

Adjuvants in Allergen-Specific Immunotherapy: Modulating and Enhancing the Immune Response

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■ Abstract

Allergen-specific immunotherapy (AIT) is the only treatment that can affect the natural course of allergic diseases such as allergic asthma, allergic rhinitis, and IgE-mediated food allergy. Adjuvants are used to induce a quicker, more potent, and longer-lasting immune response. Only 4 compounds are used as adjuvants in currently marketed AIT products: aluminum hydroxide, calcium phosphate, microcrystalline tyrosine (MCT), and monophosphoryl lipid A (MPL). The first 3 adjuvants are delivery systems with a depot effect, although they may also have immunomodulatory properties. These first-generation adjuvants are still widely used, especially aluminum hydroxide. However, aluminum is subject to limitations. MCT is the depot formulation of L-tyrosine; it enhances IgG production without inducing a significant increase in IgE, is biodegradable, and has good local and systemic tolerability. In turn, MPL is an immunostimulatory agent that is the only second-generation adjuvant currently used for AIT. In addition, multiple adjuvants are currently being studied, including immunostimulatory sequences (ISSs), nanoparticles (liposomes, virus-like particles, and biodegradable polymers), and phosphatidylserine derivatives.

In a murine model of allergic bronchial inflammation by sensitization to olive pollen, the specific IgE level was significantly higher in sensitized mice treated with olive pollen and aluminum hydroxide. However, specific IgE levels were significantly reduced and bronchial hyperreactivity significantly improved in sensitized mice treated with olive pollen and bacterial derivatives (MPL or ISSs).

Key words: Asthma. Allergen-specific immunotherapy. Adjuvant. Aluminum hydroxide. Calcium phosphate. Microcrystalline tyrosine. Monophosphoryl lipid A. Immunostimulatory sequences. Nanoparticles.

■ Resumen

La inmunoterapia específica con alérgenos (ITE) es el único tratamiento con potencial para modificar la evolución natural de enfermedades alérgicas como el asma alérgica, la rinitis alérgica y la alergia a alimentos mediada por IgE. Los adyuvantes se usan para provocar una respuesta inmune más rápida, más potente y de mayor duración.

Hasta ahora, solo cuatro compuestos se usan como adyuvantes en los productos de ITE comercializados actualmente: hidróxido de aluminio, fosfato cálcico, tirosina microcristalina (MCT) y monofosforil lípido A (MPL). Los tres primeros son sistemas de liberación retardada (efecto *depot*), aunque también podrían tener propiedades inmunomoduladoras. Estos adyuvantes de primera generación todavía se usan ampliamente, sobre todo el hidróxido de aluminio. Sin embargo, el aluminio tiene algunas limitaciones. MCT es la formulación de liberación retardada de la L-tirosina; aumenta la producción de IgG sin provocar un incremento significativo de IgE, es biodegradable y tiene una buena tolerabilidad local y sistémica. A su vez, MPL es un inmunoestimulador y es el único adyuvante de segunda generación usado actualmente en ITE. Además, hay múltiples adyuvantes en investigación, como las secuencias inmunoestimuladoras (SIE), nanopartículas (liposomas, partículas similares a virus y polímeros biodegradables) y derivados de la fosfatidilserina.

En un modelo murino de inflamación bronquial alérgica por sensibilización al polen de olivo, el nivel de IgE específica fue significativamente mayor en los animales sensibilizados tratados con polen de olivo e hidróxido de aluminio. Sin embargo, en los animales sensibilizados tratados con polen de olivo y derivados bacterianos (MPL o SIE) se observó una disminución significativa del nivel de IgE específica y una mejoría significativa de la hiperreactividad bronquial.

Palabras clave: Asma. Inmunoterapia específica con alérgenos. Adyuvante. Hidróxido de aluminio. Fosfato cálcico. Tirosina microcristalina. Monofosforil lípido A. Secuencias inmunoestimuladoras. Nanopartículas.

1. Introduction

Allergen-specific immunotherapy (AIT) is the only disease-modifying therapy for allergic asthma, allergic rhinoconjunctivitis, and other allergic conditions [1]. Its aim is to induce a tolerogenic response against the allergen of interest [2]. Recent systematic reviews and meta-analyses have confirmed that AIT is effective in reducing symptoms and medication needs in patients with allergic asthma [3] and rhinoconjunctivitis [4]. Moreover, AIT reduces the risk of developing asthma, at least in the short term, in patients with allergic rhinitis [5]. AIT is also effective in patients with IgE-mediated food allergy [6] and insect venom allergy [7].

