ADJUVANTS IN ALLERGEN-SPECIFIC IMMUNOTHERAPY: MODULATING AND ENHANCING THE IMMUNE RESPONSE

RUNNING TITLE: Adjuvants in allergen immunotherapy

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ABSTRACT

Allergen-specific immunotherapy (AIT) is the only treatment that may 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 AIT immune response.

Up to now, only four compounds are used as adjuvants in currently marketed AIT products: aluminum hydroxide, calcium phosphate, microcrystalline tyrosine (MCT), and monophosphoryl lipid A (MPL). The three first adjuvants are delivery systems with depot effect, although they also may have immunomodulatory properties. These first-generation adjuvants are still widely used, especially aluminum hydroxide. However, aluminum has some limitations. MCT is the depot formulation of L-tyrosine; it enhances IgG production withoutinducing a significant IgE increase, 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, there are multiple adjuvants under research, including immunostimulatory sequences (ISS), nanoparticles (liposomes, virus-like particlesand biodegradable polymers), and phosphatidylserine derivatives.

In a murine model of allergic bronchial inflammation by sensitization to olive pollen, specific IgE level was significantly higher in sensitized mice treated with olive pollen and aluminum hydroxide. However, sensitized mice treated with olive pollen and bacterial derivatives (MPL or ISS) showed a significant reduction of specific IgE levels and a significant improvement of bronchial hyperreactivity.

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KEY WORDS: Asthma. Allergen-specific immunotherapy. Adjuvant. Aluminum hydroxide. Calcium phosphate.Microcrystallinetyrosine. Monophosphoryllipid A. Immunostimulatorysequences. 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. Tirosinamicrocristalina. Monofosforillípido A. Secuenciasinmunoestimuladoras. Nanopartículas.

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INTRODUCTION

Allergen-specific immunotherapy (AIT) is the onlydisease-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].

MECHANISM OF ACTION OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

AIT mechanisms arestill not fully understood. Based on current knowledge, effective AITsequentially activates multiple mechanisms and induces cellular and molecular changes (Figure 1). This complex mechanism of action of AIT has been definedto occur in three 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 a rapid upregulation of histamine type 2 receptor.The second phase, early tolerance, includes a decrease in interleukin (IL)-4 secreting Th2 cells and increases of IL-10 secreting Treg cells and Breg cells. There is a switch from a Th2-type response to a Th1-type, with increases in IL-10 and transforming growth factor (TGF)-β production. There is also an increase of Treg cells that correlates with clinical improvement. Finally, sustained toleranceimplies that Treg cells stimulate B

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cells to produce allergen-specific IgG4, atolerogenichigh-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].

ADJUVANTS IN ALLERGEN-SPECIFIC IMMUNOTHERAPY

In allergy, an adjuvant is a substance or compound that is co-administered along with the allergen extract and has the ability to increase the allergen immunogenicity and/or to modulate the immune response[9].Adjuvants are used to induce a quicker, more potent, and longer-lasting AIT immune response (Figure 2) [10].

Theyhave been widely used to improve and simplify AIT,because they allow to reduce the number of needed doses. Furthermore, research is currently focused on finding more effective and safer compounds [9].

The ideal adjuvant should be biodegradable, stable, sustained, non-toxic, 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 alsocombine optimal physiochemical properties(e.g. particle morphology and adsorption capacity) along with some biological activity properties (e.g. enhancing IgG4 antibody titers and avoiding the Th2-type immune response)[12].In addition, the European Medicines Agency released a guideline on adjuvants in vaccines for human use; the quality and non-clinical (including toxicity) chapters are applicable to AIT [13].

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Different classifications of adjuvants have been proposed according to the compound nature 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. However, 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 clinical development. Up to now, only four compounds are used in currently marketed AIT products: aluminum hydroxide, calcium phosphate, microcrystalline tyrosine (MCT), and monophosphoryl lipid A (MPL). The three first adjuvants are considered to be delivery systems, although they also may have immunomodulatoryproperties. In turn, MPL is an immunostimulatory agent. Mechanisms of action of each adjuvant are described below.

