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Adjuvants for allergy vaccines

Philippe Moingeon

Research and Development; Stallergenes; Antony, France

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Allergen-specific immunotherapy is currently performed via either the subcutaneous or sublingual routes as a treatment for type I (IgE dependent) allergies. Aluminum hydroxide or calcium phosphate are broadly used as adjuvants for subcutaneous allergy vaccines, whereas commercial sublingual vaccines rely upon high doses of aqueous allergen extracts in the absence of any immunopotentiator. Adjuvants to be included in the future in products for allergen specific immunotherapy should ideally enhance Th1 and CD4+ regulatory T cell responses. Imunomodulators impacting dendritic or T cell functions to induce IL10, IL12 and IFNγ production are being investigated in preclinical allergy models. Such candidate adjuvants encompass synthetic or biological immunopotentiators such as glucocorticoids, 1,25-dihydroxy vitamin D3, selected probiotic strains (e.g., *Lactobacillus* and *Bifidobacterium* species) as well as TLR2 (Pam3CSK4), TLR4 (monophosphoryl lipid A, synthetic lipid A analogs) or TLR9 (CpGs) ligands. Furthermore, the use of vector systems such as mucoadhesive particules, virus-like particles or liposomes are being considered to enhance allergen uptake by tolerogenic antigen presenting cells present in mucosal tissues.

Introduction

Current trends in allergen specific immunotherapy. Following the pioneer studies by Noon, Freeman et al.[1,2] more than a century ago, allergen-specific immunotherapy (AIT) has been progressively established as a reference treatment for type I allergies, most particularly allergic rhinoconjunctivitis with or without moderate asthma induced by common respiratory allergens.[3] In contrast to symptomatic treatments, AIT can be curative, due to its capacity to reorient inappropriate allergen-specific humoral and cellular immune responses from a Th2 to a mixed Th1/T reg pattern.[4] Allergen-specific immunotherapy is commonly performed via the subcutaneous route as a treatment for allergies to grass and tree pollens, house dust mites, insect venoms, molds, dog and cat dander or even synthetic drugs.[3,5] Subcutaneous immunotherapy (SCIT) is performed with soluble allergens in North America, whereas allergen extracts are rather adsorbed on aluminum hydroxide or calcium phosphate as adjuvants in Europe.[3,5,6] As will be reviewed below, numerous other candidate adjuvants are being evaluated in preclinical models or in humans, with the aim to reduce the number of injections needed for subcutaneous desensitization.

In the past 20 years, other routes of allergen administration (e.g., intranasal, oral, sublingual, intralymphatic, epicutaneous) have been and are being explored as an alternative to SCIT.[8] Most particularly, among non invasive routes, sublingual immunotherapy (SLIT) is now considered as a safe and efficacious alternative to SCIT for respiratory allergies associated with either grass, tree pollens or house dust mites.[9–11] Commercial sublingual vaccines are based on aqueous allergen extracts presented either as drops or more recently as fast disolving tablets or lyocs. During SLIT, high doses (usually 50- to 100-fold the doses used for SCIT) of the allergen extract are kept under the tongue for 1 to 2 min prior to being swallowed, as per the so called sublingual-swallow procedure. As of today, none of the commercially available sublingual vaccines contain any adjuvant.[9]

Irrespective of the route of immunization used, current allergy vaccines rely upon either natural or modified allergen extracts. The latter can for example be obtained following treatment with glutaraldehyde to form polymers with altered structure, thus precluding IgE recognition, as is the case for allergoids.[3] New allergen presentation platforms are needed to improve the efficacy of such existing subcutaneous and sublingual allergy vaccines.[12, 13]

In addition, in the last few years, second generation treatments based on recombinant allergens (in a natural or hypoallergenic conformation), fusions proteins, mix of peptides, plasmid DNA have raised considerable interest, even if none of those molecular allergens have reached commercialization.[14–16] However, in contrast to natural extracts which often possess an intrinsic adjuvant activity (e.g., Th2-inducing activity ascribed to the Der p 1 mite allergen, endotoxin, phytoprostanes),[17,18] purified allergens provided under such a well defined molecular form are usually poorly immunogenic. Thus, such second generation vaccines will also require novel immunopotentiators and vector systems (collectively referred to as adjuvants in the present review).