2. Mechanism of Action of Allergen-Specific Immunotherapy

AIT mechanisms are not fully understood. Based on current knowledge, effective AIT sequentially activates multiple mechanisms and induces cellular and molecular changes (Figure 1). This complex mechanism of action of AIT occurs in 3 phases: rapid desensitization, early tolerance, and sustained tolerance [8]. Rapid desensitization is characterized by an early fall in degranulation of mast cells and basophils, probably due to rapid upregulation of histamine type 2 receptor. The second phase, early tolerance, includes a decrease in interleukin (IL) 4-secreting T_H2 cells and increases in IL-10-secreting Treg cells and Breg cells. There is a switch from a T_H2 -type response to a T_H1 -type response, with increases in IL-10 and transforming growth factor β production. There is also an increase in Treg cells that correlates with clinical improvement. Finally, sustained tolerance implies that Treg cells stimulate B cells to produce allergen-specific IgG4, a tolerogenic high-affinity blocking antibody that competes with allergen-specific IgE, thus avoiding the allergen-induced release of mediators by mast cells and basophils. These sequentially activated mechanisms induce immune tolerance that attenuates or even abolishes both the acute (early) phase of allergic reaction and any subsequent immunologic event [8].

3. Adjuvants in Allergen-Specific Immunotherapy

In allergy, an adjuvant is a substance or compound that is co-administered with the allergen extract and has the ability to increase allergen immunogenicity and/or to modulate the immune response [9]. Adjuvants are used to

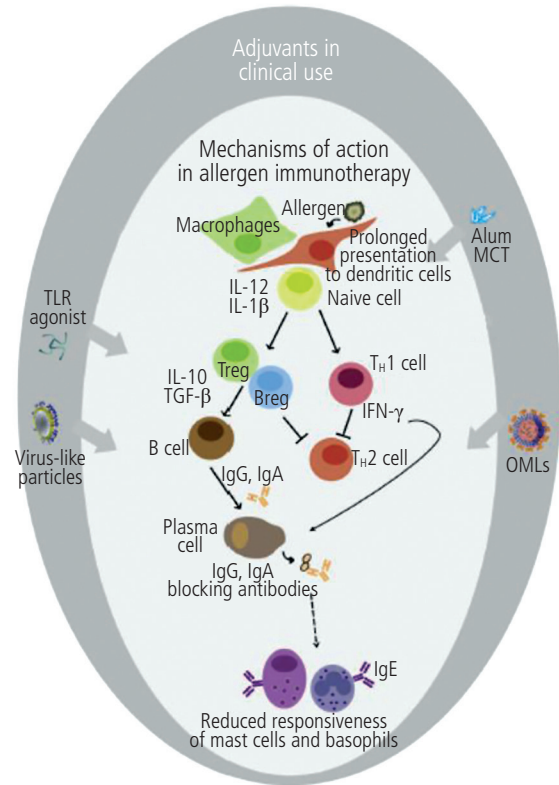


Figure 1. Immune mechanisms of allergen-specific immunotherapy and adjuvants. Modified from Chesné et al, 2016 [49]. MCT indicates microcrystalline tyrosine; TLR, Toll-like receptor; OML, oligomannose-coated liposomes.