DELIVERY SYSTEMS

These adjuvants have been widely used for many years and are first-generation adjuvants. They have very different structures and compositions but quite similar mechanisms of action [16]. Traditionally, they have been considered as particulate carriers that deliver allergens to immune cells and prolong allergenpresence at the injection site [14]. This slow release of allergen (depot effect) increasesthe exposure time of the allergen to the immune system, stimulating the production of high and

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sustained antibody titers. However, it has been suggested that the depot effect is not theprimary mode of action, although it is related to adjuvant tolerability [12].

Aluminum hydroxide

Aluminum compounds have been used as adjuvants for almost a century [9] and nowadays they remain the most frequently used adjuvants in vaccination and immunotherapy [17]. Aluminum as adjuvant in AIT enhances allergen immunogenicity and tolerability, but also raises IgG and IgE titers [12].However, in spite of its wide use, its exact mechanism of action as adjuvant is still unknown. Available experimental evidence is scarce, but three mechanisms have been suggested to explain the increase of humoral immunity by aluminum adjuvants. The first mechanism is the formation of a sustained antigen release depot, which would enhance antibody production[18]and could contribute to AIT safety [17].The second mechanism is the conversion of a soluble antigen into a particulate form and the subsequent phagocytosis by antigen-presenting cells (APCs). The third suggested mechanism is the induction of inflammation, which results inthe 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 innate immune system (monocytes, eosinophils, neutrophils, and others) to the injection site. Moreover, damaged tissue releasesthe endogenous danger signal uric acid. Subsequently, monocytes react to aluminum and uric acid through activation of thenucleotide-binding and oligomerization domain-, leucine-rich

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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 themajor histocompatibility complex (MHC) I and MHC II molecules while migrating to the draining lymph nodes [19].

However, aluminum has some limitations, which are related to its potential adverse effects reviewed elsewhere [19]. It can induce a Th2 response [17]; due to this property, it is used for designing experimental animal models of allergic asthma, allergic rhinitis, and eosinophilic esophagitis [19]. This Th2 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 ofa large observational study[20]. With regard to systemic adverse events, aluminum has a low biodegradability and it could accumulate after repeated administration in AIT [21]. In addition, it had been suggested that aluminum adjuvants could be related to the autoimmune/autoinflammatory syndromeinduced 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 so far, routine use of aluminum adjuvants in AIT can be questionable [23]. There is an increasing concern about chronic aluminum toxicity and authorities from different countries are inspecting aluminum safety [17].

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Calcium phosphate

Calcium phosphate is another mineral salt that is used as a depot adjuvant in AIT. It is less commonly used than aluminum hydroxide [12], but it may offer some advantages because it is naturally present in the organism andit is also 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 compared with aluminum [25].

Microcrystalline tyrosine (MCT)

L-tyrosine is a biodegradable aminoacid of natural origin with ideal adjuvant properties[26]. The patent of its depot formulation (MCT) is registered for use as animmunomodulator and adjuvant.

Antigen-adjuvant adsorption affects vaccine potency, and MCT has shown adsorption capacities higher than 95% for allergens and MPL, with favourable stability capabilities [27]. MCT half-life is 48 hours [26], thus allergen release is sustained and immune exposure is prolonged. MCT is naturally metabolized (biodegradable):L-tyrosine is metabolizedafter liberation from the injection site. This reduces the risk of granulomas observed with other depot adjuvants [26]. Moreover, MCT enhances IgG production with limitedIgE increase [26].

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MCT immunogenicity was studied in BALB/c mice immunized with ovalbumin adsorbed to MCT or aluminum. Upon a second exposure to ovalbumin, the increase of Th1 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 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].

