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Review Article

Nanoparticulate Adjuvants and Delivery Systems for Allergen Immunotherapy

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In the last decades, significant progress in research and clinics has been made to offer possible innovative therapeutics for the management of allergic diseases. However, current allergen immunotherapy shows limitations concerning the long-term efficacy and safety due to local side effects and risk of anaphylaxis. Thus, effective and safe vaccines with reduced dose of allergen have been developed using adjuvants. Nevertheless, the use of adjuvants still has several disadvantages, which limits its use in human vaccines. In this context, several novel adjuvants for allergen immunotherapy are currently being investigated and developed. Currently, nanoparticles-based allergen-delivery systems have received much interest as potential adjuvants for allergen immunotherapy. It has been demonstrated that the incorporation of allergens into a delivery system plays an important role in the efficacy of allergy vaccines. Several nanoparticles-based delivery systems have been described, including biodegradable and nondegradable polymeric carriers. Therefore, this paper provides an overview of the current adjuvants used for allergen immunotherapy. Furthermore, nanoparticles-based allergen-delivery systems are focused as a novel and promising strategy for allergy vaccines.

1. Introduction

Over the past few years, a large increase in the prevalence of allergic diseases has been reported [1]. Epidemiological studies report that allergic sensitization is detected in more than 25% of the general population, particularly in industrialized countries [2]. Nowadays, *allergy* is defined as immediate reactions (also known as type I hypersensitivity) against foreign antigens, accompanied by a high IgE stimulus [3]. Various environmental proteins, namely, allergens, are recognized as foreign elements by the immune system of some individuals. Allergic diseases include rhinitis (hay fever), asthma, food allergy, allergic skin inflammation, ocular allergy, and anaphylaxis [4].

The etiology of allergic immune responses is complex, and several factors, including environmental and genetics, have been described as triggers of allergic diseases [5]. Recently, different gene polymorphisms were identified, and they also affect the individual predisposition to develop allergy, known as atopy [6–9]. However, a genetic predisposition alone does not explain the increased prevalence of these diseases in the last decades. It has been proposed that this increase seems to be attributed to environmental factors acting on a genetic basis [7].

Since the first description of allergy in early 1900s, significant progress to research and clinics has been made in offer possible innovative therapeutics for the management of allergic diseases [10–13]. Remarkable advances in allergen immunotherapy include the use of purified allergens-based vaccines [14], blocking antibodies [15], the recombinant interleukins administration [16, 17], DNA vaccines [18], and gene therapy [19]. However, current approaches show limitations concerning the long-term efficacy and safety due to local side effects and risk of anaphylaxis. Effective and safe vaccines require the use of effective and safe adjuvants [20–23]. Therefore, this paper provides an overview of

