergies.^{9–11} In this approach, the allergen extract is kept under the tongue for at least 1 to 2 minutes, to allow capture by oral dendritic cells before being swallowed. SLIT significantly reduces rhinoconjunctivitis symptoms as well as symptomatic medication uptake in patients allergic to grass, tree pollens, or house dust mites (HDM).^{9–11} SLIT has mostly been conducted with allergen extracts presented as either drops or, more recently, as solid tablets. In all circumstances, high doses (usually 50–100 times the doses used for SCIT) of the allergen(s) are needed to reach clinical efficacy because SLIT is performed in the absence of any immunopotentiator.⁹

Irrespective of the route of immunization used, allergy vaccines currently available are based on either natural or modified allergen extracts, but second-generation

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treatments relying on recombinant proteins are being developed.¹² Given that such highly purified proteins are usually poorly immunogenic, those vaccines require new antigen presentation platforms. In this context, novel adjuvants and vector systems are needed to further improve the efficacy of subcutaneous and sublingual allergy vaccines.^{13,14} Although an intrinsic adjuvant activity can be associated with the allergen itself (eg, Th2-inducing activity ascribed to Der p 1, natural adjuvants present in allergen extracts),^{15–17} this review only focuses on biologic or synthetic candidate adjuvants, as well as vector systems to be used in combination with the allergen(s).

MECHANISMS OF ALLERGEN-SPECIFIC IMMUNOTHERAPY: IMPLICATIONS FOR SELECTING ADJUVANTS AND VECTOR SYSTEMS

Both SCIT and SLIT are associated with a decrease in serum allergen-specific immunoglobulin E (IgE) concomitantly with an upregulation of immunoglobulin G (IgG), most particularly IgG1 and IgG4s,^{18–20} some of which may exhibit a potential blocking activity.^{21,22} Such changes in the antibody balance are believed to downregulate IgE-mediated histamine release by basophils and mast cells but also to decrease IgE-mediated antigen presentation to CD4⁺ T lymphocytes.^{18–20} In a limited number of studies, an increase in allergen-specific immunoglobulin A (IgA) antibodies has also been documented in serum as well as in mucosal tissues, at least for SLIT.^{23,24} Successful immunotherapy also inhibits the recruitment and activation of proinflammatory cells, such as mast cells, basophils, and eosinophils in the skin, as well as nasal or bronchial mucosae.^{25–27}

In the current model of allergen-specific immunotherapy, a prominent role is attributed to CD4⁺ T lymphocytes in controlling the various immune effector mechanisms contributing to allergic inflammation.^{18,27,28} Although allergic patients mount allergen-specific Th2 responses associated with the secretion of interleukin (IL)-4, IL-5, and IL-13 cytokines by CD4⁺ T cells, subcutaneous immunotherapy has been shown to redirect those allergen-specific T cell responses toward both (1) a Th1 type with an increased production of interferon- γ (IFN γ) within the blood and target organs (immunodeviation),^{26,29} and (2) CD4⁺ Tregs capable of downregulating Th2 responses through the production of IL-10 and/or transforming growth factor β (TGF β), or following cell-cell contact (immunosuppression).^{28,30,31}

Recent studies confirmed that SLIT also induces IFN γ -producing Th1 cells as well as IL-10 or TGF β -secreting Tregs.^{27,31–34} The observed bias of the oral immune system toward tolerance induction as opposed to proinflammatory responses is explained by the peculiar tolerogenic phenotype of oral dendritic cells (DCs) observed both in murine and humans.^{35–37} Specifically, 3 predominant subsets of oral DC have been described including (1) Langerhans cells found in the mucosa itself, (2) myeloid DCs along the lamina propria, and (3) plasmacytoid DCs located within subepithelial tissues.³⁷ All those DC subsets are prone to produce IL-10, IL-12, and possibly TGF β in the presence of the allergen, thus supporting mixed Th1/regulatory CD4⁺ T cell responses in draining cervical lymph nodes.^{35–37} Those DCs express high levels of Toll-like receptors (TLR) such as TLR2 and TLR4, allowing modulation of the function of those cells using specific ligands.^{35,37}

Based on our current understanding of immune mechanisms leading to tolerance in the course of immunotherapy, appropriate adjuvants and/or vector systems likely to enhance the efficacy of allergy vaccines include the following:

1. Synthetic or biologic adjuvants providing cosignals to immune cells, most particularly antigen-presenting cells (APCs). Such immunopotentiators should ideally reinforce allergen-specific Th1 and/or Treg responses (**Table 1**)

Table 1
Adjuvants for allergy vaccines

Immunomodulators	Comments	References
Mineral adjuvants	Aluminum hydroxide and calcium phosphate are commonly used as adjuvants in subcutaneous allergy vaccines. They work through both a depot effect (ie, slow release of the allergen, presentation as particles to target APCs) as well as an interaction with the innate immune system (eg, activation of the inflammasome)	7,13,38
Probiotics	In mice, mucosal (ie, intranasal or sublingual) administration of commensal bacteria such as <i>Lactococcus lactis</i> , <i>Lactobacillus plantarum</i> or <i>Bifidobacterium bifidum</i> together with the allergen(s) induces Th1 and/or Tregs as well as asthma improvement. Efficacious probiotics seem to interact with DCSign3 and may exhibit specific forms of teichoic acids in their wall composition, thus inducing a strong IL-10 production by immune cells. In humans, selected probiotics used as a stand-alone therapy were shown to protect children against eczema, in link with the induction of Th1 responses	39–44
Attenuated mycobacteria	In mice, heat-killed <i>Mycobacterium vaccae</i> induces Treg cells secreting IL-10 and TGF β , conferring protection against airway inflammation. Some efficacy has also been observed in children with atopic dermatitis or asthma after intradermal administration of <i>M vaccae</i> or BCG. No synergy has been observed in humans between BCG and SLIT when using distinct administration sites	45–51
Bacterial toxins (CT, LT, CTB, LTB)	Genetically detoxified CT and LT (or B subunits without ADP-ribosyl transferase activity) retain a strong capacity to induce serum and mucosal IgA responses. The antigen/allergen can be mixed, fused, or chemically conjugated with the toxin moiety. In mice, sublingual administration of the allergen conjugated to CTB enhances tolerance induction	52,53
TLR ligands	Ligands for TLR2 (including lipopeptides, Pam3csk4), TLR4 (MPL, RC 529, OM-294-BA-MP), TLR7 (imidazoquinolines), TLR9 (CpGs) have shown some efficacy in murine models (decrease of both airway inflammation and Th2 responses, with induction of Th1 and/or Treg responses). Synthetic oligonucleotides containing CpG motifs (CpG ODN) are potent Th1 adjuvants via both the systemic and mucosal routes. Intradermal immunization with Amb a 1 fused to CpG oligonucleotides prevents allergen-induced hyperresponsiveness in mice. In humans, grass pollen allergens combined with MPL induce a strong production of specific IgG1 and IgG4 antibodies through the subcutaneous route. MPL is sometimes associated with tyrosine-absorbed glutaraldehyde modified allergen extracts. Following SLIT in grass pollen allergic patients, MPL enhanced specific IgG responses and decreased reactivity to nasal allergen challenge. A conjugate Amb a1-CpG vaccine has been tested in ragweed allergic humans through the subcutaneous route, with some level of clinical efficacy. The effect of CpG is believed to be associated with the induction of stronger Th1 responses, and possibly CD25 ⁺ Treg cells	54–63 64–68
Small synthetic molecules	In murine models, dihydroxyvitamin D ₃ plus glucocorticoids, calcineurin inhibitors (cyclosporin A, FK 506), rapamycin, aspirin, and mycophenolate mofetil all enhance IL-10 production by CD4 ⁺ T cells. Dexamethasone plus dihydroxyvitamin D ₃ enhance SLIT efficacy in a murine asthma model when combined with the allergen. No synergy was observed between fluticasone and SLIT in humans when using distinct administration routes	69–77

Abbreviations: BCG, bacille Calmette-Guérin; CT, cholera toxin; CTB, cholera toxin B subunit; LT, lymphotoxin; LTB, lymphotoxin subunit B; MPL, monophosphoryl lipid A; SLIT, sublingual immunotherapy.

2. Vector systems targeting the allergen to tolerogenic APCs. Specifically, mucoadhesive particulate vector systems seem well adapted to the sublingual (and other mucosal) routes where they enhance the duration of contact between the allergen(s) and the mucosa. Furthermore, presenting the allergen as a particle significantly improves the uptake by dendritic cells and macrophages with phagocytic activity (**Table 2**).