Like for any vaccines, adjuvants to be associated with allergens are expected to allow simplifying immunization regimens, and reaching efficacy faster and for a longer duration. Although allergy vaccines are usually well tolerated, an additional expected benefit of adjuvants in this field is to help lowering the allergen dose, thus improving the safety profile with less local reactions to the site of administration. Importantly, allergy vaccines are therapeutic vaccines, used in patients prone to mounting Th2 responses. Thus, appropriate adjuvants should rather orient the immune response toward Th1 or regulatory mechanisms both known to downregulate Th2 cells.[12]

Correspondence to: Philippe Moingeon;
Email: pmoingeon@stallergenes.com
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Immune mechanisms associated with allergen-specific immunotherapy. Our current understanding of immune mechanisms involved in allergen-specific immunotherapy emphasizes a prominent role for $CD4^+$ T lymphocytes in controlling all effector immune mechanisms linked with allergic inflammation.[4,19,20] Specifically, most allergic patients exhibit allergen-specific Th2 responses associated with the secretion of IL-4, IL-5 and IL-13 cytokines by $CD4^+$ T cells. During subcutaneous or sublingual immunotherapy, such allergen-specific $CD4^+$ T cell responses are rather redirected toward both a Th1 type with an increased production of IFNγ (immunodeviation) as well as IL10-producing $CD4^+$ regulatory T cells (immunosuppression).[4,19,20] Such changes in the polarization of T cell responses have been documented both in peripheral blood and in respiratory mucosae[21,22]

As a consequence of such a change in the cytokine milieu, both SCIT and SLIT are associated with a decrease in seric allergen-specific IgEs, concomitantly with an upregulation of IgG1, IgG4, IgA antibodies,[4,20,23] some of which are thought to mediate a "blocking" anti-inflammatory activity.[24] Successful immunotherapy also leads to a substantial decrease in the recruitment and activation of proinflammatory cells, including basophils, mast cells and eosinophils in the skin, as well as nasal or bronchial mucosae.[25]

Based upon the above afore mentioned immune mechanisms thought to be critical for tolerance induction during immunotherapy, adjuvants expected to enhance the efficacy of allergy vaccines include:

(1) Synthetic or biological immunopotentiators capable to reinforce allergen-specific Th1 and/or regulatory T cell responses (**Table 1**). These molecules could act directly on $CD4^+$ T cells, but also on dendritic or even epithelial cells.

(2) Vector systems facilitating allergen uptake by tolerogenic APCs such as the dendritic cells found in oral tissues[26,27] (**Table 2**).

Adjuvants for Allergy Vaccines Administered via the Subcutaneous (and Other Parenteral) Route(s)

Immunopotentiators. Mineral adjuvant molecules such as calcium phosphate or aluminum hydroxide are broadly used in humans as adjuvants for subcutaneous allergic vaccines[7, 12] (**Table 1**). Aluminum salts are commonly included in vaccines against infectious pathogens with the aim to elicit proinflammatory responses following activation of the inflammasome. In addition however, those compounds also decrease established Th2 responses.[7] Beyond such mineral adjuvants, various immunopotentiators were shown in murine models to downregulate Th2 responses when administered via parenteral routes (**Table 1**). For example, TLR ligands (e.g., monophosphoryl lipid A or MPL, imidazoquinolines, CpGs), living or heat-killed bacteria (e.g., *Lactobacillus plantarum, Lactococcus lactis, Mycobacterium vaccae*), small molecules such as the active 1.25-dihydroxy Vitamin D3 metabolite all contribute in decreasing airway inflammation in murine models of asthma, when used as adjuvants.[28–33]

Several of those immunopotentiators impacting Th1 or Treg responses have been investigated in humans during SCIT. For example, the Th1 adjuvant MPL injected together with a grass pollen extract enhanced allergen-specific IgG1 and IgG4 responses, raising the possibility to reduce the number of preseasonal injections to control allergic symptoms.[34,35] Also, a synthetic CpG oligonucleotide acting as a Th1 adjuvant was chemically conjugated with the purified Amb a 1 allergen from short ragweed. This vaccine was shown to improve rhinoconjunctivitis symptoms in patients with allergy to ragweed pollen.[36] The parallel use of oral steroids (prednisone) with or without Vitamin D3 (both of which were reported as potential inducers of regulatory T cells) during SCIT did not improve efficacy in asthmatic children allergic to house dust mites.[37]

