The Use of Adjuvants for Enhancing Allergen Immunotherapy Efficacy

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KEYWORDS

• Allergen-specific immunotherapy • Immune tolerance • Adjuvants

KEY POINTS

- Allergen-specific immunotherapy currently represents the only curative treatment for allergy, but its broader application requires safer and more efficacious treatment protocols.
- Adjuvants can improve the efficacy of allergen-specific immunotherapy, and a variety of promising immunomodulatory adjuvants are currently being developed.
- Innovative strategies have been proposed to simplify immunization and to achieve long-term tolerance.

INTRODUCTION

Allergic diseases are characterized by the seasonally recurring production of T helper 2 (T_H2) cytokines (eg, Interleukin [IL]-4, IL-5, and IL-13), which drive the production of allergen-specific type-E immunoglobulin (IgE) by B cells and the recruitment and sensitization of effector cells such as eosinophils, basophils, and mast cells.[1] Long-time surviving specific memory T cells and B cells generate a pool of cells that quickly expand upon rechallenge thereby forming an immunologic memory that allows quick responses against pathogens but unfortunately also seasonal recurrence of allergic symptoms. A key factor in the early-phase symptoms is that allergen-specific IgE binds to the high affinity IgE receptor, FcεR1, on the surface of mast cells, basophils,

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eosinophils, and dendritic cells (DCs) resulting in the rapid release of proinflammatory mediators such as histamine, prostaglandins, and leukotrienes, which elicit allergic symptoms such as itching, swelling and bronchoconstriction.[1] Late-phase reactions are mediated by infiltrating T cells that release T_H2 cytokines triggering additional tissue inflammation.

Allergen-specific immunotherapy (SIT) is currently the only curative treatment able to change the seasonal recurring natural course of IgE-mediated allergies and to induce long-term remission.[2] By exposing allergic patients to increasing doses of allergen, this therapeutic strategy aims to re-educate the immune system to promote a tolerogenic response toward a specific allergen.[3] This tolerogenic response is thought to be mediated by a change in immunologic memory. Effective immunotherapy is associated with the induction of distinct subsets of regulatory T cells (Tregs) that induce peripheral tolerance by increased secretion of IL-10 and transforming growth factor (TGF)-β, which increase the production of IgG4 and IgA antibodies.[4] In clinical settings, successful SIT is defined by a marked reduction in symptom duration and severity at the time of allergen exposure, a decrease in the use of antiallergic drugs, and an overall improvement in the quality of life of affected patients.[1]

However, despite great progress in the last decade, SIT faces several problems regarding its efficacy, side effects, low patient adherence, and the high cost owing to the long duration (3–5 years) of treatment.[5] It is estimated that less than 5% of all allergic patients, who could potentially benefit from allergy immunotherapy, actually undergo this treatment. Thus, finding new strategies to enhance SIT safety, more compact treatment regimens, and improved efficacy represents major objectives of current research efforts, which will be instrumental for improving a broader implementation of SIT in the clinic.

Factors that influence the safety and efficacy of SIT include the pattern of sensitization, the nature of the allergen preparation (allergen extracts, adjuvants, and conjugated molecules), and the route of administration (subcutaneous or sublingual). Thus, the optimization of allergen/adjuvant formulations and their mode of administration is currently a bottle neck in specific immunotherapy.[6] Subcutaneous immunotherapy (SCIT) is the most commonly used form of SIT and is found to be effective in adults and children suffering from allergies to house dust mites, animal dander, and pollen.[7] However SCIT requires frequent injections and can be associated with allergic side effects, including fatal airway obstruction and anaphylaxis. Other alternative methods of delivery such as epicutaneous, intralymphatic, oral, or sublingual immunotherapies have been proposed and are currently being evaluated.[8] Because of its noninvasive character and good efficacy, sublingual immunotherapy is now considered a promising alternative to SCIT for respiratory allergies to grass and tree pollen or house dust mite allergens.[9]

In recent years, considerable research effort has been put into the chemical modification of allergens to improve the efficacy and safety of SIT, but also the demand of drug authorities to standardize allergen preparations has consolidated the available allergen preparations. Various strategies have been developed to modify allergenic molecules into safer hypoallergenic derivatives to limit adverse IgE-mediated reactions while maintaining their immunogenic properties.[10–13] Current research shows that allergenic peptides and various forms of recombinant allergens (hypoallergens, dimers, trimers, fusion proteins) can be efficient in controlling allergic inflammation and inhibiting symptoms of asthma notably by inducing the production of inhibitory antibodies.[12,14,15]

The use of appropriate immunomodulatory adjuvants is a particularly promising strategy to improve the safety and efficacy of current SIT protocols, because a

stimulated immune system may require less allergen and thus reduce side effects by avoiding allergen–IgE complexes. In the treatment of infectious diseases, adjuvants traditionally are added to vaccines to boost immunization against purified or recombinant pathogen antigens. Because adjuvants can skew the immune response toward T helper 1 (T_H1) known to downregulate allergic T_H2 inflammation, their use in SIT may provide a clear benefit.[16] An optimal adjuvant for specific immunotherapy should reduce the allergen dose, thereby decreasing local side effects and improving the overall safety of SIT, does not induce T_H2 cells, and provides long-lasting tolerogenic memory.

