



Adjuvants for immunotherapy

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Purpose of review

This article gives an overview about the current state of preclinical and clinical studies using different kinds of adjuvants and their effect on allergen-specific immunotherapy (SIT).

Recent findings

Recently, vectors such as liposomes and microspheres and adjuvants such as Toll-like-receptor agonists [e.g. nonmethylated cytosine–guanine dinucleotide (CpG) motifs derived from bacterial DNA or monophosphoryl lipid A (MPL A)] have been used in clinical phase II and III trials demonstrating encouraging clinical effects.

Summary

SIT has been optimized for more than 100 years with different approaches. Among these, adjuvants have been shown to amplify the effect of SIT by modulating the immune response to this therapy. In the first part, this article reviews the immunological mechanisms underlying the use of adjuvants targeting key cells of the innate immune system such as dendritic cells. In the second part, it overviews first clinical trials which have been reported so far in both subcutaneous and sublingual allergen-specific immunotherapy investigating the therapeutic potential of adjuvanted extracts. Most of these clinical trials revealed both clinical efficacy and immunological effects. However, more studies are warranted to further focus the specific role of adjuvants in the process of tolerance induction in allergic patients.

Keywords

adjuvants in vaccines, depot carrier, immune system, subcutaneous immunotherapy, sublingual immunotherapy

INTRODUCTION

The prevalence of IgE-mediated allergic diseases reaches up to 35% in industrialized countries [1,2] and is estimated to further increase in the next decades [3,4]. Allergen-specific immunotherapy (SIT) was first reported by Noon in 1911 [5] and still represents the gold standard of therapy in patients with allergic rhinitis, allergic conjunctivitis, allergic asthma and hymenoptera venom allergy [3,6].

However, the clinical efficacy of SIT might still be improved in certain patients and rare, but very serious side-effects have been reported because of the activation of the immune system of sensitized patients [7,8]. Hence, the research on SIT in the course of the previous decades has mainly been focused on improving the clinical efficacy on one hand, while diminishing the potential of harmful side-effects on the other [3]. Many innovations and modifications of this therapy have been reported for both the subcutaneous immunotherapy (SCIT) and the sublingual immunotherapy (SLIT) route [9,10]. Among these, adjuvants have been partly shown to become encouraging candidates in improving the clinical efficacy of SIT by enhancing the nonallergic

part of the immune system (Th1 cells) or tolerance-inducing immunological mechanisms (e.g. regulatory T cells) [11,12]. As per definition, adjuvants are substances that have the potential of enhancing the immunogenicity of antigens or allergens. However, they are themselves pharmacologically inactive and develop an immunomodulatory effect only in combination with a specific allergen or antigen [12]. Moreover, approaches have been made to use vaccine vectors such as polymers (microspheres) or liposomes to increase the uptake of vector-bound antigens to key immune cells [13].

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KEY POINTS

- Adjuvants added to allergen SIT or vector systems to ease allergen delivery to key cells of the immune system may enhance efficacy and increase tolerability of this 'causal' therapy.
- Adjuvants target the key cells of the innate immune system such as dendritic cells.
- Though promising candidates for both SLIT and SCIT, the specific immunological mechanisms underlying the therapeutical potential of adjuvants and vector systems will be further investigated in upcoming trials.

The following section overviews the immunological mechanisms involved in allergen-specific immunotherapy which may be enhanced by adjuvants and vector systems and, hereafter, focuses on the clinical data of adjuvanted SCIT and SLIT in published studies [12,13[■],14].

IMMUNOLOGICAL MECHANISMS UNDERLYING THE USE OF ADJUVANTS IN ALLERGEN-SPECIFIC IMMUNOTHERAPY

The final success of immunotherapy rests in its possibility to change the deviated, Th2-skewed and overstimulated immune response of the allergic patient to a more tolerogenic and balanced Th1–Th2 response. As a result, successful SIT is accompanied by a reduction of Th2 cytokines (IL-4, IL-5 and IL-13) and a reduced number and activity of allergen effector cells – mast cells and eosinophils, while tolerogenic (IL-10 and TGF- β) and Th1 (INF- γ) cytokines are upregulated [15]. These changes go hand in hand with a shift in the specific immunoglobulin pattern produced: a stable or reduced quantity of IgE makes room for a rise in protective and tolerogenic immunoglobulins, such as IgG4 and IgA [16,17[■]].