POTENTIAL ADJUVANTS AND VECTOR SYSTEMS FOR ALLERGY VACCINES

Adjuvants/Vector Systems for the Subcutaneous (and Other Parenteral) Routes

Preclinical studies

Mineral adjuvant molecules (eg, calcium phosphate or aluminum hydroxide) are well established as adjuvants for subcutaneous allergic vaccines (see **Table 1**).^{7,13} Although aluminum salts activate the inflammasome and, as such, are broadly used by the vaccine industry to elicit proinflammatory responses, it is also clear that alum decreases established Th2 responses.⁷ In addition to these mineral adjuvants, numerous candidate immunopotentiators have been shown in murine models to downregulate Th2 responses via parenteral routes (see **Table 1**). For example, in murine models of asthma, living or heat-killed bacteria (eg, *Lactobacillus plantarum*, *Lactococcus lactis*, *Mycobacterium vaccae*) as well as TLR ligands (eg, monophosphoryl lipid A [MPL], imidazoquinolines, CpGs) showed clear benefits when used as adjuvants.^{45,54–57} The active 1,25-dihydroxy vitamin D₃ metabolite increased the effect of immunotherapy in a mouse asthma model, following induction of IL-10 and TGFβ by immune cells.⁶⁹

Various vector systems have also been considered to target allergens to dendritic cells (see **Table 2**). For example, plasmid DNA or DNA absorbed on microparticles markedly enhance systemic and mucosal immune responses following injection.⁸² In addition, fusion proteins combining the Fel d 1 allergen and the Fcγ immunoglobulin fragment have been designed. Those bifunctional molecules coengage FcεRI and FcγRII receptors, thus preventing acute reactivity to Fel d 1.⁹³ A recombinant fusion protein associating Bet v 1 to the bacterial S layer protein SbpA was also shown to form particles and, as a consequence, to facilitate allergen capture by APCs with subsequent induction of Th1 and Tregs.⁹⁴

Human studies

Some new adjuvants are also being explored in humans to potentiate antigen-specific Th1 or Treg responses during SCIT (see **Table 1**). For example, the Th1 adjuvant MPL has been tested via the subcutaneous route in association with a grass pollen vaccine, with evidence for enhanced specific IgG1 and IgG4 responses leading to a potential reduction in number of preseasonal injections needed to reduce allergic symptoms.^{64,65} Also, a synthetic CpG oligonucleotide (known to represent a powerful Th1 adjuvant) conjugated with purified natural Amb a 1 was shown to improve symptoms in patients with ragweed pollen allergy.⁶⁶ Administration of oral steroids (prednisone) with or without vitamin D₃ had no beneficial effect on SCIT efficacy in asthmatic children allergic to HDM.⁷⁰ In this study, a potential immunosuppressive effect of prednisone was corrected by vitamin D₃.

For vectors tested in humans via parental routes, nonreplicating viruslike particles (VLPs) generated by the spontaneous assembly of viral capsid proteins also represent an attractive alternative to express either the whole antigen/allergen, or adjuvants such as CpG oligonucleotides.^{85–88} Specifically, a recombinant fusion protein associating a peptide derived from Phl p 1 with a rhinovirus-derived VP1 protein has been designed as a candidate vaccine for grass pollen allergies.⁸⁵ Association of