Several adjuvants, from alum (aluminum hydroxide) adjuvants to toll-like receptor (TLR) agonists, probiotics, and nanoparticles were developed and studied in the last decades.[17] This review describes adjuvants currently in use or under development and their role in enhancing the efficacy of allergen immunotherapy. This review summarizes immunologic mechanisms involved in successful allergen-specific immunotherapy and thus targets of adjuvant action and discusses therapeutic effects of current adjuvants and their role in the establishment of allergen tolerance.

IMMUNOLOGIC MECHANISMS OF SUCCESSFUL ALLERGEN-SPECIFIC IMMUNOTHERAPY

Allergen-specific immunotherapy can modulate cellular and humoral responses leading to a significant reduction in the recruitment and activation of inflammatory effector cells at sites of allergen encounter.[18] The long-term allergen-specific immune tolerance triggered by SIT can be divided into rapid, intermediate, and late treatment responses (**Fig. 1**).[4,19]

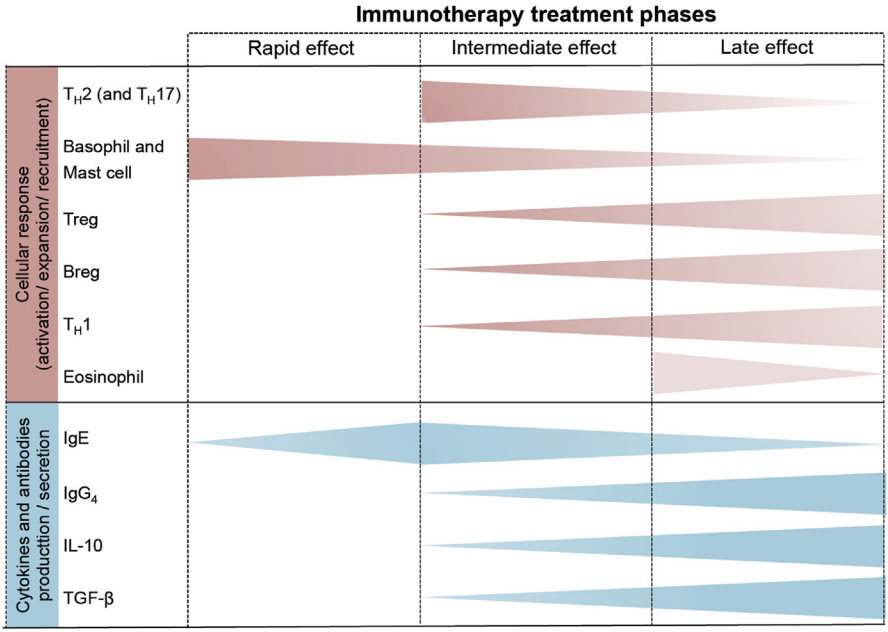

Fig. 1. Immunotherapy treatment phases.

Rapid Treatment Response

The first allergen administration during SIT induces a decrease in basophil and mast cell activation (ie, degranulation and proinflammatory mediator production), thereby reducing the capacity of these cells to induce anaphylaxis.[20,21] During this early phase, mast cells and basophils become unable to respond to environmental proteins despite the presence of specific IgE.[4,22] In a process referred to as *desensitization*, mast cells are rendered hyporesponsive to an activating challenge by exposure to low or high doses of allergen.[4] However, the molecular mechanisms responsible for this rapid desensitization are not yet completely elucidated. A recent study described the rapid upregulation of the histamine receptor 2 (H2R) on the surface of basophils from patients undergoing venom immunotherapy.[23] H2R was found to strongly suppress FcεR1-induced basophil activation and mediator release. Thus, a rapid increase in the surface expression of immunoregulatory receptors such as H2R might be instrumental in early protective mechanisms of SIT, particularly within the first few hours during the repetitive administration of venom protein (build-up phase).

Intermediate Treatment Response

The administration of progressively increasing allergen doses during the course of SIT will then influence adaptive immune responses, resulting in the generation of allergen-specific regulatory T (Treg) and B (Breg) cells and the simultaneous decrease in T_H2 cells. In the last 2 decades, several studies found that Tregs play a major role in the induction and the maintenance of tolerance during SIT.[24–26] After allergen-specific immunotherapy and subsequent allergen challenge, $FOXP3^+IL\text{-}10^+$ T cells along with single $FOXP3^+IL\text{-}10^-$ or $FOXP3^-IL\text{-}10^+$ T cells are detectable in the nasal tissue and are absent in the placebo-treated group.[26] The generation of these Tregs during SIT is associated with the production of the anti-inflammatory cytokines TGF-β and IL-10, which have the capacity to inhibit the activation and migration of effector T_H2 and T helper 17 (T_H17) cells, which are central players in allergic reactions.[27–34]