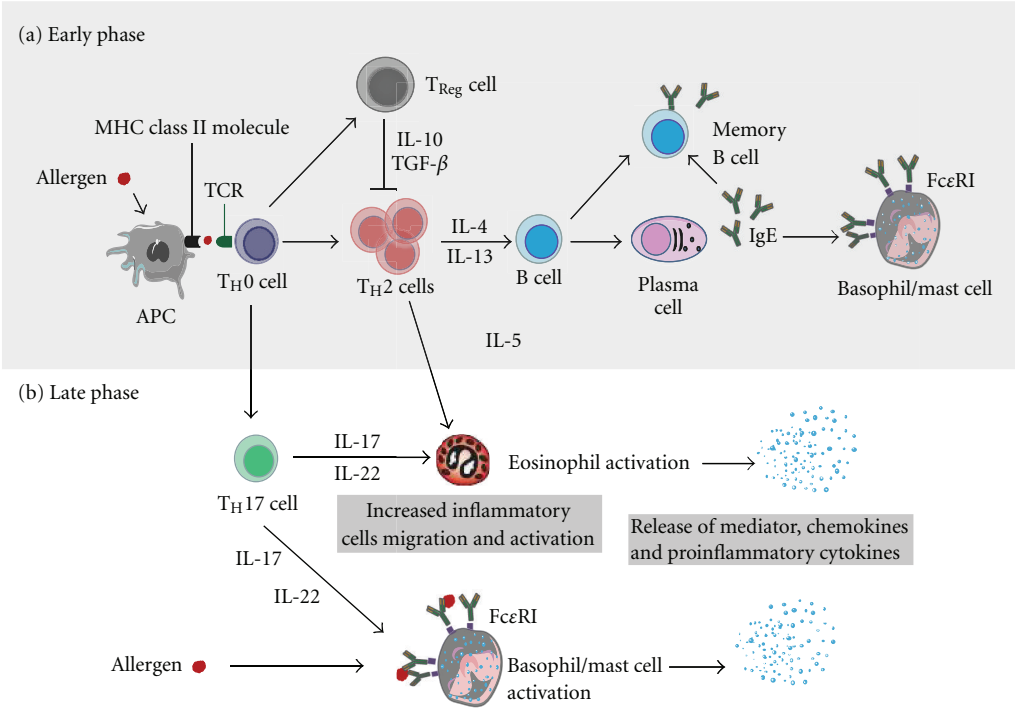

Figure 1: Mechanisms of allergic responses. Allergic response is constituted by two phases: an early phase (a) of initial sensitization and memory activation and a later phase response (b) after re-exposure to allergen, resulting in release of inflammatory mediators as a consequence of allergen cross-linking basophil/mast cell-bound specific IgE.

the currently adjuvants used for allergen immunotherapy. Furthermore, nanoparticles-based allergen-delivery systems are focused and discussed as a novel and promising strategy for allergy vaccines.

2. Immunological Aspects of Allergic Diseases

Allergic diseases are immunologic disorders characterized by an imbalance in the responses of activation of $CD4^+$ T helper (T_H1 and T_H2) and T regulatory (T_{Reg}) cells [24]. Among the many cells involved in allergic reaction, $CD4^+$ T cells play a central role in the immune response against allergens. In atopic individuals, functional polarization of allergen-specific response of $CD4^+$ T cells and cytokine profile is T_H2-polarized (Figure 1). In contrast, activated allergen-specific T cells from nonatopic subjects are T_H1 polarized accompanied by secretion of IFN-γ and IL-2. Allergic inflammatory cytokines are secreted, particularly IL-4, IL-5, and/or IL-13. This latter is an important mediator of allergic inflammation. IL-13 promotes immunoglobulin class switching for IgE production and increases both recruitment and activation of inflammatory cells such as eosinophils, mast cells, and basophils [25, 26]. Recently, several studies suggest that T_H17 and T_{Reg} also have a significant role in the development of allergic diseases [27, 28]. Current studies have demonstrated that these novel T cells, producing IL-17 and IL-10, regulate innate immunity by signal transduction, which mitigates their proinflammatory function (Figure 1) [29].

The mechanism of the allergic reaction is characterized by two phases: the initial sensitization phase, which is followed by the second phase that is characterized by immediate hypersensitivity symptoms. After an initial exposure (Figure 1(a)), T_H2 pathway is initiated by the uptake of allergens by professional antigen-presenting cells (APCs) that present peptides on MHC class II molecules to naive $CD4^+$ T cells, which activate a cell response. Thereafter, high amounts of specific IgE antibodies are produced and bind to high affinity IgE receptor (FcεRI) in membranes of circulating basophils and mast cells that reside in skin and mucous membranes. Upon re-exposure to the allergen (Figure 1(b)), an immediate hypersensitivity response is triggered as consequence of cross-linking of the allergen with two molecules of mast cell-bound IgE. This signal stimulates the release of histamine and other inflammatory mediators such as serine proteases, platelet activating factor, cytokines, leukotrienes and prostaglandins. These mediators increase vascular permeability and promote mucus production, which are responsible for the symptoms and signs of allergic diseases [25, 26].

3. Allergen-Specific Immunotherapy

Allergen-specific immunotherapy (SIT) involves the administration of increasing doses of allergen(s) in order to obtain a hyposensitization and long-term relief of symptoms occurring after natural allergen exposure. SIT