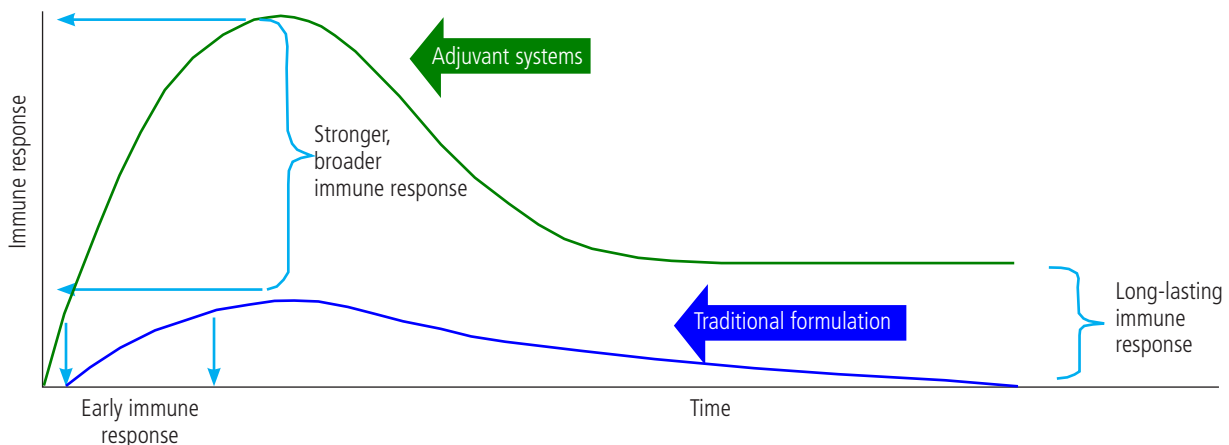


Figure 2. Advantages of adjuvant-formulated vaccines. Modified from Pulendran et al, 2006 [10].

Table 1. Adjuvants in Allergen-Specific Immunotherapy

DELIVERY SYSTEMS
Aluminum hydroxide
Calcium phosphate
Microcrystalline tyrosine
IMMUNOMODULATORY AGENTS
Monophosphoryl lipid A
ADJUVANTS UNDER RESEARCH
Immunostimulatory sequences
Nanoparticles
Liposomes
Virus-like particles
Immunostimulatory complexes
Biodegradable polymers
Phosphatidylserine

induce a quicker, more potent, and longer-lasting immune response to AIT (Figure 2) [10]. They have been widely used to improve and simplify AIT, because they make it possible to reduce the number of doses needed. Furthermore, research is currently focused on finding more effective and safer compounds [9].

The ideal adjuvant should be biodegradable, stable, sustainable, nontoxic, and cost-effective. It should also promote an appropriate immune response [11]. Currently, the desired characteristics of the ideal adjuvant for AIT have been extended: it should also combine optimal physicochemical properties (eg, particle morphology and adsorption capacity) with biological activity properties (eg, enhancing IgG4 antibody titers and avoiding the T_H2 -type immune response) [12]. In addition, the European Medicines Agency released a guideline on adjuvants in vaccines for human use; the quality and nonclinical (including toxicity) chapters are applicable to AIT [13].

Different classifications of adjuvants have been proposed according to the nature of the compound or the mechanisms of action. According to authors such as O'Hagan et al [14], Klimek et al [12], and Moreno et al [15], from a functional point of view, adjuvants can be categorized as delivery systems, which modulate antigen presentation, and immunomodulatory agents, which are direct modulators of the immune response, although some adjuvants could be included in both categories [14].

Many compounds have proved their potential as adjuvants. However, a few have been studied for AIT (Table 1), and an even smaller number have reached the clinical development stage. Only 4 compounds are used in currently marketed AIT products: aluminum hydroxide, calcium phosphate, microcrystalline tyrosine (MCT), and monophosphoryl lipid A (MPL). The first 3 adjuvants are considered to be delivery systems, although they may also have immunomodulatory properties. In turn, MPL is an immunostimulatory agent. The mechanisms of action of each adjuvant are described below.

4. Delivery Systems

These systems have been widely used for many years and are considered first-generation adjuvants. They have very different structures and compositions but quite similar mechanisms of action [16]. Traditionally, they have been considered particulate carriers that deliver allergens to immune cells and prolong allergen presence at the injection site [14]. This slow release of allergen (depot effect) increases the exposure time of the allergen to the immune system, stimulating the production of high and sustained antibody titers. However, it has been suggested that the depot effect is not the primary mode of action, although it is related to adjuvant tolerability [12].