More recently, a TGF-β and IL-10–producing B-cell subset was identified and found to play an important role in suppressing T_H2 as well as T_H17 immunity while promoting the maintenance of Tregs.[4,35,36] Of note, this regulatory B-cell population has been implicated in the induction of tolerance during SIT in the context of bee venom allergy.[37] Expansion of IL-10 secreting suppressive B cells can promote the emergence of IgG4, an inhibitory antibody isotype suspected to play a key role in the establishment of allergen tolerance.[7,19] One study further suggested that the production of IgG4 is restricted to the regulatory B cells compartment.[37] IgG4 class switch recombination in B cells is caused by co-stimulation with IL-4 and IL-10, whereby IL-10 decreases IL-4–induced IgE class switching, but increases IL-4-induced IgG4 production.[38,39]

The emergence of IgG4 antibodies is of particular importance for SIT because this isotype is found to dampen FcεR1-mediated degranulation of mast cells, basophils, and eosinophils.[18,40] The competition of allergen-specific IgG4 and mast cell–bound–specific IgE antibodies for allergen has been proposed as a central mechanism underlying the efficacy of SIT.[19,41] By competing with IgE, allergen-specific IgG4 antibodies may also decrease the reactivity of antigen-presenting B cells to allergen, thereby reducing antigen presentation to T cells.[41] As serum IgG4 levels dramatically increase after prolonged SIT and remain stable over years after SIT treatment, high-avidity IgG4 has been proposed as a marker of SIT-induced immune tolerance[42];

however generally allergen-specific IgG4 of all avidities is not correlating with clinical success of the therapy.

Late Treatment Response

The long-term efficacy of SIT is associated not only with a significantly reduced immediate response to allergen provocation but also with a blunted late-phase response (LPR) in the affected end organ (eg, skin or respiratory tract).[4,43] The mechanisms driving the late-phase response differ from the mast cell–mediated early-phase response and involve a strong bronchial or nasal hyperreactivity and the recruitment and activation of eosinophils and effector T cells at sites of allergen exposure.[44] This late phase of the allergic response can be modulated by IL-10–producing regulatory B cells and T cells able to inhibit the recruitment and activation of eosinophils and the differentiation and proliferation of T_H2 cells.[42] Because T_H2 cells and eosinophils are central players in tissue remodeling, SIT approaches should induce mechanisms to efficiently control these cells or limit their clonal expansion, thereby avoiding the development of irreversible end organ hyperreactivity.[4]

ADJUVANTS AS MEANS TO OVERCOME LIMITATIONS OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

SIT has been a controversial treatment for allergic diseases.[6] Despite proven efficacy in many clinical studies,[45] concerns regarding the long-term efficacy and safety of SIT currently limit its clinical use.

Because SIT requires the application of high allergen doses and repeated injections (in the case of SCIT), local side effects but also systemic allergic reactions with the risk of severe or fatal anaphylaxis can occur. To reduce the administered allergen dose, allergens can be applied in combination with an adjuvant to boost the immunologic response toward the allergen. Adjuvants are traditionally added to vaccines to reduce the frequency of injections or the dose of antigen owing to their capacity to induce strong and sustained immunity against pathogens. An adjuvant (Latin, *adiuvare:* to aid) is functionally defined as a compound that enhances the specific immune response against an antigen in vivo.[46] On a cellular level it is anticipated that allergen vaccination is inducing de novo T-cell differentiation of naïve T cells into the helper T cells (Th1, T_H2, Tregs etc). In allergy, co-administration of antigen (allergen) with adjuvants can drive T_H1 responses to compete with T_H2-mediated hypersensitivity, actively suppress T_H2 type inflammation. The choice of adjuvant crucially depends on the antigen (allergen) as well as the route of immunization and is limited by the extent of adverse reactions in response to the chosen combination. Ideally, an adjuvant should be cost effective, biodegradable, non-toxic, stable for extended periods of time *in vivo* and induce an appropriate immune response.[47] Unfortunately, the use of adjuvants is hampered by issues such us toxicity and side-effects, limiting a broad application in immunotherapy. Thus, a major aim of current research is to design and develop new adjuvants with improved safety and efficacy profiles.

CURRENT ADJUVANTS AND THEIR EFFECTS ON ALLERGEN-SPECIFIC IMMUNOTHERAPY

To date, alum, an aluminum-based compound discovered more than 80 years ago, still remains the predominant adjuvant for human vaccines. However recent advances in the understanding of immunologic mechanisms underlying immunotherapy may foster the design of new effective adjuvants with improved immunologic profiles. Current adjuvants able to enhance the efficacy of allergy immunotherapy include (!)

Table 1
Immunopotentiators for allergy immunotherapy

Adjuvants	Allergen	Route	Properties	Application in Clinic (Yes/No)
Aluminium hydroxide	Various clinical vaccines	Subcutaneous	Aluminium triggers a depot effect (slow release of the allergen) favoring the interaction with the immune system. Alum facilitates the shift away from T_H2 response through the generation of inhibitory antibodies and Treg response Alum acts as a danger signal and induces IL-1 family cytokines.	Yes (the most used in SIT)
TLRs agonists (MPL, CpG-ODNs, others)	OVA, Amb a 1, grass pollen	Subcutaneous, intradermal, sublingual	CpG-ODNs (TLR9) have good efficacy in immunotherapy mouse models (subcutaneous, intradermally) with ragweed/grass pollen allergens. In mice and humans, a synthetic CpG conjugated to Amb a 1 showed good efficacy when used during SCIT. Other ligands particularly Pam3Csk4 and LP40 (TLR2), imidazoquinolines (TLR7, 8) are found to induce Treg and T_H1 responses in preclinical models	Yes (MPLs, CpGs)