IMMUNOMODULATORY AGENTS

Immunomodulatory agentsconsist ofa heterogeneous group of products that act directly on immune cells modulating their antigen response. Their mechanism of action is based on the activation of innate immune receptors on macrophages, dendritic cells (DCs), 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 pattern molecules (DAMPs). PAMPs are absent in the host and are exogenous danger signals that induce signals related with innate immunity. Major PAMPs are microbial nucleic acids, lipoproteins, surface glycoproteins, and membrane components such as lipopolysaccharide (LPS). Pathogen recognition receptors (PRRs) recognize PAMPs and include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and others

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[29]. TLRs recognize PAMPs and activate many intracellular signaling pathways that produce proinflammatory and antimicrobial responses that ultimately result in the production of cytokines, chemokines, cell adhesion molecules and immunoreceptors[29].Immune response can be different according to the TLR-mediated activated pathway: Th1 response for TLR-4 and TLR-9, and Th2 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 depot effect, but immunomodulators that potentiate immune cells/pathways [12]. The most studied second-generation adjuvants in AIT are MPL and immunostimulating sequences (ISS) of synthetic oligodeoxynucleotides (ODN) containing unmethylated cytosine-phosphrothionate-guanine (CpG) motifs.

Monophosphoryl lipid A (MPL)

The only second-generation adjuvant approved for AIT is MPL (GSK, United Kingdom), a detoxified derivative of LPS of *Salmonella minnesota* R595. LPS molecules are found in the outer membrane of Gram-negative bacteria and are another example of PAMPs. LPS is recognized by PRRs of APCs, eliciting a potent immune response [12]. Immunological effects of LPS are mediated by its diphosphoryl lipid A moiety, which is toxic to most animal species. However, MPL is obtained by a hydrolytic process and later chromatography and it has one phosphoryl group and six acyl side chains (lower fatty acid content), but no polysaccharide side group. This way, MPL retains the immunomodulatory properties of LPS but without its toxicity [12].

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MPL was the first TLR agonist included in a licensed human vaccine [16]. It has been employed in many vaccine formulations, notably in hepatitis B and human papilloma virus (HPV) vaccines [31],and it 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 incorporated MPL as adjuvant[32].MPL has also been included in clinical trials of *Plasmodium falciparum*[33] and herpes simplex virus (HSV) type 2 [34] vaccines. Moreover, the FDA has 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, DCs mature and produce cytokines, especially IL-12, which activate T cells to mature into Th1 phenotype [36].In a*in vitro* study peripheral blood mononuclear cells(PBMCs), obtained from 13 patients with grass pollen (*Phleum pratense*) allergy, were cultured with a*P. pratense* extract and MPL. Allergen-induced IFN-γ production increased ($p<0.001$) while IL-5 production decreased ($p<0.01$). When a neutralizing antibody against IL-12 was added, a 95% inhibition of MPL-induced IFN-γ production was observed. Thisstudy showed that MPL deviated Th2 cell responses to Th1 responses in an IL-12 and monocyte-dependent way [37].

ADJUVANTS UNDER RESEARCH

Immunostimulatory sequences (ISS)

Bacterial DNA is an overt example of PAMP. It contains unmethylated cytosine-phosphodiester-guanine (CpG) motifs, which are rare in human DNA. During infection, these motifs are recognized and a protective immune response is triggered. Synthetic

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oligodeoxynucleotides containing unmethylatedCpG motifs (CpG-ODNs) are immunostimulatory sequences (ISS) that mimic the immunostimulatory activity of bacterial DNA [38]. CpG-ODNs are recognized by the PRR TLR-9on B-cells, DCs and other cell types, promoting Th1 and Treg responses [39].Although a relatively old concept, it has emerged recently as immunotherapy adjuvant and it has been used for different types of immunotherapies[40–42].