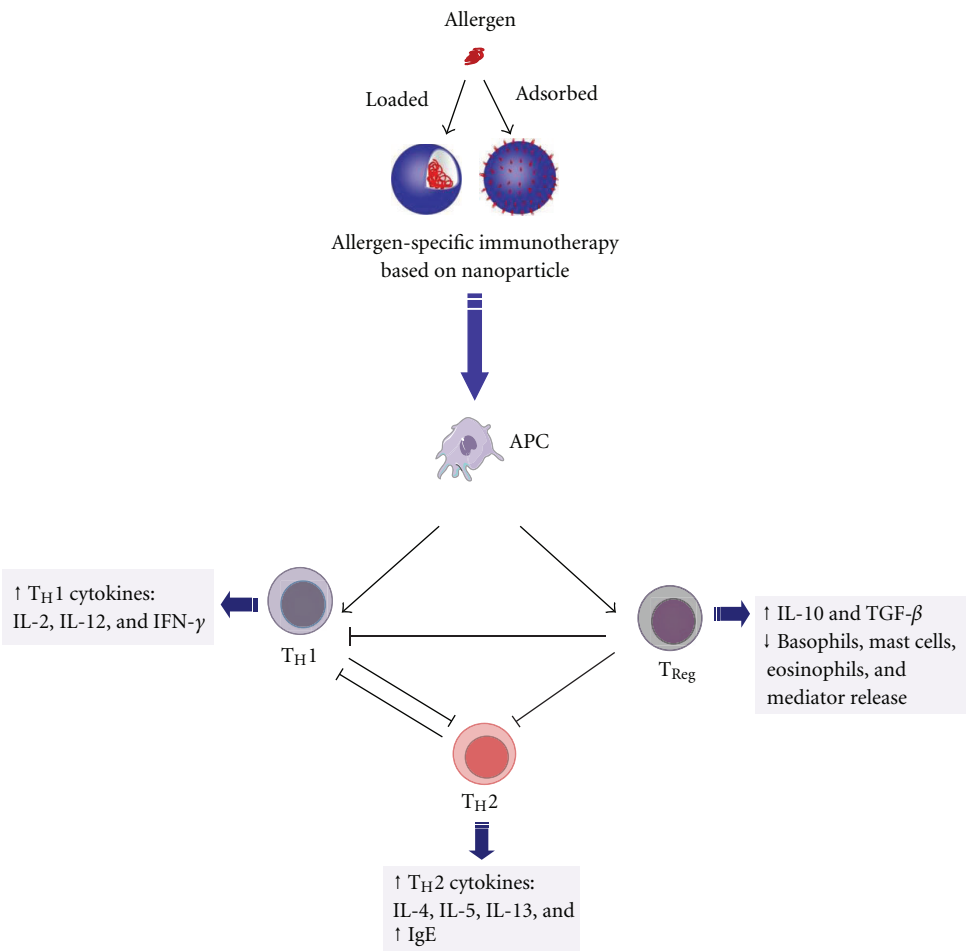

Figure 2: Immunomodulation of allergic diseases by using nanoparticle-based vaccines. Immunological changes after successful allergen specific-immunotherapy are indicated by whole arrows; truncated arrows indicate inhibitory effects. Redirection of allergic-T_H2 responses, in favour of T regulatory cell induction or/and T_H1 activation, are depicted.

have been considered an efficient, safe, and long-term-benefit approach, which may be combined with appropriate allergen-avoidance strategies. However, discovery of allergen-specific immunotherapy raised a number of crucial questions regarding the route of administration, the dose response relationship, and the intervals between administrations [30].

Nowadays, several immunotherapeutic strategies have been achieved to modulate the immune system by different pathways [13]. Advances in the standardization of allergenic composition of vaccines, optimal dose of allergen and, above all, clinical studies that support their effectiveness are crucial not only to achieve more effective and safe vaccines, but also to provide greater dosing convenience [31].

3.1. Mechanisms of Allergen-Specific Immunotherapy. It has been established that an immune-tolerant state represents an essential step for a successful immunotherapy [32]. Several findings suggest that SIT acts through an immunomodulatory activity (Figure 2), changing the T_H1, T_H2, T_H17, and T_{Reg} cell differentiation [33–35]. Following SIT, an increase in $CD8^+$ cells and T_H1/T_H0 ratio are observed. Also, a decrease in T_H2/T_H0 ratio takes place. Additionally, a change in cytokine response with production of IL-4 and IFN-γ (IL-4 to IFN-γ) is observed as result of downregulation of T_H2 or increased T_H1 response. In this context, the generation of allergen-specific T_{Reg} cells (producing IL-10 and TGF-β) suppressed proliferative and cytokine responses, initiating peripheral T-cell tolerance. In addition, the number of T_H2 cells such as basophils and eosinophils is reduced at the allergen exposition sites (e.g., mucosa and skin), which reduces the IgE-mediated release of histamine by basophils [32–34].