Probiotics	OVA, Bet v1, peanut	Sublingual, oral	Bacteria such as *L plantarum* or *Bifidobacterium plantarum* showed efficacy in the treatment of allergic mice against Bet v1 and OVA. A recent clinical trial found a reduction of IgE and induction of IgG4 in peanut allergic children after oral immunotherapy in combination with probiotics.	Yes (with the probiotic *Lactobacillus rhamnosus*)
Bacteria products (*M vaccae*, *CTB*)	Mite, birch pollen	Intradermal, subcutaneous, mucosal (oral or nasal, intratracheal)	*M vaccae and CTB* decrease airway inflammation and modulate the immune system (Treg, T_H1, IgA) in mice when injected with antigen (house dust mite or birch pollen). Although some benefits have been found in mice, no clinical trials have yet evaluated the effect of *CTB* and *M vaccae* during immunotherapy in humans.	No
Vit D	OVA, grass pollen, house dust mite	Sublingual, subcutaneous	The administration of Vit D alone or in combination with glucocorticosteroids during OVA-specific immunotherapy in mice reduces T_H2-driven airway inflammation and airway hyperreactivity. An expansion of Treg and activation of DCs has been reported in response to Vit D. Preliminary promising results have been described after treatment with Vit D in combination with SCIT in mite allergic patients.	Yes (with mite allergen)

Abbreviations: CpG-ODNs, CpG oligodeoxynucleotides; *CTB*, *Cholera toxin B*; MPL, Monophosphoryl lipid A; OVA, Ovalbumin.

synthetic or biological immunopotentiators reinforcing allergen-specific T_H1 or regulatory T cell responses through a direct activation of T cells, dendritic cells, or epithelial cells (**Table 1**) and (2) nanoparticle delivery systems promoting allergen uptake and presentation by antigen presenting cells (APC) in the oral mucosa but also protecting the encapsulated active product (allergen) (**Table 2**).[9]

Immunopotentiators

Aluminium-based adjuvants

Aluminium-based adjuvants have been used successfully in prophylactic vaccinations for almost 100 years. Aluminum hydroxide or aluminum phosphate are the most widely used adjuvants in allergy immunotherapy to boost immune responses to the injected allergens.[48] Approximately 75% of all adjuvant-based therapies include an aluminum salt.[49] The vaccine preparation is primarily composed of micrometer-sized clusters of nano-sized primary particles of the aluminum salt to which the antigen is adsorbed.[50] The injection of alum-adsorbed allergens has proven efficacious and safe in a variety of SCIT studies and in studies using other parenteral routes.[49] Classically, alum adjuvants have been proposed to promote immune responses through the so-called depot effect, that is, by allowing for the slow release of the antigen from the salt particles, thereby maintaining the stimulation of the immune response at the inoculation site. Several hypotheses regarding alum's mechanisms of action have been proposed.[51] The first immunization with alum-adsorbed allergen was further shown to induce an early cytokine response, including the IL-1 family member IL-18, facilitating IL-4 production.[52] The induction of IL-18 by alum adjuvants has been shown to depend on the activation of the NLRP3 inflammasome.[53,54] After prolonged and repeated immunization, alum was reported to skew the immune response toward a Treg/T_H1 response and to reduce T_H2 activation by increasing IgE-blocking IgG antibodies.[51] Thus, the use of aluminum adjuvants in allergen immunotherapy is a useful tool to trigger a modified T_H2 response with protective character.[55] To date, the use of alum-adsorbed allergen extracts has improved symptoms in the case of aeroallergens and venom immunotherapy. However, aluminum adjuvants have important limitations, particularly with regard to their profound T_H2-biasing effects and their potential implication in neurologic pathologies.[50,56–58] The T_H2 induction is considered an unwanted side effect, possibly related to an increase of allergen-specific IgE after the initiation of immunotherapy, before a blunted IgE response in the following season and decrease after prolonged immunotherapy. Current research aims to develop new adjuvant formulations able to induce tolerance by avoiding the induction or amplification of T_H2 responses.

Toll-like receptor agonists

TLRs are key components of the innate immune system able to elicit an inflammatory response in response to pathogen-associated molecular patterns. Pathogen-associated molecular patterns include lipid, protein, lipoprotein, carbohydrate, and nucleic acidstructures found exclusively on microbes (bacteria, viruses, fungi, and parasites) but absent from host cells. The addition of TLR agonists to immunotherapy has shown some benefits, which were linked to their potential to induce mixed T_H1/Treg responses, reversing established allergic inflammation.[59] The prototypic bacterial lipopolysaccharide (LPS) induces the TLR4-dependent activation of APCs resulting in the induction of a strong specific T_H1 response against co-administered antigens.[60] However, because of the toxicity of LPS, it remains a poor choice for a therapeutic adjuvant.[17] MPL, a derivative of LPS, has been shown to exhibit reduced toxicity while maintaining immunomodulatory properties.[61]