Antigen-presenting cells (APCs), such as the different types of dendritic cells, play a fundamental role in these immunological mechanisms involved in SIT, as they can specifically stimulate a Th1 or T regulatory cell response [8,15]. Dendritic cells are part of the first-line defense of the body and bridge the innate and adaptive arm of the immune system [18,19]. As such, they are widely spread throughout all body tissues and blood, especially in superficial tissues such as skin and mucosal surfaces, where dendritic cells are critical in detecting all kinds of pathogens entering the organism [20–22]. Skin dendritic cells as well as oral dendritic cells (e.g. Langerhans cells) have the ability to take up

and process antigens via several mechanisms such as receptor-mediated endocytosis or macropinocytosis [22]. Upon activation, they obtain the capacity to migrate into the tissue lymphoid organs in order to present the antigens to T cells and to induce an antigen-specific immune response [23,24]. In this context, dendritic cells are able to mediate either a proinflammatory or a protolerogenic immune reaction, depending on several factors like expression of costimulatory or coinhibitory molecules and other signals from the microenvironment [22,25[■]]. Concerning the latter, dendritic cells can be activated in at least two different ways [26,27]. Firstly, several receptors allow the dendritic cells to sense the environment and directly ligate the molecules on the surface of microorganisms. These are the so-called pattern-recognition receptors (PRRs), Toll-like receptors (TLRs) are one example. Some TLRs are embedded into the dendritic cells' membrane, while others are situated intracellularly ([19], Fig. 1). Activation of the dendritic cells via the TLR generally leads to the induction of a Th1 or Treg response. Secondly, dendritic cells can be activated in an indirect way by cytokines derived from the inflammatory process [28,29]. In this case, the stimulation of the T cells can be toward a Th1, Th2 or Treg response, depending on the kind of cytokines released.

The mammalian TLR represents a residue system for the recognition of antigens and signaling [21]. There are only 10 functional TLR genes with a limited specificity, in comparison to the adaptive immune system [20,29,30]. As such, each TLR will only recognize a specific set of ligands: certain pathogen-associated molecular patterns (PAMPs), characteristic for different components of pathogens and infectious agents ([20,21], see Table 1).

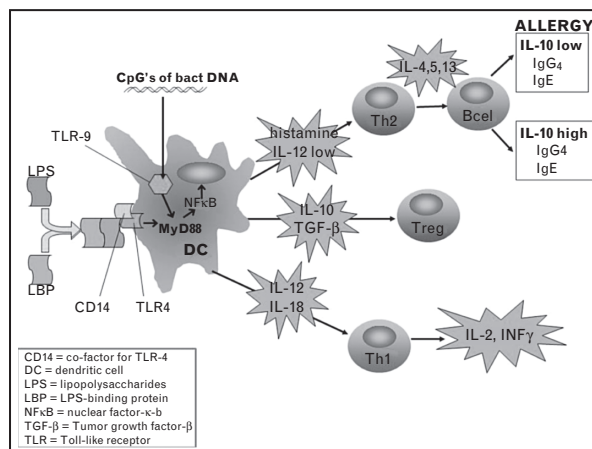


FIGURE 1. Activation of the dendritic cells on the cell-membrane (TLR-4) or intracellularly (TLR-9) induces Th1 or Treg responses. Adapted with permission [19].

The discovery that many adjuvants, historically used to enhance the immune response in vaccines, can bind to TLRs has been the basis for the search for new adjuvants that might be of use in distinct vaccine therapies, including (allergen-) specific immunotherapy by targeting TLR-4 receptors with lipopolysaccharides (LPS) or TLR-9 receptors with unmethylated CpG dinucleotides, characteristic of prokaryotic DNA (CpG motifs) [11,13^{*,}31–33].