4.1. Aluminum Hydroxide

Aluminum compounds have been used as adjuvants for almost a century [9] and remain the most frequently used adjuvants in vaccination and immunotherapy [17]. Aluminum as an adjuvant in AIT enhances allergen immunogenicity and tolerability but also raises IgG and IgE titers [12]. However, despite its widespread use, its exact mechanism of action as an adjuvant remains unknown. Available experimental evidence is scarce, although the increased humoral immunity caused by aluminum adjuvants can be explained by 3 mechanisms. The first is the formation of a sustained antigen release depot, which would enhance antibody production [18] and could contribute to the safety of AIT [17]. The second mechanism is the conversion of a soluble antigen into a particulate form and subsequent phagocytosis by antigen-presenting cells (APCs). The third mechanism is the induction of inflammation, which results in the recruitment and activation of APCs and antigen capture [18]. When injected, the aluminum-formulated allergen causes inflammation at the injection site. Immediately after injection, cells release chemokines and cytokines that recruit cells of the innate immune system (monocytes, eosinophils, neutrophils, and others) to the injection site. Moreover, damaged tissue releases the endogenous danger signal uric acid. Subsequently, monocytes react to aluminum and uric acid through activation of the nucleotide-binding and oligomerization domain-, leucine-rich repeat- and pyrin domain-containing 3 (NLRP3, also known as NALP3) inflammasome [19], which is a caspase-1 activating complex that induces inflammation. Monocytes capture the allergen and process it in conjunction with the major histocompatibility complex (MHC) I and MHC II molecules, while migrating to the draining lymph nodes [19].

However, aluminum is subject to limitations, which are related to its potential adverse effects reviewed elsewhere [19]. Since it can induce a T_H2 response [17], it is used in experimental animal models of allergic asthma, allergic rhinitis, and eosinophilic esophagitis [19]. This T_H2 polarization may constitute a suboptimal effect in AIT.

Other limitations of aluminum are potential acute and chronic inflammation at the injection site [17], as observed in more than 15% of patients of a large observational study [20]. With regard to systemic adverse events, aluminum has low biodegradability and could accumulate after repeated administration in AIT [21]. In addition, it has been suggested

that aluminum adjuvants could be related to the autoimmune/ autoinflammatory syndrome induced by adjuvants (ASIA). However, current evidence does not support a relationship between aluminum and ASIA [22]. Although no clear associations between vaccinations using aluminum adjuvants and serious adverse events have been found to date, routine use of aluminum adjuvants in AIT is questionable [23]. There is increasing concern about chronic aluminum toxicity, and authorities from different countries are inspecting the safety of aluminum [17].

4.2. Calcium Phosphate

Calcium phosphate is a mineral salt that is used as a depot adjuvant in AIT. It is used less commonly than aluminum hydroxide [12], although it may offer some advantages because it is naturally present in the body and is biodegradable and biocompatible. Furthermore, results of studies in humans suggest that calcium phosphate is able to adsorb antigens, does not induce IgE production, and markedly increases IgG levels [24].

Calcium phosphate could be an alternative to aluminum adjuvants [24]. However, in animal models, it also induced local adverse reactions and showed lower adjuvant activity than aluminum [25].

4.3. Microcrystalline Tyrosine

L-tyrosine is a biodegradable amino acid of natural origin with ideal adjuvant properties [26]. The patent of its depot formulation, MCT, is registered for use as an immunomodulator and adjuvant.

Antigen-adjuvant adsorption affects vaccine potency, and MCT has shown adsorption capacities higher than 95% for allergens and MPL, with favorable stability [27]. Given that the half-life of MCT is 48 hours [26], allergen release is sustained and immune exposure is prolonged. MCT is naturally metabolized (biodegradable): L-tyrosine is metabolized after release from the injection site, thus reducing the risk of granulomas observed with other depot adjuvants [26]. Moreover, MCT enhances IgG production with limited increases in IgE levels [26].

MCT immunogenicity was studied in BALB/c mice immunized with ovalbumin adsorbed to MCT or aluminum. Upon a second exposure to ovalbumin, the increase in T_H1 cytokines (interferon γ [IFN- γ]) and IL-10 was higher with MCT. IgG1 and IgG2a production was similar with both adjuvants, whereas IgE production was higher with aluminum. Thus MCT was considered a biodegradable alternative to aluminum [28].

Finally, preclinical and clinical studies have suggested that L-tyrosine is safe as an adjuvant in humans. No genotoxicity, mutagenicity, carcinogenicity, or teratogenicity has been observed. MCT has shown good local and systemic tolerability in children and adults. It is contraindicated in tyrosine metabolism disorders [26].

5. Immunomodulatory Agents

Immunomodulatory agents consist of a heterogeneous group of products that act directly on immune cells to modulate

their antigen response. Their mechanism of action is based on the activation of innate immune receptors on macrophages, dendritic cells, and other APCs [14].