There are several types of synthetic CpG-ODNs with different structure and biological properties [38]. The ISS 5'-TGACTGTGAACGTTCGAGATGA-3', namedISS-1018, has been studied as 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 of IFN-γ and the reduction of 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].Later, a vaccine composed of Amb a 1 conjugated with ISS-1018 was assessed in a randomized double-blind placebo-controlled phase II study. Patients with ragweed allergy (n = 25) received six weekly injections of vaccine or placebo before the first ragweed season and were followed for two 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 IIb 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].Anyway, ISSsare still under investigation as adjuvants in AIT. As an example, the promising results of sublingual AIT using ODN-CpG as adjuvants in a murine model of food allergy [46].

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Nanoparticles

Nanoparticles (NPs) are under research as new adjuvants/delivery systems in AIT. NPs protect the allergen from degradation, achieve high concentration at the site of action, and prevent from IgE recognition. They have a strong immunogenic effect with low allergenic potency[47,48]. They also combine the potential of optimal allergen presentation [9] along with intrinsic adjuvant properties [12].

Encapsulation of allergens into NPs has great potential to enhance 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 and temperatures[49]. Encapsulation also prevents allergen recognition by IgE from mast cells or basophils, which should reduce the risk of adverse events [48]. It also offers the possibility of co-delivering the allergen and immunostimulatory agents such as CpG motifs or LPS derivatives [9]. Finally, it limits allergen capture to those cells that have the ability to phagocyte the NP [49].NPs have also shown an advantage as adjuvants in oral immunotherapy against food allergens in animal models[50–52].

Other adjuvants that are also under research are liposomes, virus-like particles, and polymers.Here we briefly describe their main features.

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

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liposome-encapsulated extract of *D. pteronyssinus*, vaccinated patients showed increases ofspecific IgG, IgG1 and IgG4,a decrease of sputum eosinophils, and lower clinical scores [53]. Newer liposome formulations are promising [48], but there are no safety data so far[54].

Virus-like particles

Virus-like particles (VLPs) are formed by a high number of copies of a viral capsid protein that mimic 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 Qβ coat proteins. After administration of the Der p 1-VLP to healthy volunteers, a significant increase of specific IgG was observed [56]. Then, house dust mite (HDM) allergen preparation combined with a VLP adjuvant consisting of the bacteriophage Qβ coat proteins filled with the CpG G10 (QbG10) was administered to patients with HDM allergy. Specific IgG also increased and symptoms of rhinitis and allergic asthma were significantly reduced[57]. In a third study, 299 patients with HDM allergy received QbG10 aloneor placebo without co-administration of allergen. However, rhinoconjunctivitis score and quality of life were significantly better in patients treated with a high dose of this allergen-free immunomodulator compared with placebo [58]. In a more recent double-blind, randomized study inpatients with mild-to-moderate persistent allergic asthma, treatment with QbG10 improved asthma symptoms, salbutamol use, and Asthma Control Questionnaire score [59]. These results have suggested that allergens might not be needed for AIT [55]. Other studies

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of VLP in AIT are underway, such as a trial of a VLP-containing peanut allergy vaccine [60]. In addition, AIT with VLPs has been safe and well tolerated in clinical trials [55].

Biodegradable polymers

Polymeric NPs, 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 non-toxic. It increases the penetration of macromolecules across the mucosa [12]. In turn, the synthetic biodegradable polymer more extensively studied in AITis the polylactide-*co*-glycolic acid (PLGA), a polyester used for NP preparation.PLGAis biocompatible, biodegradable and safe [12]. However, chitosan and PLGA have been only studied in animal models [49].

Phosphatidylserine

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

Three PS derivatives could potentially be useful in AIT: dioleoyl PS (DOPS), lysooleoyl PS (LOPS) and stearoylarachidonoyl PS (SAPS). Their effects on immunoglobulins are different: DOPS stimulates IgG and IgA, LOPS stimulates IgA, and SAPS inhibits IgE. They have been shown not to be mutagenic or cytotoxic [12].