In addition to these adjuvants aimed to enhance the immune reaction by stimulating APCs via specific TLRs, some vector systems such as ‘liposomes or microspheres’ have been described as being able to improve the uptake of allergens into APCs: APCs recognize particles much easier; thus when an allergen is presented as a particle to an APC, its uptake is significantly enhanced [13^{*,}34].

ADJUVANTS IN SUBCUTANEOUS IMMUNOTHERAPY CLINICAL TRIALS

The following overviews the current preclinical and clinical reports on adjuvanted vaccines in SCIT.

Microspheres

Microspheres are polymers like PLGA (poly-D,L-lactic-coglycolic) acid widely used in medicine [35]. These polymers can be assembled to microscopic pellets, feasible to coat proteins with controlled release [36]. Scholl *et al.* [37] were able to demonstrate the tolerogenic effect of Bet v1 allergen coated by microspheres in mouse models with increased IFN-gamma and IL-10 production in isolated T-cells. However, clinical data on this promising approach are still pending.

Table 1. Toll-like receptors and their possible ligands

Possible TLRs on the dendritic cell side	Possible ligands
TLR-1:TLR-2 heterodimer	Peptidoglycan lipoproteins
TLR-2:TLR-6 heterodimer	Lipoarabinomannan (mycobacteria)
TLR-3	dsRNA
TLR-4	LPS (Gram-negative bacteria)
	Lipoteichoic acids (Gram-positive bacteria)
TLR-5	Flagellin
TLR-7	ssRNA
TLR-8	G-rich oligonucleotides
TLR-9	Unmethylated CpG DNA

Toll-like receptors and their possible ligands (adapted from Janeway's Immunobiology, Seventh Edition by Murphy *et al.* Reproduced by permission of Garland Science/Taylor & Francis LLC Copyright 2008 [21] and Barton GM, Medzhitov R. Toll-like receptors and their ligands. *Curr Top Microbiol Immunol.* 2002;270:81–92[20].

Liposomes

Liposomes are composed of a lipid bilayer and are able to encapsulate an aqueous solution inside their hydrophobic membrane [38], a property that qualifies liposomes for being perfect vectors for the administration of all kinds of pharmaceutical drugs. Some early animal experiments implied that liposomes could have a modulatory effect on the allergen-specific immune response in SIT [39]. Recently, it has been shown that immunization with oligomannose-coated liposomes may be an effective immunotherapy for food allergies, as it induced a subset of regulatory CD8(+) T cells as well as CD4(+) CD25(+) Foxp3(+) T cell and modulates humoral immune responses in allergen-sensitized mice [40].

A very promising phase II, double-blind and placebo-controlled study [41] performed by a Spanish group investigated the safety and efficacy of SCIT with the major allergen Der p 1 encapsulated in liposomes in 55 asthmatic patients sensitized to mite allergens over a 12-month period. The study demonstrated a significant clinical benefit in the active group receiving SCIT with a dramatic decrease in the symptom medication score, together with reduction of skin test reactivity in skin prick test and intracutaneous testing as well as a statistically significant decrease in bronchial allergen-specific reactivity.

To date, a phase III study on SCIT with allergen-adjuvanted liposomes in comparison to a conventional SIT without liposomes is still pending.

Aluminum hydroxide as mineral adjuvant

Aluminum hydroxide as a mineral adjuvant may be considered as the adjuvant with the longest clinical experience. Eighty years ago, Glenny *et al.* [42] were able to demonstrate that diphtheria toxoid adjunct with aluminum salt induces a better immunogenicity compared to the nonadjunct formulation. This was the basis for aluminum being used as an immunological adjuvant in different vaccines like diphtheria, pertussis, tetanus and typhus [11,43].