Usually, SIT induces a transient increase in serum IgE, which decreases during the course of the treatment. Successful SIT is also associated with a high increase (10 to 100-fold) in IgG blocking antibodies such as IgG4 and IgG1. IgG4 acts by capturing the allergen before it crosslinks with the IgE that is bound on the surface IgE receptors of mast cells and basophils, inhibiting its activation. Still, IgG4 antibodies have anti-inflammatory activity through inhibition of the production of other IgG subtypes [35].

Recently, the counterregulatory role of IL-10 has been demonstrated. It is secreted by T_{Reg} cells during SIT, which modulates isotype formation and also change the response from an IgE to an IgG4-dominated phenotype. Other indirect function of T_{Reg} cells is the suppression of T_H17 cells, accompanied by a decrease in IL-17 secretion [36].

3.2. Routes of Administration. Lately, several routes for allergen delivery have been assessed in immunotherapy. Since its discovery, the traditional SIT has been commonly given subcutaneously with high clinical efficacy [30]. However, subcutaneous immunotherapy (SCIT) is associated with a significant risk of severe adverse events [37, 38]. Thus, efforts have been done towards alternative routes (local and noninjection) for allergen delivery [39].

In the 1980s, the sublingual route appeared as a promising noninjection route [40]. Sublingual immunotherapy (SLIT) was regarded as an efficient and safe route. Usually, SLIT is recommended for patients with severe adverse reactions to conventional SCIT [41]. Similarly, local nasal immunotherapy (LNIT) proved to be effective and safe. However, the exact mechanisms of action and optimal dose of both SLIT and LNIT has not been established yet [39]. These noninjection routes were proposed by the World Health Organization (WHO) as viable alternatives to the subcutaneous route [42]. On the other hand, clinical efficacy of oral immunotherapy (OIT) was achieved with high doses of allergen. This has induced to a major research on the development of new mucosal adjuvants, discussed in the following section.

4. Adjuvants for Allergen Immunotherapy

The overall goal in allergen vaccine development is improving both clinical efficacy and safety. Nevertheless, the use of high amount of allergen on allergen immunotherapy is limited by a significant risk of allergic reactions [42]. Thus, effective and safe vaccines with reduced dose of allergen have been developed using adjuvants. Adjuvants (from Latin, *adjuvare,* aid) are defined as heterogeneous compounds that enhance the immune response against to coadministered antigens [43].

Ideally, adjuvants for allergy immunotherapy should stimulate a T_H1 immune response without inducing autoimmunity and should not be mutagenic, carcinogenic, and teratogenic. Besides, optimal adjuvants need to be apyrogenic and stable in the vaccine formulation [44]. Despite the undeniable progress in this area, the use of adjuvants still has several disadvantages, which limits its use in human vaccines. Therefore, the benefits and risks related to the use of adjuvants for allergy immunotherapy need to be counterbalanced [45]. In this context, several novel adjuvants for allergen immunotherapy are currently being investigated and developed [43].

4.1. Mechanisms of Action. Traditionally, adjuvants exert their effects in different ways: the depot effect, the targeting to antigen-presenting cells, and the nonspecific modulation of immune system [43]. The use of allergen extracts adsorbed to adjuvants protects the antigen from enzymatic degradation. On the other hand, depot formation entraps the antigen and provides its slow release. The persistence of the antigen increases the recruitment of APCs in the injection site, which triggers a prolonged inflammatory response. Then, the recruitment of competent cells activates innate and adaptive immune system.

Adjuvants can be divided into two groups according to their mechanisms of action as delivery systems and immunomodulatory adjuvants. However, some compounds can act by both mechanisms simultaneously [45–47].

4.2. Traditional Adjuvants. Hundreds of compounds and molecules have been extensively evaluated as adjuvants [45]. Aluminium salts and emulsions are traditionally used as general immunologic adjuvants. Recently, liposomes, immunostimulating complexes (ISCOMs), oligonucleotides, and microorganisms-derived adjuvants (i.e., MPL) have been introduced as novel adjuvants in allergy vaccines [44, 46–48].

4.2.1. Aluminium Hydroxide. Aluminium salts (alum) represents the most commonly used adjuvant in human vaccines. Francis and Durham showed that alum-precipitated diphtheria toxoid was more immunogenic than an aqueous toxoid [44]. Alum-adsorbed allergen extracts induce a strong T_H2 response by a depot effect and also stimulates the activation of APCs, independent of Toll-like receptor (TLR) signalling, but dependent of NLR (NALP3) inflammasome [46]. Alum is the most common and safe adjuvant for injection immunotherapy in humans. However, some problems were reported after use of alum in allergic and prophylactic vaccines. These drawbacks include the enhanced sensitivity to alum and local granuloma formation at injection sites. Yet, tolerance induction has been observed after the use of aluminium. Thus, it seems to be rational to consider replacing alum compounds with other more inert molecules for the treatment of type I hypersensitivity [49].