The so-called danger signals trigger innate and adaptive responses by the immune system. These signals include pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns. PAMPs are absent in the host and are exogenous danger signals that induce signals associated with innate immunity. Major PAMPs are microbial nucleic acids, lipoproteins, surface glycoproteins, and membrane components such as lipopolysaccharide. Pathogen recognition receptors recognize PAMPs and include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors, and other types of receptor [29]. TLRs recognize PAMPs and activate many intracellular signaling pathways that produce proinflammatory and antimicrobial responses, resulting in the production of cytokines, chemokines, cell adhesion molecules, and immunoreceptors [29]. The immune response differs according to the TLR-mediated activated pathway: T_H1 response for TLR-4 and TLR-9, and T_H2 response for TLR-2 [30]. Second-generation adjuvants are synthetically bacterial derivatives that interact with TLRs on immune cells. They are not delivery systems with a depot effect, but immunomodulators that potentiate immune cells/pathways [12]. The most studied second-generation adjuvants in AIT are MPL and immunostimulating sequences (ISSs) of synthetic oligodeoxynucleotides (ODNs) containing unmethylated cytosine-phosphorothioate-guanine (CpG) motifs.

5.1. Monophosphoryl Lipid A

The only second-generation adjuvant approved for AIT is MPL (GSK, United Kingdom), a detoxified derivative of lipopolysaccharide of *Salmonella minnesota* R595. Lipopolysaccharide molecules are found in the outer membrane of gram-negative bacteria and are another example of PAMPs. Recognition of lipopolysaccharide by pathogen recognition receptors of APCs elicits a potent immune response [12]. The immunological effects of lipopolysaccharide are mediated by its diphosphoryl lipid A moiety, which is toxic to most animal species. However, MPL is obtained by a hydrolytic process followed by chromatography and has 1 phosphoryl group and 6 acyl side chains (lower fatty acid content) but no polysaccharide side group. Thus, MPL retains the immunomodulatory properties of lipopolysaccharide but without its toxicity [12].

MPL was the first TLR agonist included in a licensed human vaccine [16]. It has been used in many vaccine formulations, notably in hepatitis B and human papillomavirus vaccines [31], and has been delivered in millions of licensed vaccines with a low incidence of adverse events. In addition, since 1999, allergic patients have been treated with allergy vaccines that incorporate MPL as an adjuvant [32]. MPL has also been included in clinical trials with *Plasmodium falciparum* vaccines [33] and herpes simplex virus type 2 vaccines [34]. Moreover, the United States Food and Drug Administration recently approved an MPL-containing vaccine for the prevention of herpes zoster [35].

MPL interacts with TLR-4. After stimulation of these receptors by MPL, dendritic cells mature and produce

cytokines, especially IL-12, which activate T cells to mature into the T_H1 phenotype [36]. In an in vitro study, peripheral blood mononuclear cells obtained from 13 patients with grass pollen allergy (*Phleum pratense*) were cultured with a *P. pratense* extract and MPL. Allergen-induced IFN- γ production increased ($P < .001$), while IL-5 production decreased ($P < .01$). When a neutralizing antibody against IL-12 was added, 95% inhibition of MPL-induced IFN- γ production was observed. This study showed that MPL deviated T_H2 cell responses to T_H1 responses in an IL-12 and monocyte-dependent way [37].

6. Adjuvants Under Research

6.1. Immunostimulatory Sequences

Bacterial DNA is a clear example of a PAMP. It contains unmethylated CpG motifs, which are rare in human DNA. During infection, these motifs are recognized, and a protective immune response is triggered. Synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG-ODNs) are ISSs that mimic the immunostimulatory activity of bacterial DNA [38]. CpG-ODNs are recognized by the pathogen recognition receptor TLR-9 on B-cells, dendritic cells, and other cell types and promote T_H1 and Treg responses [39]. Although a relatively old concept, CpG-ODNs have emerged recently as adjuvants in immunotherapy and have been used for different types of immunotherapies [40-42].