In SIT, aluminum hydroxide is applied to obtain a prolonged release of therapeutic allergens resulting in a reduced peak allergen concentration, though the immune stimulation is maintained [3,44]. In addition to this ‘depot effect’ of mineral salts, there are several presumptions regarding further immunological effects of these depot carriers. For example, a possible interaction with the innate immune system might cause the activation of inflammasomes or enhance activation of APCs, as well as modulate the APC–T cell interaction

[13[■],15,45]. Nevertheless, there is an unmet need for further research to reveal the exact mechanisms behind this phenomenon.

Early flow cytometry investigations by Durham and colleagues of presensitized mononuclear cells suggested an enhancement in the shift from a pro-allergic T_H2 to a more balanced T_H1 response through the costimulatory administration of aluminum hydroxide [45].

A large, multicenter, clinical, DBPC-controlled, dose-finding study (UK Immunotherapy Study) [46] with 410 patients suffering from a grass pollen allergy showed a clear dose-dependent clinical effect of SIT with aluminum hydroxide-adjuvanted grass pollen extracts. However, this trial was not designed to investigate the specific role of aluminum hydroxide as mineral adjuvant, as the dose of both allergen and adjuvant were reduced or augmented in the low versus the high-dose group, respectively.

A recently published trial of our group in 400 grass-pollen-allergic patients revealed immunological effects (IgE-blocking factor and IgG4) of a short-time preseasonal SCIT of approximately 10 weeks using a vaccine with an optimized ratio between natural grass pollen extract and adjuvanted aluminum hydroxide [47]. The specific role of adjuvanted aluminum hydroxide in SCIT would be best clarified in a head-to-head trial of natural allergen aluminum hydroxide-adjuvanted SCIT versus natural allergen SCIT and placebo with clinical and immunological outcomes. A trial such as this has not yet been performed.

Immunomodulatory sequences DNA (ISS, CpG motifs)

CpG motifs are conformed by nonmethylated cytosine–guanine dinucleotides derived from bacterial DNA. CpG motifs bind and activate Toll-like receptor 9 (TLR-9, see Fig. 1) [9]. These are mainly found on plasmacytoid dendritic cells (pDCs) as well as on B cells [48], which are involved in stimulating the immune response, especially to viruses, toward a Th1 pattern. Phase I studies already demonstrated that CpG motifs potentially enhance the immune response in the treatment of several tumors like glioblastoma [49], non-Hodgkin lymphoma [50], as well as in vaccination against the Hepatitis B or influenza viruses [51,52]. This Th1-skewed immunomodulating capacity makes CpG motifs promising candidates for adjuvants in SIT. After animal experiments revealed a promising shift toward a T_H1-response by conjugating the major allergen of ragweed pollen (Amb-a-1) to CpG motifs, this adjuvanted SIT was tested in clinical trials [53].

The first clinical, placebo-controlled, phase I trial revealed encouraging immunological effects as well as a good safety profile in a short-term SIT of only six injections of purified Amb a 1 linked to the 22-base long immunostimulatory phosphorothioate oligodeoxyribonucleotide 1018 [Amb a 1-immunostimulatory DNA sequence conjugate (AIC)] [54]. On the basis of the data, subsequent phase II trials were initiated. One of the studies [55] tested a preseasonal short-term SIT of 6 weeks in a group of 57 adults with a ragweed allergy. A predominant T_H1 and a decreased T_H2 cytokine profile as well as a diminished increase in eosinophilia in nasal biopsies was demonstrated after postseasonal nasal challenge. A subsequent phase II trial by Creticos *et al.* [56] demonstrated clinical efficacy of CpG-adjuvanted Ragweed SCIT (symptom score as well as RQLQ) combined with immunological effects such as an increase of Amb a 1-specific IgG antibody levels. However, the results of this trial were limited by a low number of treated patients.