4.2.2. Emulsions. Depot adjuvants based on Freund's adjuvants have been used in experimental studies and introduced in the clinic. Usually, the allergens are incorporated in a water-phase followed by the addition of oil and an emulsifying agent to form an emulsion. This allergen dosage forms have often a good immunogenic profile, with high efficacy, and prevented treatment-induced anaphylactic side effects. Nevertheless, frequent local reactions have limited the use of emulsions on long-term human prophylactic vaccines [46].

4.2.3. Liposomes. Liposomes are synthetic spheres based on a bilayer structure of phospholipids, which allow the encapsulation of hydrophilic antigens [50]. Liposomes act as both delivery carrier and immunomodulators. However, their low stability and manufacturing problems limited the use of these systems as adjuvants in human vaccines [46, 48].

4.2.4. Monophosphoryl Lipid A. Currently, novel adjuvants derived from Gram-negative bacterial cell components have been investigated. Monophosphoryl lipid A (MPL; Coriza, USA), a purified lipopolysaccharide extracted from *Salmonella minnesota*, has been used as adjuvant in allergy vaccines [44]. MPL increases the activation of dentritic cells and T cells, inducing a shift in cytokine production with a potent T_H1 response [45]. In addition, MPL has been shown to enhance both mucosal and systemic immunity after intranasal administration. Clinically, SIT with MPL has shown good efficacy and attenuated toxicity [44, 51].

4.2.5. ISCOMs. Immune stimulating complexes (ISCOMs) are spherical complexes of about 40 nm, with a strong negative charge, composed of saponin, cholesterol and phospholipids. The micelles consist of three layers, hydrophilic-hydrophobic-hydrophilic. ISCOMs-incorporated antigens can trigger humoral, mucosal, and cellular immune responses [48, 52].

4.2.6. Oligonucleotides. Synthetic oligonucleotides containing immunostimulatory CpG (ISS) motifs present a strong induction of T_H1 response. Conjugation of ISS with allergen showed enhancement in its immunogenicity and also reduced its allergenicity [53]. Moreover, it caused an increase in the activation of both dendritic and T cells, which induced a shift in cytokine production to a T_H1 profile [54].

4.2.7. Particulate Delivery Systems. Particulate delivery systems belong to the category of adjuvants that facilitate the antigen uptake by APCs or by increasing the influx of professional APCs into the injection site. Among the different types of particulated delivery systems, polymer nanoparticles are a group of delivery systems with interesting abilities as adjuvants for both conventional and mucosal vaccination, since they can enhance the delivery of the loaded antigen to the gut lymphoid cells due to their ability to be captured and internalized by cells of the GALT. We will discuss in more detail the use of polymeric nanoparticles as adjuvants in the following section.

5. Nanoparticles-Based Allergen-Delivery Systems

Currently, nanoparticles-based allergen-delivery systems have received much interest as potential adjuvants for allergen immunotherapy [55]. It has been demonstrated that incorporation of allergens into a delivery system plays an important role in the efficacy of allergy vaccines. In the last years, several nanoparticles-based delivery systems have been described, including biodegradable and nonbiodegradable polymeric carriers [44, 45].

5.1. Biodegradable Polymeric Nanoparticles. Polymeric nanoparticles are colloidal carriers that vary in size from 10 to 1000 nm [56]. They can be divided into two categories: nanocapsules and nanospheres. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a polymer membrane, whereas nanospheres are polymeric matrix in which the drug is physically and uniformly dispersed. Obtaining one or another type of nanoparticle depends on the preparation method used [57].

Over the last thirty years, different types of polymers and copolymers have been used to design nanoparticles. Among them, biodegradable polymers are the most used as great promise the field of drug-delivery systems. These types of nanoparticles provide controlled/sustained release properties, subcellular size, and biocompatibility with tissues and cells [58], and they are well established carrier systems with high potential for the delivery of bioactive macromolecules, including peptides, proteins, and nucleic acid vaccines [59]. Encapsulation in the polymers allows maintaining the integrity and activity of these biomolecules, protecting them from exposure to extreme pH conditions, bile and panctreatic secretions, and augments the immunopotentiating effect of the antigens [60].