Table 2
Vector systems for allergy vaccines

Vectors/Delivery Systems	Comments	References
Liposomes, virosomes ISCOMs PLGA	Lipid-based vehicles such as liposomes can incorporate functional viral envelope proteins (leading to virosomes). Mucosal administration of such particulate antigen formulations increases specific IgG and IgA antibodies. Nasal administration of plasmid DNA complexes formulated with cationic lipids induces specific secretory IgA antibodies in the mucosa as well as serum IgG and IgA antibodies. Intranasal immunization with the antigen formulated in liposomes together with immunopotentiators (eg, LPS, MPL, or LTb) induces strong systemic and mucosal responses (with IgG and IgA in the lung and the nose). These spherical particles (30–100 nm diameter) comprise the saponin-adjuvant Quil A, cholesterol, and phospholipids mixed with the antigen. ISCOMs induce a strong systemic and mucosal Th1 adjuvant activity. ISCOM particles target both the endosomal and cytosolic compartments, thus leading to class I and class II restricted antigen presentation to T cells. Poly (D,L) lactic-co-glycolic acid can be used to form nanoparticles or microparticles to present the antigen/allergen, thus facilitating APC capture. Subcutaneous administration in mice of PLGA-Bet v 1 induced IgG2, Th1 and Treg responses while preventing hypersensitivity to Bet v 1	77–79 80,81 82–84
VLPs	VLPs are formed following the spontaneous assembly of capsid proteins. VLPs stimulate both humoral and cellular immune responses, leading to systemic and mucosal responses in mice and in humans. Potential VLP delivery systems include the porcine parvovirus, Norwalk virus as well as human papilloma viruses, which specifically target immature DCs. VLPs made from the Q β phage proteins have been successfully tested in humans with mite allergies in association with the Der p 1 allergen or a CpG adjuvant	78,85–89
Carbohydrate-particles	Particles made of synthetic polymers represent a powerful means to address the antigen/allergen onto phagocytic APCs. Nanoparticles or microparticles made of maltodextrin or chitosan polymers have been shown in murine models to improve targeting of the allergen onto oral DCs and enhance tolerance induction following sublingual administration	14,23,90–92
DC targeting vectors Plasmid DNA	In mice, anti-DEC-205 antibodies coupled to ovalbumin target the antigen to DCs and induce strong specific T cell responses. Antigens coupled to kpOmpA target CD11⁺c mucosal (eg, nasal) DCs through Toll-like receptor 2. CyaA from <i>Bordetella pertussis</i> binds specifically to CD11b and as such has been used to target the allergen to oral DCs, thus enhancing tolerance induction via the sublingual route in a murine asthma model. STxB also targets DCs, leading to strong specific Th1 and humoral responses. Fusion proteins assembling allergens with Ig FcRs or with S layer bacterial product also enhance capture by human dendritic cells. Plasmid DNA has been used in preclinical models, both via parenteral and mucosal routes, alone or adsorbed on microparticles	93–99 82,100

Abbreviations: CyaA, adenylate cyclase; DC, dendritic cell; FcR, Fc fragment; ISCOMs, immunostimulating complexes; kpOmpA, outer membrane protein A from *Klebsiella pneumoniae*; LPS, lipopolysaccharide; PLGA, D,L-lactic-co-glycolic acid; STxB, shiga toxin B subunit; VLP, viruslike particle.

Fel d 1 to VLPs prevents allergenicity and enhances its capacity to elicit protective IgGs.⁸⁶ Recently, VLPs made from capsid proteins obtained from the Q β phage coupled to a Der p 1–derived peptide were shown to be highly immunogenic in healthy volunteers,⁸⁷ with the induction of strong IgG responses after a single injection. When tested in mite allergic patients, Q β G10 VLPs containing a synthetic oligonucleotide as a Th1 adjuvant significantly decreased rhinoconjunctivitis and asthma symptoms after 6 weekly injections.⁸⁸ Fusion of the Fel d 1 allergen to a modular antigen transporter (MAT) facilitates capture, processing, and efficient presentation of the allergen by APCs in association with major histocompatibility complex (MHC) class II molecules.⁷⁸ This vaccine is currently considered for clinical evaluation in humans. Other potential particulate allergen formulations that could be tested in humans include liposomes, virosomes, D,L-lactic-co-glycolic acid (PLGA), or immunostimulating complexes (ISCOMS).^{80,83}

Adjuvants/Vector Systems for the Sublingual (and Other Mucosal) Route(s)

Preclinical studies

Mucosal adjuvants tested in murine models to modulate T cell polarization include bacterial toxins, such as cholera toxin (CT) and the closely related *Escherichia coli* heat-labile enterotoxin (LT), as well as their nontoxic derivatives (ie, genetically detoxified forms or B subunits without ADP-ribosyl transferase activity) (see **Table 1**). These bacterial toxins can be coadministered as a mix, conjugated or fused with soluble antigens/allergens.^{52,53} Triggering TLR2 or TLR4 with either Pam3CSK4 or biologic/synthetic lipid A analogues (eg, MPL and OM-294-BA-MP), respectively, has proved effective in inducing Th1 and Treg responses via the nasal or sublingual routes.^{58–61} Similarly, synthetic drugs (eg, glucocorticoids plus 1,25-dihydroxy vitamin D₃) also represent potential Treg adjuvants for sublingual immunotherapy because they are powerful inducers of IL-10 production by immune cells, such as dendritic cells and CD4⁺ T lymphocytes.⁷¹ Both glucocorticoids and vitamin D₃ inhibit the maturation of monocyte-derived DC, as shown by an impaired upregulation of costimulatory and MHC molecules and a diminished capacity to stimulate allogeneic T cells.^{71–75} Other potential adjuvants for the sublingual (and other mucosal) routes include probiotics selected for their capacity to induce a strong IL-10 and IL-12 production by mucosal DCs. In this regard, a combination of the allergen with either *L plantarum* or *Bifidobacterium bifidum* strains enhances both allergen-specific Th1 and Treg responses as well as tolerance induction via the sublingual route.^{39,71} In murine asthma models, pure Th1 adjuvants do not promote significant tolerance induction during SLIT.^{39,71}