In another phase II/III trial including 462 patients with ragweed allergic rhinitis, only a weak improvement in the primary outcome parameter [total nasal symptom score (TNSS)] was observed not reaching statistical significance [57]. On the basis of these results, Dynavax-Technologies Corporation ceased any further development in the field of CpG motifs as adjuvants in SIT (www.dynavax.com).

Other working groups tried to conjugate the A-type CpG motif to virus-like particles (VLPs) in order to achieve a prolonged degradation of the conjugate, a faster transport to the peripheral lymphoid organs, a better recognition by TLR-9-expressing cells and more effective uptake in the endosomal compartment, as well as providing a better therapeutic safety [58,59]. Kündig *et al.* [60] conducted a study with a Q β Vlp coupled to a synthetic 16-amino acid sequence of Der p 1, which was applied to 24 healthy volunteers. Only mild local reactions were observed. A rapid immunological response with increases of IgG (especially IgG 1 and IgG3) and high specific anti-Der p1 and anti Q β antibodies was induced. IgE antibodies did not measurably increase.

In a recently published phase I/IIa controlled, but open trial by the same study group tolerability, as well as clinical and immunological effects of a 10-week SIT with house dust mite allergens conjugated to VLPs were investigated [61]. The study revealed a significant reduction in the asthma and rhinitis score and an increased threshold in the titrated conjunctival challenge test as soon as 2 weeks after the end of the therapy course, combined with long-term effects even 36 weeks later. Furthermore,

Der p1 and Der f1 IgG1 and IgG4 levels increased only 10–12 weeks after the initiation of therapy.

Taken together, preliminary data from the first clinical trial indicate immunological and clinical effects as well as a good safety profile of a short-term SCIT with VLP-conjugated CpG motifs. However, these encouraging results will have to be confirmed in larger Phase II and III clinical trials.

Another phase IIb, dose-finding trial of our group on 299 patients sensitized to house dust mite with perennial allergy aimed to assess tolerability and clinical efficacy of an allergen-free modulator (CpG motif packaged into VLP without conjugation to mite allergen) in a short-term SCIT schedule [62²²]. For the high-dose vaccine, a significant improvement for symptom and medications scores as well as for Rhinitis-Quality-of-life-(RQLQ) scores was observed indicating this (nonallergen-specific) treatment as a promising candidate for a new immunotherapeutic.

Monophosphoryl lipid A

LPS is a component of the cell wall of Gram-negative bacteria with adjuvant capacities [11,63]. However, its use is limited by its toxicity. Small amounts of LPS can induce a state of shock and inflammation that mimics a Gram-negative sepsis when injected. A derivative of LPS, monophosphoryl lipid A (MPL) from salmonella Minnesota R595, partly achieves this retaining adjuvant effect but is associated with much lower toxicity than LPS [64]. Its adjuvant immunological effects are mediated by TLR-2 and TLR-4.

Today, MPL-containing preparations are widely used for various cancer or Hepatitis-B vaccines (www.gsk.com), but may also be promising candidates for adjuvanted SIT. In the first in-vitro study, Puggioni *et al.* [65] analyzed *in vitro* T_H1-associated and T_H2-associated cytokine production in mononuclear cells from grass-pollen-allergic donors with a timothy (*Phleum pratense*) extract alone or adjuvanted with MPL. Interestingly, there was a stronger T_H1 immune response, a higher IFN- γ and a diminished IL-5 production with MPL-adjuvanted vaccine in comparison to the MPL-free preparation. Moreover, other similar in-vitro trials found an increased production of the T_H1-associated cytokines IL-10 and IL-12 [66].

In an early, placebo-controlled, double-blind study [67] of our group that included 14 patients suffering from a tree pollen allergy, who received a preseasonal short-term therapy with MPL-adjuvanted birch-pollen allergoid, an enhanced production of IFN- γ and a suppression of the T_H2-related

cytokines IL-4 and IL-5 during the season in comparison to the untreated placebo group were demonstrated.