It is well known that properties of nanoparticles such as size, surface charge, hydrophobicity/hydrophilicity, and steric effects of particle coating can determine its compatibility with the immune system [61–63].

Furthermore, nanoparticles can also be designed to provoke an immune response, by either direct immunostimulation of antigen presenting cells or delivering antigens to specific cellular compartments [64].

For the obtention of the desired therapeutic response, size particle control is important since microparticles are rapidly cleared by reticuloendothelial system, while nanoparticles have prolonged circulation time and are efficient drug, enzyme, and protein carriers by any route of administration [65, 66].

Furthermore, in order to achieve the desired therapeutic response with these biodegradable polymeric devices, it is also important, to select the right polymer to be used as an encapsulating agent, since its nature significantly influences the size and the release profile of the nanoparticles [67]. These biodegradable polymers can be either natural (chitosan, alginate, carrageenan, albumin, gelatin, collagen, among others) or synthetic [poly(lactic acids), PLA), poly(lactide-co-glycolic acids), PLGA), poly(methyl methacrylate), PMMA), poly(ε-caprolactone), PCL), poly(alkylcyanoacrylates), PACA), and copolymers]. The former generally provide a relatively quick drug release, while the latter enable extended drug release over periods from days to several weeks [67, 68]. However, the use of synthetic polymer can be limited due to the need of organic solvents and harsh formulation conditions [58, 68]. The most commonly and extensively used polymeric nanoparticles for vaccine purposes are described in this section.

5.1.1. Polyesters. Polyesters are thermoplastic polymers with hydrolytically labile aliphatic ester linkages in their structure. Although all polyesters are theoretically degradable, only aliphatic ones with short aliphatic chains between ester bonds can degrade in the time required for most of the biomedical application [69]. These polymers are the most used biodegradable polymers as drug-delivery systems and

have attracted considerable attention as drug carriers due to their biocompatibility and biodegradability.

(1) Polylactides. For vaccination purposes, polyesters such as PLA and PLGA (of variable MW and composition) have been the most popular materials for the preparation of polymer nanoparticles [70]. Due to its well-documented biocompatibility, safety, and biodegradability, these polymers are FDA approved for several clinical applications in humans [59].

PLGA is a polyester composed by of one or more of three different hydroxy acid monomers, d-lactic, l-lactic, and/or glycolic acids [71]. These copolymers undergo hydrolysis of its ester groups in the presence of water, and the degradation time depends on the LA : GA monomer ratio (50 : 50 up to 100 : 0), molecular mass, end functional group (free or esterified carboxylic acid), and the shape and structure of the matrix [69]. These parameters also determine the encapsulation efficiency and release rate of drugs and antigens from this material [72]. For instance, it has been demonstrated that nanoparticles prepared from a 75 : 25 PLGA copolymer (a slow degradation rate polymer) presented slower release rate of antigen than those prepared from a 50 : 50 PLGA copolymer (a fast degradation rate polymer) [73].

The application of these polymeric particles to antigen delivery has been widely investigated from the early 90s [59]. Several authors have reported that due to its ability to efficiently target professional antigen presenting cells (APCs), the effects of PLGA entrapped antigen on the cellular and humoral immune response have several times been shown to be superior to application of soluble, free antigen [74–77].

Thus, both nano- and microparticles of PLGA can be used to increase and modulate immune responses against encapsulates antigens and to facilitate appropriate processing and presenting antigens to T cells [78–80]. Therefore, in recent years, these polymers have been studied for allergen-delivery use, finding that the PLGA nanoparticles are of great interest for therapy of allergies [81–83]. As an example, PLGA nanoparticles loaded with Bet v1 (the major allergen of birch pollen) reduce the predominance of the T_H2 response, increasing the Bet v1 IgG2a levels, concomitant with an increase in IFN-γ and IL-10 levels [82, 83].

Other less commonly used polylactide particles include poly(DL-lactide glycolide) or PLG microparticles that are also able to induce $CD8^+$ T cell responses [84]. These particles have been used as a vehicle for Ole e 1, the main olive pollen allergen, in the desighning of allergen-specific vaccine [85].

Despite its obvious interest as nanoparticulate adjuvants and delivery systems for immunotherapy, the use of these polymers as peptide or protein delivery may negatively affect the stability of the loaded compound due to the bulk degradation mechanism of the polymer and the acidic degradation obtained products [59, 69, 86–89]. Another limitation of these nanoparticles is their insufficient stability and penetration capacity upon mucosal administration [59].