Vector systems considered for mucosal routes should protect the allergen(s) from degradation by local proteases while targeting mucosal APCs (see **Table 2**). To this end, bacterial subunits such as the outer membrane protein A from *Klebsiella pneumoniae* (OmpA) or the shiga toxin B subunit have been conjugated to antigens/allergens to target APCs (see **Table 2**).^{95–97} Among those, the adenylate cyclase protein from *Bordetella pertussis* conjugated to ovalbumin (OVA) has been shown to enhance tolerance induction during SLIT in mice with OVA-induced asthma, as a consequence of a specific addressing of the allergen to oral CD11b⁺ tolerogenic myeloid DCs.^{37,98} In addition, positively charged carbohydrate polymers have been used to generate mucoadhesive particles capable of binding efficiently to negatively charged epithelial cells, thus enhancing the duration of allergen contact with the mucosa.²³ The advantage of particulate formulations such as nanoparticles made from polymerized maltodextrin or chitosan-based microparticles is to enhance in vitro and in vivo uptake

of the allergen(s) by dendritic cells, thus resulting in superior tolerance induction via the sublingual route when administered to asthmatic mice (see **Table 2**).^{23,90}

Human studies

In recent years, the use of selected lactic acid bacterial strains in the prevention of allergy has also been suggested in humans (see **Table 1**). For example, oral administration of lactic acid bacteria led to reduced atopic dermatitis in children with a positive family history of type I allergy,⁴⁰ suggesting that such probiotics could also be used as adjuvants for mucosal vaccines. However, to date, no probiotics have been administered jointly with the allergen in allergic patients. The Th1 adjuvant MPL has been tested in grass pollen allergic patients through the sublingual route.⁶⁷ When used at a high dose, MPL enhanced specific IgG responses and reduced reactivity to a subsequent nasal allergen challenge (see **Table 1**). Bacille Calmette-Guérin (BCG) has also been tested in children asthmatic to mite allergens in association with SLIT, with no effect on clinical outcome.⁴⁶ One drawback in the latter study is that the adjuvant was administered via the intradermal route, whereas allergens were given sublingually. No vector systems have been tested to improve allergen delivery via the sublingual route in humans, but maltodextrin-based mucoadhesive nanoparticles have been shown to elicit strong mucosal IgA responses when administered intranasally as part of an experimental flu vaccine (see **Table 2**).

SUMMARY

A better understanding of immune mechanisms involved in the induction and maintenance of antigen-specific immune tolerance provides opportunities to design and develop appropriate adjuvants and vector systems leading to improved allergy vaccines. A guiding hypothesis in this regard is that allergy vaccines should induce both allergen-specific Th1 as well as regulatory CD4⁺ T lymphocytes able to produce suppressive cytokines (eg, IL-10 and/or TGF β). Identifying appropriate allergen presentation platforms could help increase the clinical efficacy of allergy vaccines and simplify immunization schemes. In addition, an expected benefit of adjuvants and vector systems modulating/targeting appropriate immune cells is to help lower the allergen dose, thus improving the safety profile. Ample evidence is now available confirming that multiple adjuvants are efficacious in murine allergy models, via both systemic and mucosal routes. Some of those adjuvants (eg, MPL, CpGs) have been shown to enhance Th1 responses in humans, but their effect on clinical efficacy remains to be firmly established. With respect to vector systems, viruslike particles represent a promising platform to present allergens and/or adjuvants to the immune system, with encouraging results obtained during SCIT of mite allergic patients. Mucoadhesive particulate vectors seem most particularly suitable to develop a new generation of safer and more efficient allergy vaccines to be administered via mucosal (ie, sublingual) routes.

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