A larger DBPC-phase III study [68] with 141 patients suffering from a grass pollen allergy could reveal a clinical efficacy in case of SCIT using a grass pollen allergoid bound to tyrosine as depot carrier and MPL as adjuvant in comparison to the placebo group. In this trial, there was a significant improvement in the nasal and conjunctival symptom score as well as in the combined symptom medication score after a preseasonal short-term therapy of only four injections. Furthermore, active treated patients showed diminished skin reactivity. The increase in grass-pollen-specific IgG and the corresponding suppression of the seasonal IgE rise (blunting) found in this trial underline the immunological mechanisms involved in this adjuvanted SCIT. A recently published clinical trial on more than 1000 allergic patients mainly performed in the USA and Canada confirmed the efficacy of a preseasonal short-time SCIT with MPL-adjuvanted grass-pollen SCIT [69].

However, data on the distinctive role of MPL as an adjuvant in SCIT (as directly compared to SCIT with an adjuvant-free allergen vaccine) are still lacking but should be investigated in future trials.

Heydenreich *et al.* [70²²] recently detected a complex mixture of bacteria and molds on grass pollen. Contact of immature dendritic cells from grass-pollen-allergic donors with supernatants of homogenized Gram-positive bacteria induced maturation of dendritic cells as measured by upregulation of CD80, CD83 and CD86 and by enhanced production of IL-6, IL-12p40 and TNF- α , which was less pronounced compared with effects induced by LPS. Consequently, stimulation of autologous CD4(+) T cells with supernatants of homogenized Gram-positive bacteria plus grass-pollen allergen-pulsed dendritic cells led to an enhanced proliferation and production of IL-4, IL-13, IL-10, IL-17, IL-22 and IFN- γ production compared with T cells that were stimulated with allergen-pulsed immature dendritic cells alone, whereas production of the transcription factor for regulatory T cells Foxp3 was not significantly affected [70²²]. These data indicate that grass pollen is colonized by several microorganisms that influence the immune response differently. Similarly to LPS, supernatants of homogenized Gram-positive bacteria may serve as adjuvants by augmenting dendritic cells' maturation and inflammatory Th1, Th2 and Th17 responses helping to initiate allergic immune responses. Taken together, these results indicate that the blocking of such microbial augmentation

Table 2. Overview on different types of adjuvants and vector systems investigated in preclinical studies and clinical trials

Type of adjuvant	Immunological effects	Current phase of clinical trial	Species	Key results	Group
1 Bet v1 coated by microspheres	Increase in INF-gamma and IL-10 production in isolated T-cells	–	Animal (mouse model)	Tolerogenic effect of Bet v1 allergen coated by microspheres	Scholl <i>et al.</i> 2004 [37]
2 Oligomannose-coated liposomes	Induction of CD8 ⁺ and CD4 ⁺ Tregs and modulating humoral immune response	–	Animal (mouse model)	Possible immunotherapy for food allergies	Kawakita <i>et al.</i> 2012 [40]
3 Der p 1 encapsulates in liposomes	Increase of serum levels of specific IgG, only transient (initial) increase of specific IgE	Phase II DBPC study	55 Asthmatic patients sensitized to mite allergens	Significant decrease in the symptom medication score, reduction of skin test-reactivity and decrease in bronchial allergen-specific reactivity	Basomba <i>et al.</i> 2002 [41]
5 AH-Adjuvanted grass pollen extracts	–	Multicenter, DBPC-controlled, dose-finding study	410 Grass-pollen-allergic patient	Dose-dependent clinical effects of fixed combination of SIT with AH-Adjuvanted grass pollen extracts	'UK Immunotherapy Study', Frew <i>et al.</i> [46]
6 Optimized ratio between natural grass pollen extracts and AH	Induction of IgE-blocking factor and IgG4	Phase II Multicenter DBPC-controlled short-term (10 weeks) SCIT	400 Grass-pollen-allergic patient	Significant immunological effects with acceptable safety profile	Pfaar <i>et al.</i> [47]
7 Immunomodulatory sequences DNA (ISS, CpG motifs)	Predominant Th1 and decreased Th2 cytokine profile as well as a diminished increase in eosinophilia in nasal biopsy	Phase II study preseasonal short-term (6 weeks) SCIT	57 Ragweed-allergic adult patients	Significant immunological effects as 'proof-of-concept'	Tulic <i>et al.</i> 2004 [55]
8 Immunomodulatory sequences DNA (ISS, CpG motifs)	–	Phase II/III multicenter DBPC trial	462 Ragweed-allergic adult patients	Weak improvement in the TNSS in first season, not reaching statistical difference between active and placebo treatment	Busse <i>et al.</i> 2006 [57]
9 Q β VLP coupled to a synthetic 16 amino acid sequence of Der p 1	Rapid Increase of IgG (IgG1 and IgG3) and anti-Der p1 and anti-Q β antibodies	Phase I	24 Healthy volunteers	Rapid immunological effects in healthy volunteers. Only mild local reactions	Kündig <i>et al.</i> 2006 [60]
10 House dust mite allergens conjugated to VLPs	Increase of Der p1 and f1 IgG1 and IgG4 – serum levels	Single-center, Phase I/IIa controlled open trial (10 weeks lasting)	20 House dust mite allergic patients	Immunological effects and significant reduction in the asthma and rhinitis score. Increased threshold in the conjunctival challenge test (CPT)	Senti <i>et al.</i> 2009 [61]