Thus, recent research has been focused on the search for additional strategies to improve the efficiency of these nanoparticles by addressing its limitations. Overall, most of these strategies involve the modification of the surface of the particles by using a suitable stabilizer. The substance most commonly used for this purpose is poly(ethylene glycol) (PEG) [59], which is a hydrophilic, non-ionic, and biocompatible polymer that can be added to the particles by different routes, including covalent bonding and mixing during nanoparticle preparation or surface adsorption [90]. For instance, it was demonstrated that PLA-PEG nanoparticles, with a high PEG coating density and small size, are significantly better transporters across the nasal mucosa than noncoated PLA and even than PLA-PEG nanoparticles with a lower coating density [91].

Different studies have been performed using these nanoparticles for vaccine purposes. For example, it has been demonstrated that recombinant hepatitis B surface antigen encapsulated within PEGylated PLGA nanoparticles allows generating very fast immune responses compared to the non encapsulated antigen counterpart [92]. Other authors achieved an enhancement of the transport of the PEG-PLA nanoparticles encapsulated tetanus toxin across the intestinal barrier [93, 94].

(2) Poly(ε-Caprolactone). Poly(ε-caprolactone) (PCL) is a biocompatible, bioerodable, biodegradable and semicrystalline polyester that can be used for the formulation of nanoparticles [68]. Due to the semicrystallinity and hydrophobicity, the *in vivo* degradation of PCL is much slower than PLGA, making it more appropriate for long-term delivery systems, extending over a period of more than one year [58, 95]. Furthermore, PCL particles, unlike polylactides, do not generate an acidic environment that could negatively affect the antigenicity of the encapsulated antigens or allergens [96].

However, most of the PCL studies found in the field of immunotherapy have been performed using PCL microparticles/microspheres [95]. Studies that use this polymer as nanoparticles for allergen immunotherapy have not been found and it has been little used as nanoparticulate adjuvant system for vaccine development. For example, PCL nanospheres together with mucoadhesive polymers (alginate or chitosan) were used as a potential carrier for *Streptococcus equi* surface proteins. Serum IgG antibody levels of animals vaccinated with *S. equi* antigens encapsulated or adsorbed onto PCL particles were significantly higher than those caused by free antigens or even free antigens adjuvanted with cholera toxin B subunit [97]. Thus, although no examples of the use of PCL nanoparticles for allergen immunotherapy were found, the good results obtained by different authors by using these PCL-nanoparticles in the field of vaccination suggest these systems as potential adjuvants in allergen immunotherapy.

5.1.2. Poly(Anhydrides). In contrast to polylactide nanoparticles, the degradation products of polyanhydrides are non-cytotoxic and less acidic than those of polyesters, which can improve the stability of encapsulated antigen [88]. The copolymers between methyl vinyl ether and maleic

anhydride (PVMA) (commercialized as Gantrez AN from ISP, Corp.) are a good example of these polyanhydrides. Due to the presence of reactive anhydride groups which do not need time-consuming chemical activation, this copolymer allows to easily prepare nanoparticles under mild conditions, using a solvent displacement method [98]. Moreover, a recent research concluded that the use of Gantrez AN 119 nanoparticles prepared by using reduced amount of organic solvent (acetone) facilitates the encapsulation of the antigen and offers a mild irritation at mucosal epithelia when orally unstabilised nanoparticles are used [99].

One of the most important properties of the Gantrez nanoparticles is their ability to develop strong bioadhesive interactions with components of the gut mucosa [100]. In addition, their surface can be easily modified by simple incubation with different excipients or ligands in order to modify their *in vivo* distribution [101, 102], and even to increase its affinity for the intestinal mucosa [100]. This makes Gantrez nanoparticles good candidates for oral immunotherapy treatments, which have many evident advantages (patient compliance, safest route, least expensive route etc). In fact, some studies have demonstrated the efficacy of these particles by this route. For example, Gómez and coworkers [103] found enhancements in both T_H1 and T_H2 markers (IgG2a and IgG1, resp.) after oral administration Gantrez AN nanoparticles loaded with ovalbumin as allergen model. Moreover, these carriers were able to protect a model of sensitized mice to ovalbumin from anaphylactic shock. Thus, these PVMA nanoparticles have been widely used as carriers for controlled delivery of antigens such as *Lolium perenne* (allergenic proteins of rye-grass pollen) (N), [88, 103–106].