Table 2
Nanoparticle delivery systems for allergy immunotherapy

Adjuvants	Allergen	Route	Properties	Application in Clinic (Yes/No)
Liposomes (OML, nanoliposome)	OVA, Cry j 1, grass pollen, Der p1	Intranasal, intradermal	OML loaded with OVA or Cry j 1 (pollen) improves the allergic features in allergic mouse models by modulating the humoral (control of IgE elevation) and cellular immunity (Tregs CD4+ and Tregs CD8+, T_H1). In humans, cutaneous administration of liposomes loaded with allergen has shown benefits in asthmatic patients (high specific IgGs levels; reduction of sputum eosinophils and serum ECP levels). However, prolonged immunization causes side effects.	Yes (grass pollen, Der p1)
VLPs	Der p 1, Phl 1	Subcutaneous, intramuscular	VLPs are described as effective adjuvants in immunotherapy against mite and grass pollen. VLPs loaded with CpGs only (without allergen) are immunomodulatory.	Yes (Phl p 1, Der p1)
ISCOMs	OVA, PLA2	Subcutaneous, intranasal	In animals, the administration of ISCOMs together with allergen elicits humoral and cellular responses. This system is powerful in activating DCs and inducing antigen-specific cytotoxic CD8+ T cells. ISCOM-based vaccines are found to promote long-lasting immune responses.	No (preclinical)
Polymeric nanoparticles (Chitosan NPs, PLGA, others)	OVA, mite, Bet v1, profilin, peanut	Intranasal, sublingual, subcutaneous, intravenous, oral, intradermal	The therapeutic effect of chitosan-based NPs loaded with allergen or with plasmids encoding allergen has been proven in allergic mice during immunotherapy. Subcutaneously or intravenously administered PLGA NPs containing allergen alone (Bet v1, profilin) or CpG-allergen (mite) in mice enhances the tolerance. Other polymeric NPs are described in mice as potential adjuvants for OIT: (1) Gantrez NPs combined with LPS from *Brucella ovis* stimulating the production of IgG2a antibodies and IL-10; (2) PVM/MA NPs formulated with peanut protein triggering a pro-T_H1 immune response; (3) the copolymer PHEA loaded with a hybrid molecule expressing the pollen allergens (Par j1 and 2) promoting a high T_H1/T_H2 ratio	No (preclinical)

Abbreviations: copolymer PHEA, α,β-Poly(N-2-hydroxyethyl)-d,l-aspartamide; ECP, Eosinophil cationic protein; NPs, nanoparticles; PLA2, phospholipase A2; PLGA, poly(lactic-co-glycolic acid); PVM/MA, poly(methyl vinyl ether-co-maleic anhydride).

Through TLR4 signaling, MPL is able to induce potent immune deviation toward T_H1/Treg response.[62] The use of MPL as adjuvant has been tested in clinical phase II and III studies and was approved for subcutaneous and sublingual immunotherapy (Pollinex Quattro).[63] The formulation allergen-MPL (especially grass pollen) is well tolerated and results in a significant reduction of symptom scores and serum IgE levels while increasing the T_H1 polarization and the production of allergen-specific IgG4.[63]

DNA or CpG-ODNs have been described as immune modulators and as potential vaccine adjuvants.[64] CpG-ODNs interact with TLR9 localized in the endosomes of APCs resulting in the production of a series of cytokines including Interferon-γ and IL-12 favoring the shift of human allergen-specific T_H2 cells toward a T_H1/T_H0 phenotype.[65] In subcutaneously sensitized mice, injection of a mix of grass pollen/CpG-ODN reduced T_H2 inflammation and IgE secretion with conflicting effects on T_H1 stimulation.[66] The clinical use of CpG-ODNs in SIT against ragweed allergy has shown variable effects possibly owing to differences in the efficacy of different types of CpG.[17] Recently, an innovative approach has been developed based on the conjugation of immunostimulatory DNA to allergenic proteins. In both animal and human studies, a synthetic CpG oligonucleotide conjugated to the major allergen of ragweed Amb a 1 has been shown to improve allergic symptoms after subcutaneous administration.[67,68]

Other TLR agonists have been identified in vitro or in vivo as potential adjuvants in allergen immunotherapy. Among these the TLR7 agonists imiquimod (R-837) and resiquimod (R848) (imidazoquinoline class) are of special interest in allergy immunotherapy because in addition to inhibiting T_H2 cell differentiation, they can induce T_H1 responses.[69] However, as imidazoquinolines induce serious side effects (vomiting, alteration of blood cells), their use has been limited to experimental models.[61] Other natural adjuvants signaling through TLR 7, 8, or 9 are currently being investigated, for example, modified 8 OH-adenines as a future adjuvant for use in oral immunotherapy.[70] Additionally, the use of lipopeptides such as the TLR2 agonists Pam3CSK4 and LP40 in SIT has been proposed because of the potential of these compounds to induce Treg and T_H1 responses.[71,72]