Table 2 (Continued)

Type of adjuvant	Immunological effects	Current phase of clinical trial	Species	Key results	Group
11 CpG motif packaged into VLP without allergen	–	DBPC, phase IIb, dose-finding trial	299 House dust mite allergic patients	Significant improvement for symptom and medication scores as well as rhinitis-quality-of-life score, increase in CPT threshold	Klimek <i>et al.</i> 2011 [62 [■]]
12 MPL adjuvanted to timothy grass pollen extract	Stronger Th1 response, higher INF- γ and a diminished IL-5 production	Preclinical, in-vitro study comparing extracts with and without MPL as adjuvant	PBMCs from grass-pollen-allergic patients	Immunological effects in PBMCs indicating additional value of MPL	Puggioni <i>et al.</i> 2005 [65]
13 Grass pollen allergoid bound to tyrosine as depot carrier and MPL as adjuvant	Increase in grass pollen-specific IgG and suppression of the seasonal IgE rise (blunting)	DBPC-phase III study	141 Grass-pollen-allergic patients	Significant improvement in the nasal, conjunctival and combined symptom medication score	Drachenberg <i>et al.</i> 2001 [68]
14 Grass pollen allergoid bound to tyrosine as depot carrier and MPL as adjuvant	Significant differences in IgG levels between active and placebo treatment	DBPC-phase III study	1028 (!) Grass-pollen-allergic patients	Significant, but relatively small improvement in combined symptom-medication-score in 4 peak pollen weeks	Dubuske <i>et al.</i> 2011 [69]
16 Supernatants of homogenized Gram-positive bacteria	Induced maturation of DC as measured by upregulation of CD80, CD83 and CD86 and by enhanced production of IL-6, IL-12p40 and TNF- α	Preclinical, in-vitro study	Immature DC from grass-pollen-allergic donors	Supernatants of homogenized Gram-positive bacteria may serve as adjuvants similar to LPS	Heydenreich <i>et al.</i> 2012 [70 [■]]
17 MPL-adjuvanted SLIT	Dose-dependent increases in grass pollen-specific IgG levels	Phase I/II dose-ranging placebo-controlled 'proof-of-concept' clinical trial of 10 weeks	80 Grass-pollen-allergic patients	Good tolerability to all investigated MPL doses, with possible immunological and clinical effects (NCT)	Pfaar <i>et al.</i> 2011 [83]

AH, aluminum hydroxide; DC, dendritic cell.