Vector systems. Various vector systems have also been used successfully to formulate allergens administered via parenteral routes in animal models (**Table 2**). The latter include liposomes, virosomes, D, L-lactic-co-glycolic acid (PLGA) or immunostimulating complexes (ISCOMS).[38,39] Also, plasmid DNA or DNA absorbed on microparticules were shown to enhance systemic and mucosal immune responses.[40] Fusion proteins associating the Fel d 1 cat allergen and the Fcγ immunoglobulin fragment were also designed to co-crosslink FcεRI and FcγRII receptors, thus downregulating immunoreactivity to Fel d 1.[41] Another fusion protein combining the birch pollen Bet v 1 allergen to the bacterial S layer protein SbpA was shown to form particules facilitating allergen uptake by dendritic cells, with subsequent induction of Th1 and regulatory T cells.[42] Fusing the allergen with the TLR5 ligand flagellin was also shown to reduce Th2 responses.[43]

As of today, only a limited number of vector systems have been evaluated in humans via parental routes. For example, non-replicating virus-like particles (VLPs) obtained following assembly of capsid proteins from the Qβ phage were coupled to peptides derived from the Der p 1 mite allergen. Those recombinant Der p 1 VLPs induce strong IgG responses after a single injection in healthy volonteers.[44] Conjugation of Fel d 1 to such VLPs was shown to prevent IgE reactivity while enhancing IgG induction.[45] QβG10 VLPs containing a synthetic oligonucleotide as a Th1 adjuvant were also tested in mite allergic patients, leading in a significant reduction of rhinoconjunctivitis and asthma symptoms after six weekly injections.[46] More recently, a recombinant fusion protein associating a peptide derived from the Phl p 1 grass pollen allergen with a rhinovirus-derived VP1 protein was also proposed as a candidate vaccine for grass pollen allergies.[47] Another fusion protein associating the Fel d 1 allergen to a modular antigen transporter (MAT) was designed to facilitate capture, processing and efficient presentation of the allergen by APCs in association with MHC class II molecules.[48] When administered in humans, this vaccine elicited strong IgG4 responses to the allergen.

Adjuvants for Allergy Vaccines Administered via the Sublingual (and Other Mucosal) Route(s)

Immunopotentiators. Various mucosal immunopotentiators have been tested in preclinical tolerance models. The latter include bacterial toxins, such as cholera toxin (CT) or *E. coli* heat-labile enterotoxin (LT), (as well as genetically detoxified forms or B-subunits without any ADP-ribosyl transferase

Table 1. Immunopotentiators for allergy vaccines

Immunopotentiators	Status	Route	Allergen	Comments
Mineral adjuvants	Clinical	Subcutaneous	Various commercial vaccines	Aluminum hydroxide and calcium phosphate are commonly used for subcutaneous allergy vaccines in Europe. Mechanisms involved include both a depot effect (ie slow release of the allergen, formulation of the allergen as particles to target APCs) as well as interaction with the innate immune system (e.g., activation of the inflammasome).
Probiotics	Clinical (stand alone) or preclinical (adjuvant)	Mucosal	OVA, Bet v 1	Mucosal (ie intranasal or sublingual) administration of commensal bacteria such as *Lactococcus lactis, Lactobacillus plantarum or Bifidobacterium bifidum* in various murine models together with the allergen(s) induces Th1 and/or regulatory T cells as well as asthma improvement. Tolerogenic IL10 inducing probiotics interact with DCSign3 and exhibit specific forms of teichoid acids in their wall composition. Selected probiotics used as a stand alone therapy protect children against eczema, following the induction of Th1 responses. or asthma after intradermal administration.
Attenuated *Mycobacteria,* bacterial products	Clinical (stand alone) or preclinical (adjuvant)	Systemic / mucosal	Mite, grass pollen	In mice, heat-killed *Mycobacterium vaccae* induces Treg cells secreting IL-10 and TGFβ, and decreases airway inflammation. Genetically detoxified cholera toxin (CT) and lymphotoxin (LT) (or B subunits without ADP ribosyl transferase activity) induce strong seric and mucosal IgAs responses. The allergen can be mixed, fused, or chemically conjuged with the toxin moiety. Sublingual administration of the allergen conjugated to CTB enhances tolerance induction in murine models. *M vaccae* and BCG exhibit some clinical efficacy in children with atopic dermatitis or asthma after intradermal administration.
TLR ligands	Clinical (for CpGs and MPL), preclinical (others)	Subcutaneous, intradermal / sublingual	OVA, Amb a 1, grass pollen	Ligands for TLR2 (including lipopeptides, Pam3Csk4), TLR4 (MPL, RC 529, OM294-BA-MP), TLR7 (imidazoquinolines), TLR9 (CpGs) have some efficacy in murine asthma models (decrease of both airway inflammation and Th2 responses, with induction of Th1 and/or T Reg responses). Intradermal immunization with Amb a 1 fused to CpG oligonucleotides prevents allergen-induced hyperresponsiveness in mice. A conjugate Amb a 1-CpG vaccine has been tested in ragweed allergic humans through the subcutaneous route, with some level of clinical efficacy, and induction of Th1 responses and $CD25^+$ T Reg cells. In humans, the TLR4 ligand monophosphoryl lipid A (MPL) with or without tyrosine-absorbed grass pollen allergens induces a strong production of IgG1 and IgG4 antibodies through the subcutaneous route. Following SLIT in grass pollen allergic patients, MPL enhanced specific IgG responses and decreased reactivity to nasal allergen challenge.
Small synthetic molecules	Clinical (fluticasone), preclinical (others)	Systemic, sublingual	OVA, grass pollen	Dihydroxyvitamin D3 plus glucocorticoids, calcineurin inhibitors (cyclosporin A, FK 506), rapamycin, aspirin and mycophenolate mofetil enhance IL10 production by $CD4^+$ T cells. Dexamethasone plus dihydroxy vit D3 enhance SLIT efficacy in a murine asthma model. No synergy between fluticasone and SLIT was observed in humans when using distinct administration routes.