Probiotics

The healthy human microbiome is increasingly recognized as a rich source of immunomodulatory compounds, with a great potential to protect from allergy, particularly early in life.[73] Thus, the use of immunomodulatory live micro-organisms (probiotics) has been suggested as a new strategy to improve SIT. Bacteria such as *Lactobacillus plantarum* or *Bifidobacterium bifidum* were found to modulate systemic cytokine production and decrease allergen-specific IgE production following sublingual immunotherapy in mice sensitized to OVA or Bet v1.[74,75] In humans, most clinical trials using selected probiotics as a stand-alone therapy have failed to show clinically relevant beneficial effects on allergy.[76,77]

Attenuated mycobacteria, bacterial products

Because of their immunostimulatory properties, bacterial toxins such as *CTB* and heat-killed *Mycobacterium vaccae* were described as potential adjuvants. The administration of *CTB* (oral or nasal route) or *M vaccae* (intratracheal, subcutaneous) along with antigen (dust mite, birch pollen) in mouse models of allergic airway inflammation is reported to result in a marked reduction in classical features of airway inflammation.[9] *M vaccae* or *CTB* adjuvant was found to prevent T_H2 response by enhancing Treg and T_H1 responses and by inducing the production

of IgA antibodies.[78–80] In clinical settings, intradermal injection of *M vaccae* has shown beneficial outcomes in children with atopic dermatitis or asthma. In contrast, *M vaccae* or *CTB* have not yet been tested in combination with allergens in allergic patients.

Vitamin D

Vitamin D is a natural hormone able to prevent various allergic diseases by stimulating tolerogenic processes of the immune system.[10] The active form of vitamin D3 (VitD3),1,25-dihydroxyvitamin D3, is found to promote the migration of DC and the development of Tregs leading to the suppression of allergen-specific T_H2 cells in vitro and in vivo.[81] Studies in mouse models have described VitD3 as a potent adjuvant with the capacity to enhance beneficial effects of immunotherapy and to promote the long-term efficacy of SIT.[82] Deficiency or insufficiency of VitD3 is correlated with an increased risk of allergy and asthma and VitD3 supplementation may enhance the efficacy of SIT.[82,83] The administration of VitD3 in combination with SCIT showed some minor favorable outcomes in asthmatic children sensitized to house dust mite.[84] Ongoing clinical trials are currently evaluating the additive effect of VitD3 in SIT in grass- and birch pollen–allergic patients.

Nanoparticle Delivery Systems

Recently, nanoparticle-based delivery systems such as viruslike particles, liposomes, immunostimulating complex (ISCOMs), and polymeric nanospheres have received attention as potential adjuvants for allergen immunotherapy.[85] These generally well-established carrier systems are found to boost the efficacy of allergy vaccines.[10] Encapsulation in the nanoparticle delivery system allows for the protection of the antigen from environmental influences (eg, pH, humidity, temperature), thereby maintaining or even enhancing the immunogenicity of the allergen cargo. Because of the particle nature the antigen-uptake is limited to those cells that are enabled to phagocytize respective particles.

Liposomes

Liposomes are synthetic spheres made of lipid bilayers that can encapsulate hydrophilic antigens and act as adjuvants.[47] Liposomes increase the half life of antigens in blood ensuring a sustained antigen exposure to APCs after vaccination. Intranasal immunization with OVA encapsulated in oligomannose-coated liposomes (OML) was found to induce a Treg response and an improvement of allergic symptoms in a murine food allergy model.[86] Similar results have been obtained after intradermal immunization of pollen-allergic mice with Cry j 1 (Japanese cedar major allergen) encapsulated in OML.[87] More recently, this system has been used to improve the delivery of other adjuvants—particularly CPG-ODN—for which extensive preclinical and clinical evaluation had previously confirmed an immunomodulatory potential in allergy. Co-encapsulation of OVA antigen with CpG-ODN adjuvant in nanoliposomes is found to profoundly increase antigen-specific T_H1 immune responses in vitro.[88] In humans, the cutaneous injection of liposome-encapsulated mite or grass pollen allergens was well tolerated[89] but found to induce delayed local reactions.[9] Furthermore, the use of liposome adjuvants in humans is limited by their low stability and manufacturing problems.[90] Liposomal delivery systems with improved stability and tailored immunologic properties are currently being developed for a future application in allergen immunotherapy. Of note, phosphatidyl serine containing liposomes mimic vesicles derived from apoptotic cells, and their phagocytosis was found to promote tolerogenic immune responses.[91]

Viruslike particles

Viruslike particles (VLP) are self-assembling nanoparticles formed of biocompatible capsid proteins.[92] VLPs have the potential to interact with the immune system while avoiding pathologic effects caused by the absence of viral pathogenicity factors. Currently, some of these vectors have been evaluated in humans via parental routes (subcutaneously or intramuscularly).[92] The administration of VLPs from Qβ phage conjugated with peptides derived from *Dermatophagoïdes* pteronyssinus (Der p) 1 mite allergen was shown to induce a strong IgG response in healthy volunteers.[93] Clinical trials have evaluated the therapeutic effect of Qβ-derived VLPs loaded with CpGs or the allergen (eg, mite, mite extracts) in adult allergic patients.[94] After 6 weekly injections, allergy symptoms improved with increased levels of IgG antibodies observed in the group receiving VLP/CpGs/allergen but also VLP/CpGs alone. The good efficacy of VLPs loaded with CpGs alone has been confirmed in allergic rhinitis patients undergoing SIT treatment.[95] More recently, a rhinovirus-derived VP1 protein loaded with peptide from Phleum pratense (Phl) 1 grass pollen has also been proposed as a candidate vaccine for grass pollen allergies.[96] Because of their good safety and efficacy profile VLP-encapsulated allergens represent promising candidates for immunotherapy of allergic disorders.