Toll-like receptors and their possible ligands (adapted from Janeway's Immunobiology, Seventh Edition by Murphy *et al.* Reproduced by permission of Garland Science/Taylor & Francis LLC Copyright 2008 [21] and Barton GM, Medzhitov R. Toll-like receptors and their ligands. *Curr Top Microbiol Immunol.* 2002;270:81–92[20].

of the allergic immune response may be a promising option for adjuvanted SIT in the future.

ADJUVANTS IN SUBLINGUAL IMMUNOTHERAPY CLINICAL TRIALS

More recently, some attempts have been undertaken to investigate the role of adjuvants in SLIT [71–73]. Oral dendritic cells (Langerhans cells) may be the ideal target cells for adjuvanted SLIT vaccines enhancing the tolerance mediated by these cells mimicking the natural contact of the individuals' immune system to allergens aimed to maintain a tolerogenic state in the oral cavity [74,75].

Probiotics as adjuvants in SLIT have been investigated first in mice models [13[■]]. In these studies, the administration of commensal bacteria like *Lactobacillus helveticus* adjuvanted to allergens at the mucosal site resulted in an increased T_H1 response that indicates potentiated tolerance induction by SLIT [76]. Moreover, in-vitro experiments with human monocyte-derived dendritic cells indicated that probiotical adjuvants such as *Lactobacillus reuteri* and *Lactobacillus casei* may ligate C-type lectin dendritic cells-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) and thereby actively prime these dendritic cells to induce Treg cells and increase the levels of IL-10 [77].

Another approach investigated the use of detoxified bacterial toxins such as cholera toxin or lymphotoxin or recombinant plague antigens as mucosal adjuvants in animal models [78,79]. Moreover, for carbohydrate polymers adjuvanted to allergens as 'microparticles', the first immunological effects via the sublingual route have been demonstrated in murine models [13[■],80].

Interestingly, preliminary in-vitro data by Allam *et al.* [81,82[■]] gave evidence that human oral dendritic cells express high levels of lipopolysaccharide receptors (CD14) as well as TLR-4 receptors on their surface. Subsequent in-vitro experiments by the same group presented an enhanced IL-10 production and a suppression of T-cell proliferation when oral dendritic cells were stimulated with TLR-4 ligands [74].

On the basis of these findings, our group performed the first phase I/II, dose-ranging, placebo-controlled, clinical trial with MPL-adjuvanted SLIT on 80 grass-pollen-allergic adult patients [83]. In addition to a good tolerability to all investigated MPL doses, patients receiving the highest amount of MPL revealed early immunological effects as well as the highest proportion of negative allergen-specific nasal challenges 10 weeks after the initiation of a 2-month SLIT course. These encouraging preliminary results on adjuvanted SLIT in

humans should now be confirmed in larger clinical phase II/III trials.

CONCLUSION

Several clinical trials in which adjuvanted immunotherapy is investigated – seemingly with success – are an encouraging example of how basic knowledge of underlying mechanisms can be applied to improve treatment. Adjuvants added to allergen SIT or vector systems to ease allergen delivery may enhance the efficacy and reduce safety problems, both for the subcutaneous and for the sublingual route. APCs are crucial for the beneficial effect of most adjuvants. Interestingly, the most suitable adjuvant might differ between both the routes, because of the unique characteristics of the APCs in the oral cavity. Although several types of adjuvanted SIT are already on the market, there is still room for improvement as the fine-tuning of dosing of the adjuvant, dosing in relation to the allergen and dose intervals has not yet been completed. Moreover, the future promises the launch of possibly even better developed adjuvanted SIT products (Table 2).

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Conflicts of interest

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Moises Calderon has acted as a consultant for ALK-Abello and a speaker for ALK-Abello, Allergy Therapeutics (UK) Ltd, MSD and GSK. He has appeared on advisory boards for Allergy Therapeutics (UK) Ltd and ActoGeniX, received honoraria from ALK-Abello, Allergy Therapeutics (UK) Ltd, Schering-Plough, and MSD, and research grants from ALK Abello and GSK.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 674).

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