The several types of synthetic CpG-ODNs differ in terms of their structures and biological properties [38]. The ISS 5'-TGACTGTGAACGTTTCGAGATGA-3', also known as ISS-1018, has been studied as an adjuvant in AIT. A protein-linked immunostimulatory sequence 1018 (PLI-1018) was created with Amb a 1, the immunodominant allergen of ragweed pollen, and ISS-1018. In cultures of peripheral blood mononuclear cells from patients with ragweed allergy, the increase in IFN- γ and the reduction in IL-4 were higher with PLI-1018 than with Amb a 1 and free ISS-1018. These results suggested that ISS-1018 has strong cytokine-modulating activity when administered together with an antigen [43]. A vaccine composed of Amb a 1 conjugated with ISS-1018 was subsequently assessed in a randomized double-blind placebo-controlled phase 2 study. Patients with ragweed allergy ($n=25$) received 6 weekly injections of vaccine or placebo before the first ragweed season and were followed for 2 consecutive seasons. Nasal scores were better in vaccinated patients during both seasons, suggesting that the vaccine had long-term clinical efficacy [44]. However, in a subsequent phase 2b study ($n=738$), no differences were found between a vaccine composed of Amb a 1 conjugated with ISS-1018 and placebo, and the project was discontinued [45]. In any case, ISSs are still under investigation as adjuvants in AIT. Promising results have been reported for sublingual AIT using CpG-ODNs as adjuvants in a murine model of food allergy [46].

6.2. Nanoparticles

Nanoparticles are under research as new adjuvants/delivery systems in AIT. Nanoparticles protect the allergen from

degradation, achieve high concentrations at the site of action, and prevent IgE recognition. They have a strong immunogenic effect with low allergenic potency [47,48] and combine the potential of optimal allergen presentation [9] with intrinsic adjuvant properties [12].

Encapsulation of allergens into nanoparticles has great potential for enhancing AIT [47]. It maintains and even enhances allergen immunogenicity because it protects allergens from hydrolysis and/or enzymatic degradation [9]. It also protects against environmental factors such as a broad range of pH values and temperatures [49]. Furthermore, encapsulation prevents allergen recognition by IgE from mast cells or basophils, thus potentially reducing the risk of adverse events [48]. In addition, it offers the possibility of co-delivering the allergen and immunostimulatory agents such as CpG motifs or lipopolysaccharide derivatives [9]. Finally, it limits allergen capture to those cells that have the ability to phagocytose the nanoparticle [49]. Nanoparticles have shown an advantage as adjuvants in oral immunotherapy against food allergens in animal models [50-52].

Other currently investigated adjuvants include liposomes, virus-like particles, and polymers. Here, we briefly describe their main features.

6.2.1. Liposomes

Liposomes are synthetic spheres composed of lipid bilayers. They can encapsulate allergens and act as both delivery systems and adjuvants. Initial clinical studies suggested that liposomes were not appropriate for AIT. However, in a randomized, double-blind, placebo-controlled trial in 55 patients with allergic asthma treated with a liposome-encapsulated extract of *Dermatophagoides pteronyssinus*, increased specific IgG, IgG1, and IgG4, decreased sputum eosinophils, and lower clinical scores were observed in vaccinated patients [53]. Newer liposome formulations are promising [48], although no safety data have been reported to date [54].

6.2.2. Virus-Like Particles

Virus-like particles (VLPs) are formed by a high number of copies of a viral capsid protein that mimics a viral scaffold with repetitive features [12]. Allergens can be conjugated onto VLPs, which are recognized as PAMPs by the human innate humoral immune system [12], even without T cell cooperation [55].

A peptide sequence of Der p 1 was covalently coupled to a VLP derived from the bacteriophage Q β coat proteins. A significant increase in specific IgG was observed after administration of Der p 1 VLPs to healthy volunteers [56]. A house dust mite allergen preparation combined with a VLP adjuvant consisting of the bacteriophage Q β coat proteins filled with the CpG G10 (QbG10) was subsequently administered to patients with house dust mite allergy. Specific IgG also increased, and symptoms of rhinitis and allergic asthma were significantly improved [57]. In a third study, 299 patients with house dust mite allergy received QbG10 alone or placebo without coadministration of allergen. However, scores for rhinoconjunctivitis and quality of life were significantly

better in patients treated with a high dose of this allergen-free immunomodulator than in those treated with placebo [58]. In a more recent double-blind, randomized study in patients with mild-to-moderate persistent allergic asthma, treatment with QbG10 improved asthma symptoms, salbutamol use, and the Asthma Control Questionnaire score [59]. These results suggest that allergens might not be needed for AIT [55]. Other ongoing studies of VLPs in AIT include a trial of a VLP-containing peanut allergy vaccine [60]. In addition, AIT with VLPs has proven safe and well-tolerated in clinical trials [55].