Immunostimulating complex

ISCOMs are spherical complexes about 40 nm in size, composed of saponin adjuvant Quil A, cholesterol, phospholipids, and protein antigen.[90] ISCOMs are able to trap the antigen by apolar interactions and to elicit strong humoral, cellular, and mucosal T_H1 responses after subcutaneous or intranasal immunization.[97] The particularity of ISCOMs is the generation of antigen-specific $CD8^+$ cytotoxic cells, observed after immunization in mice.[98] Although ISCOMs have been proposed as potential vaccine vectors in vaccination with influenza or human immunodeficiency virus, no study has yet evaluated the effect of ISCOMs as adjuvants in allergen immunotherapy.

Polymeric nanoparticles

Several NPs have been developed in recent years, and particularly biodegradable polymeric carriers have shown great potential as new drug delivery systems.[90] In addition, the application of polymeric nanoparticles has yielded promising results in systemic and mucosal immunotherapy.[99] Biodegradable polymers can be of natural (eg, collagen, albumin, chitosan) or synthetic (eg, poly [lactic acids], PLA; poly [lactide-co-glycolic acids], PLGA; poly [methyl methacrylate], PVMA; poly [anhydride] nanoparticles, PHE; poly [hydroxyethyl] aspartamide) origin.[90] Two of the most extensively investigated polymers in allergen immunotherapy is chitosan and PLGA.

Chitosan is a natural mucoadhesive polysaccharide derived from crustacean shells.[6] Chitosan-based nanoparticles have been widely studied because of their biocompatibility, biodegradability, nontoxic nature, and their ability to enhance the penetration of macromolecules across the mucosa.[90] Intranasal application of Der f entrapped in chitosan microparticles in sensitized mice attenuated bronchial hyperreactivity, lung inflammation, and mucus production.[100] The immunotherapeutic efficacy of chitosan microparticles has also been shown during sublingual immunization with OVA in a murine model of allergic airway inflammation.[100–102] Several studies found that chitosan polymers improve the uptake of antigen by mucosal DCs, thereby enhancing tolerance induction during intranasal or sublingual immunotherapy.[101,102] Other studies indicate that the oral administration of chitosan NPs formulated with plasmid DNA encoding

the dust mite allergens Der p1 and Der p2 or with the Ara h2 allergen (major peanut allergen) in mice could trigger a significant reduction in allergic symptoms.[103–105] A recent study found that the treatment of mice with chitosan DNA NPs loaded with OVA led to a transferable antigen-specific tolerance, which involved Treg cells.[106]

Poly (lactic-co-glycolic acid) (PLGA) is a polyester widely used for the preparation of NPs. This polymer has been approved for several clinical applications in humans owing to its well-established biocompatibility, safety, and biodegradability.[10] In recent years, the PLGA nanoparticles have received great attention for the therapy of allergies.[107] Subcutaneous immunization of mice with PLGA particles formulated with Bet v1 (major birch pollen allergen) was found to decrease the T_H2 response to Bet v1 and to increase immunomodulatory immunoglobulins and cytokines.[108] Similar results were obtained after the immunization of allergic mice with PLGA particles composed of recombinant birch profilin (protein identified as allergen in pollen, latex, and plant food).[109] Moreover, a recent study has reported an alternative co-delivery method resulting in the successful tolerization of mice using CpG and PLGA loaded with a major house dust mite allergen (Der p2).[110,111] This approach using PLGA particles containing CpG and Der p2 resulted in the reduction of allergen-specific IgE while enhancing class switching to IgG2a. More recently, intravenous or subcutaneous immunization using tolerogenic PLGA nanoparticles loaded with peptide or antigen and the immunosuppressant rapamycin was found to induce sustained antigen-specific tolerance in animals.[112] In this study, co-administration of rapamycin was crucial for inducing both antigen-specific humoral and cellular immunity. As an alternative to PLGA polymer particles, new polymer-based adjuvant systems are being developed, including an injectable hydrogel composite comprising PLGA-PEG (poly [ethylene glycol]) copolymers. Although this system has never been tested in allergen immunotherapy, it could be a new approach to improve the efficacy of SCIT.[113]

Other polymeric nanoparticles have recently provided positive results in allergy immunotherapy in animal models including the Gantrez nanoparticles combined with lipopolysaccharide from Brucella Ovis,[114] the PVMA nanoparticles loaded with peanut,[115] and the copolymer PHEA (α,β-poly [N-2-hydroxyethyl]-DL-aspartamide) loaded with pollen extracts.[116] Currently, none of those polymeric systems have been tested in the context of mucosal allergy vaccines in humans.