Table 2. Vector systems for allergy vaccines

Vectors/delivery systems	Status	Route	Allergen	Comments
DC targeting vectors	Preclinical, except Fel d 1 MAT (clinical)	Systemic, mucosal	OVA, Fel d 1, Bet v 1	Outer membrane protein A from Klebsiella *pneumoniae* targeting CD11^{+}c mucosal DCs through Toll-like receptor 2. Adenylate cyclase (CyaA) from *Bordetella pertussis* interacting with CD11b^{+} DCs. Anti-DEC-205 antibodies and the B subunit of shiga toxin (STxB) also target 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. A Fel d 1-MAT fusion protein elicited IgG4 in humans and protection against nasal allergen challenge.
Plasmid DNA	Preclinical	Systemic and mucosal	Fel d 1, peanut	Plasmid DNA encoding allergens has shown efficacy in various preclinical models, both via parenteral and mucosal routes, alone or adsorbed on microparticles in various murine allergy models.
Liposomes, virosomes	Clinical	Intradermal, subcutaneous	Grass pollen, mite	Lipid-based vehicles, with or without viral envelope proteins (virosomes). Such particulate antigen formulations increase the induction of specific IgG and IgA antibodies. In humans, such liposomes encapsulating mite or grass pollen allergens are well tolerated via the cutaneous route, but induce delayed local reactions subcutaneously.
Virus-like particules	Clinical	Subcutaneous	Der p 1, Fel d 1 Phl p 1	Formed by the spontaneous assembly of capsid proteins. Elicit strong systemic and mucosal responses in mice and in humans. VLPs can be based on capsid proteins from the porcine parvovirus, Norwalk virus, human papilloma viruses. VLPs based on Qβ phage protein alone, or associated with Der p 1 peptides have induced protection against nasal allergen challenge. A recombinant fusion protein combining a peptide from the Phl p 1 grass pollen allergen with the rhinovirus VP1 proteins is being tested in humans.
Carbohydrate-particles	Preclinical	Intranasal, sublingual	Mite, OVA, Bet v 1	Nano or microparticles made of maltdextrin or chitosan polymers improve in murine models the uptake of the allergen by oral DCs, thereby enhancing tolerance induction during intranasal or sublingual immunotherapy.
Other particles (ISCOMs, PLGA)	Preclinical	Subcutaneaous	OVA, PLA2	Spherical particles (30–100 nm diameter) comprising the saponin-adjuvant Quil A, cholesterol, and phospholipids. ISCOMs induce a strong systemic and mucosal Th1 adjuvant activity. Poly (D, L) lactic-co-glycolic acid forming nano or microparticles, facilitating APC capture. Subcutaneous administration of PLGA formulated allergens (or encoding DNA) induces strong IgG2, Th1 and TReg responses in mice.