6.2.3. Biodegradable Polymers

Polymeric nanoparticles, especially the biodegradable ones, have great potential as drug delivery systems [49]. The most studied natural biodegradable polymer in AIT is chitosan, a natural mucoadhesive polysaccharide derived from crustacean cells. It is biocompatible, biodegradable, and nontoxic. It increases the penetration of macromolecules across the mucosa [12]. In turn, the most extensively studied synthetic biodegradable polymer in AIT is the polylactide-co-glycolic acid (PLGA), a polyester used for preparation of nanoparticles. PLGA is biocompatible, biodegradable, and safe [12]. However, chitosan and PLGA have only been studied in animal models [49].

6.2. Phosphatidylserine

Phosphatidylserine (PS) derivatives are being studied as potential biological immunostimulatory agents. PS is known to be a surface marker for apoptosis, although it is also essential for downregulation of macrophages [12].

Three PS derivatives could potentially be useful in AIT: dioleoyl PS, lyso oleoyl PS, and stearoyl arachidonoyl PS. Their effects on immunoglobulins are different: dioleoyl PS stimulates IgG and IgA, lyso oleoyl PS stimulates IgA, and stearoyl arachidonoyl PS inhibits IgE. They have been shown not to be mutagenic or cytotoxic [12].

7. Comparison of Adjuvants in AIT

Table 2 shows the mechanisms of action and the main advantages and disadvantages of the 4 adjuvants currently used in AIT.

The clinical and immunological efficacy of different adjuvants for AIT were compared in a murine model. First, a new murine model of allergic bronchial inflammation by sensitization to olive pollen (*Olea europaea*) was developed [61]. Second, the immune response was assessed in sensitized mice treated with *O europaea* coadministered with different adjuvants (aluminum hydroxide, calcium phosphate, MPL, and ISSs). The results showed that specific IgE was significantly higher in the group of mice treated with *O europaea* and aluminum hydroxide, and significantly lower in mice treated with *O europaea* plus ISS or MPL. Moreover, IFN- γ levels were significantly higher in mice that received *O europaea* and ISSs or MPL than in those that received other adjuvants. As for bronchial hyperreactivity and cellular lung inflammation, a significant improvement was only observed in mice treated with *O europaea* and bacterial derivatives (MPL and ISSs) [62].

Table 2. Adjuvants Currently Used in Allergen-Specific Immunotherapy

Type	Mechanism of action	Advantages	Disadvantages
DELIVERY SYSTEMS			
Aluminum hydroxide	Depot effect Sustained antigen release Conversion of soluble antigen into a particulate form and subsequent phagocytosis by APCs Induction of inflammation	Widely used	Nonbiodegradable T _{H2} polarized Local adverse events Safety concerns
Calcium phosphate	Depot effect Mechanism of action: similar to aluminum hydroxide	Naturally present in the organism, biodegradable, and biocompatible	Lower adjuvant activity compared to aluminum
Microcrystalline tyrosine	Depot effect Inflammasome activation	Biodegradable No significant IgE increase Good local and systemic tolerability	Contraindicated in tyrosine metabolism disorders
IMMUNOMODULATORY AGENTS			
Monophosphoryl lipid A	TLR-4 agonist	Same immunomodulatory properties of lipopolysaccharide but without its toxicity Dose sparing effects The only second generation adjuvant approved for allergen immunotherapy	Expensive, sophisticated procedure

^aAbbreviations: APC, antigen-presenting cell; TLR, Toll-like receptor.

8. Conclusion

Use of adjuvants can enhance the efficacy of AIT and simplify treatment regimens. Various adjuvants are currently available for AIT. First-generation adjuvants, ie, delivery systems with a depot effect, are widely used, although some drawbacks have been identified, especially for aluminum hydroxide. Second-generation adjuvants comprise immunomodulatory agents. MPL is the first of these new adjuvants that was approved for AIT, although various adjuvants for AIT are currently being investigated.

Adjuvant type affects the immune response to AIT and, therefore, to clinical results. On choosing the right product for AIT, we should take into account not only the characteristics of the allergen extract, but also the adjuvant added to the extract.

A better understanding of the mechanisms of both AIT and adjuvants, together with more data on safety and tolerability, will prove useful when designing new approaches to the management of allergic diseases.

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Conflicts of Interest

JMZ has received lecture fees from Stallergenes and Allergy Therapeutics. MF has no conflicts of interest. ID has received payment for lectures, including service on speakers' bureaus for Stallergenes and Allergy Therapeutics, and consultancy fees from Stallergenes. JLJ is an employee of Allergy Therapeutics Ibérica.

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