SUMMARY AND FUTURE OUTCOMES

SIT currently represents the only curative treatment for allergic diseases. Despite its proven efficacy in a considerable proportion of allergy patients, SIT has been constrained by adverse events requiring either prophylactic or rescue medications. To overcome this, innovative strategies have been proposed to simplify immunization and to achieve long-term tolerance. Among these strategies, the co-administration of immunomodulatory adjuvants during SIT is of central importance. Thus, new adjuvant systems are currently under development that will hopefully overcome the limitations of traditionally used adjuvants such as alum. Although a variety of candidate adjuvants are found to induce tolerance during SIT protocols in murine models, a limited number of these compounds have been tested in humans (**Fig. 2**). Thus, additional studies are urgently needed to show the true potential of these new adjuvants in SIT. Deeper insights into the immunologic mechanisms underlying allergy have resulted in the development and approval of targeted therapies. Monoclonal antibodies targeting IgE and T_H2 cytokines (IL-4, IL-13, IL-5) are already in clinical use for the treatment of severe allergic inflammation and have recently been proposed as an alternative to improve SIT.[117,118] Omalizumab (anti-IgE) is the first immunomodulatory drug shown to

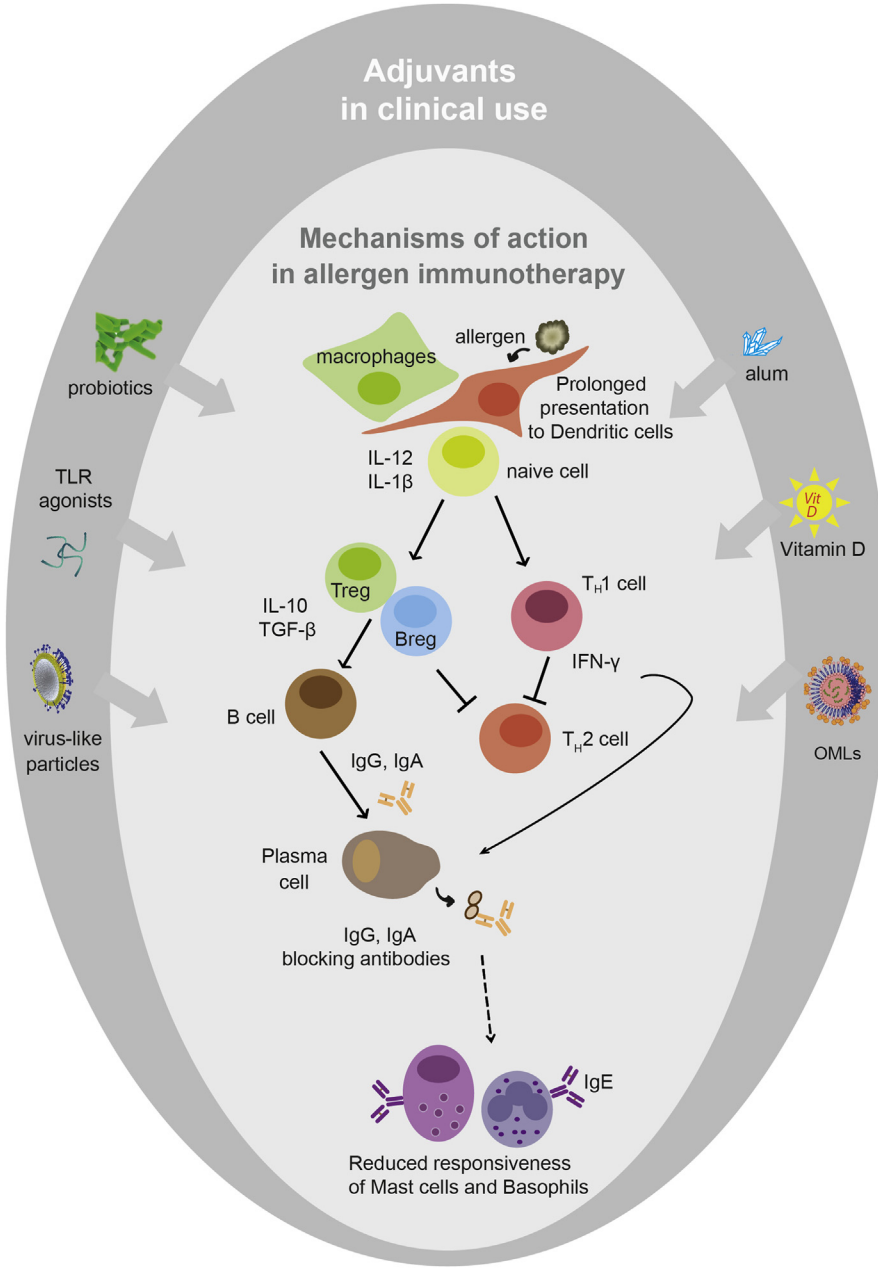

Fig. 2. Immunologic mechanisms of allergen-specific immunotherapy and the immune pontentiators or adjuvants used in clinic.

improve the efficacy and safety of SIT in humans when administered orally or subcutaneously.[119] It is hoped that future research on immunomodulatory adjuvants will result in safer and more efficacious allergen immunotherapy protocols, instrumental in fighting the ever-worsening allergy epidemic.

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