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COMPARISON OF ADJUVANTS IN AIT

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

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, immune response was assessed in sensitized mice treated with *Olea*coadministered withdifferentadjuvants(aluminum hydroxide, calcium phosphate, MPL, and ISS). The results showed that specific IgE was significantly higher in the group of mice treated with *Olea* and aluminum hydroxide, and significantly lower in mice treated with *Olea*plus ISS or MPL. Moreover, IFN-γ levels were significantly higher in mice that received *Olea* and ISS or MPL compared with other adjuvant-treated groups. Regarding bronchial hyperreactivity and cellular lung inflammation, only mice treated with *Olea* and bacterial derivatives (MPL and ISS) showed a significant improvement[62].

CONCLUSION

Use of adjuvants can enhance AIT efficacy and simplify treatment regimens. Currently, different adjuvants are available for AIT.First-generation adjuvants, delivery systems with depot effect, are widely used, although some drawbackshave been identified,especially for aluminum hydroxide. Second-generation adjuvants are immunomodulatory agents. MPL is the first of these new adjuvants that was approved for AIT, but there are various adjuvants for AIT under investigation.

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

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

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FIGURE LEGENDS

Figure 1. Immune mechanisms of allergen-specific immunotherapy and adjuvants. Modified from Chesné J et al., 2016 [49].

MCT, microcristalline tyrosine; TLR, Toll-like receptor; OML, oligomannose-coated liposomes.

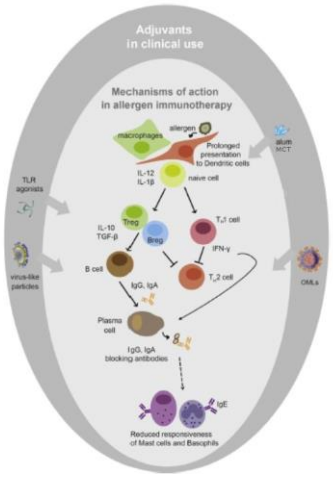

Figure 2. Advantages of adjuvant-formulated vaccines. Modified fromPulendran et al., 2006 [10].

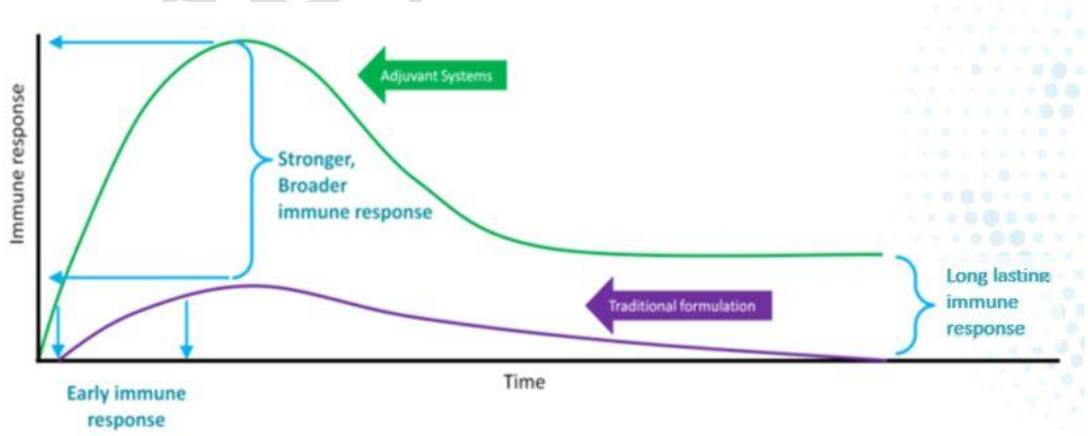

Table 1. Adjuvants in allergen-specific immunotherapy

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

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	Non-biodegradable Th2 polarised 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 (MCT)	Depot effect Inflammasome activation	Biodegradable No significant IgE increase Good local and systemic tolerability	Contraindicated in tyrosine metabolism disorders
IMMUNOMODULATORY AGENTS			
Monophosphoryl lipid A (MPL)	TLR-4 agonist	Same immunomodulatory properties of LPS but without its toxicity Dose sparing effects The only second-generation adjuvant approved for AIT	Expensive, sophisticated procedure