activity)[49,50] (**Table 1**). These toxins were either co-administered, conjugated or fused with allergens.[49, 50] TLR2 (e.g., Pam3CSK4) or TLR4 ligands (e.g., MPL and OM-294-BA-MP) were shown to strengthen Th1 and T Reg responses when used as adjuvants via the nasal or sublingual routes.[51–54] Similarly, dexamethasone associated with 1.25-dihydroxy vitamin D3 enhanced the efficacy of SLIT in a murine asthma model following induction of interleukin 10 production by immune cells, including dendritic cells and CD4^{+} T lymphocytes.[55] Another emerging class of potential adjuvants for the sublingual (and other mucosal) routes include probiotics, even if immunomodulatory properties vary considerably depending upon bacterial strains and growth conditions.[56, 57] Bacteria selected specifically for their capacity to induce a strong production of IL10 and IL12 by mucosal DCs, such as *Lactobacillus plantarum* or *Bifidobacterium bifidum*, were shown to enhance tolerance following SLIT in mice with induced asthma to OVA, at least in part by strengthening allergen-specific Th1 and T Reg responses.[58, 59] Interestingly, in preclinical models of SLIT, none of the pure Th1 adjuvants (ie without any capacity to elicit IL10 production) had any significant impact on tolerance induction.[12]

Two categories of immunopotentiators have been tested with the aim to increase SLIT efficacy in allergic human patients. High doses of the Th1 adjuvant MPL boosted allergen-specific IgG responses and reduced reactivity to a subsequent nasal allergen challenge in grass pollen allergic patients (**Table 1**).[60] BCG administered intradermally to children asthmatic to mite allergens in parallel with SLIT had no impact on clinical outcome,[61] possibly because the adjuvant and allergens were administered via distinct routes. In addition, various lactic acid bacterial strains are being considered to prevent or treat allergy in humans.[57] For example, oral administration of lactic acid bacteria reduces atopic dermatitis in children with a positive family history of type I allergy.[62] Whereas such an observation suggests that selected probiotics could be used as adjuvants for mucosal vaccines, as of today probiotics have not yet been tested in combination with allergens in allergic patients.

Vector systems. The dual interest of vector systems for mucosal vaccination is (1) to enhance the duration of contact of the allergen with the mucosa, for example by using positively charged mucoadhesive polymers binding to epithelial cells, and further (2) to target efficiently phagocytic APCs by presenting the allergen in a particulate form or by interacting with a specific surface receptor expressed by dendritic cells[12, 63] (**Table 2**). In this regard, both nanoparticles made from polymerized maltodextrin or chitosan-based microparticles were shown to enhance the uptake of allergens by oral dendritic cells, following sublingual administration to asthmatic mice, thus resulting into a superior priming of allergen-specific Tr1 cells in draining cervical lymph nodes, and consequently in a stronger decrease in airway inflammation[23,64] (**Table 2**). Still in murine models, conjugates with the outer membrane protein A from *Klebsiella pneumoniae* (OmpA) as well as the shiga toxin B subunit have been successfully used to target efficiently antigens/allergens to APCs[65–67] (**Table 2**). Similarly, the adenylate cyclase protein from *Bordetella pertussis* fused to OVA enhanced tolerance induction via the sublingual route in mice with OVA-induced asthma, following capture by $CD11b^+$ tolerogenic myeloid DCs and macrophages.[68,69]

As of today, none of those vector systems have been tested in humans in the context of mucosal allergy vaccines, although they raised the interest of the vaccine industry.[70–72] Noteworthy, maltodextrin-based mucoadhesive nanoparticles have been used to formulate an experimental flu vaccine, with evidence for the induction of strong mucosal IgA responses when administered intranasally to healthy volonteers.[12]

Conclusion

New adjuvants and vector systems are needed to improve the efficacy of allergy vaccines, reduce the dose of allergens required and simplify immunization schemes during desensitization. Our current understanding of immune mechanisms supporting the induction and maintenance of antigen-specific immune tolerance suggests an interest of adjuvants eliciting combined Th1 and regulatory $CD4^+$ T cell responses. A variety of candidate adjuvants (ie TLR ligands, bacterial toxins, probiotics) are efficacious in reducing allergic inflammation in murine models, when used in combination with the allergen via either parenteral or mucosal routes. Besides the commonly used mineral adjuvants such as Alum and calcium phosphate, only a limited number of Th1 immunopotentiators (e.g., MPL, CpGs, lactic acid bacteria) have been tested in humans, but their impact on clinical efficacy remains to be fully investigated. Among available vector systems, virus like-particles represent a highly promising platform to present allergens and/or adjuvants to the immune-system. In this regard encouraging clinical efficacy results have been obtained during SCIT of mite allergic patients. Mucoadhesive particulate vectors have not yet been tested in humans, but appear very promising based on preclinical experiments to support the development of safer and more efficient mucosal allergy vaccines. Additional studies evaluating the long-term efficacy of such vectorized allergens in large cohorts of allergic patients are needed to understand the true potential of those technologies in the field of allergen-specific immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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