Another advantage of this copolymer is that it can easily react with amino groups, which makes easy to load or link different types of immunostimulants, like proteins or even *lipopolysaccharides*.

Also, the loading of antigens into these bioadhesive nanoparticles has demonstrated to enhance the immune responses in terms of a potent T_H1 adjuvant capacity [103, 105, 107]. This strong response may be due to the effect produced by the nanoparticles that promote close interactions between antigen and antigen-presenting cells, and also act as agonists of various Toll-like receptors (TLRs), mainly TLR2 and TLR 4 [86, 108].

Other polyanhydrides based on hydrophobic moieties or on oligomeric ethylene glycol-containing anhydrides have been shown promise as novel vaccine carriers with immunomodulatory capabilities [88].

5.1.3. Poly(Gamma-Glutamic Acid). Poly(gamma-glutamic acid) (γ-PGA) is a high molecular weight polypeptide composed of γ-linked glutamic acid units and α-carboxylate side chains produced by certain strains of Bacillus [109]. Due to the amphiphilic nature of the hydrophobically modified γ-PGA copolymer, it is possible to form nanoparticles with a simple methodology [110]. In recent years, a research group has developed biodegradable nanoparticles using γ-PGA with L-phenylalanine ethyl ester and evaluated the feasibility of protein entrapment on/into this carriers as well as their biological potential. As a result of these rechearches, ovalbumin (OVA), as a model protein, was successfully encapsulated in these nanoparticles, which also did not induce any cytotoxicity against HL-60 cells [109].

Subsequent studies also showed that these particles are activators of human monocyte-derived dendritic cells and strongly stimulate the production of chemokines and inflammatory cytokines as well as upregulation of costimulatory molecules and immunomodulatory mediators involved in efficient T cell priming. Furthermore, *in vitro* studies with monocyte-derived dendritic cells and grass pollen allergen *Phleum pratense* loaded γ-PGA nanoparticles showed an increase allergen-specific IL-10 production and proliferation of autologous $CD4^+$ memory T cells [110].

Additionally, studies show that these biodegradable nanoparticles induce *in vitro* innate immune cell activation, produce antigen-specific immune responses *in vivo* through the TLR 4 and MyD88-dependent signaling pathway, and can influence innate and adaptive immune responses by first-line host sensor [111].

In conclusion, these systems seem to be a new and good adjuvants and antigen carriers for allergen-specific immunotherapy.

5.1.4. Poly(Vinylpyrrolidone). Although fewer employees than polymers described before, there are some studies that suggest the interest of using poly(vinylpyrrolidone) (PVP) for the obtention of promising allergen-delivery nanocarriers. For example, Madan and coworkers [112] showed a successful entrapment of antigens of *Aspergillus fumigatus* (pathogenic fungi responsible of several allergic diseases) and found a sustained IgG antibody levels for approximately 12 weeks in comparison to IgG levels for 7 days with free antigen after immunization of male BALB/c mice. Moreover, IgE levels of allergens loaded PVP nanoparticles were lower than observed in free allergens studies.

5.1.5. Polysaccharides. The most investigated polysaccharide for mucosal vaccine delivery is chitosan, poly(D-glucosamine). This polymer is prepared by the partial N-deacetylation of chitin, a natural polymer, the second most abundant natural polysaccharide in nature, which is derived from the cuticles of insect species or crustaceans such as crabs and shrimp [113].

Chitosan is soluble in weekly acid solutions, resulting in the formation of a cationic polymer with high charge density, and can therefore form polyelectrolyte complexes with a large variety of anionic polymers [69]. Besides, due to the presence of highly reactive amino groups along its structure, chitosan is susceptible to chemical or biological functionalization [114]. Also, the preparation of chitosan nanoparticles based on the basis this polymer can be easily done without the use of organic solvents, which is interesting to maintain the immunogenicity of the antigens [115].

Chitosan possesses other advantageous properties such as low production costs, biocompatibility, biodegradability, and nontoxicity that, along with its ability to enhance the penetration of macromolecules across the intestinal and

nasal barriers, make it a suitable candidate for the design of mucosal vaccine formulations [68, 69, 116–119]. Thus, slowed mucociliary transport as well as a transient increase in paracellular absorption should lead to an improved immunological response [115].

Several studies have reported the use of chitosan-based nanocarriers for gene delivery [120–122]. CS-based nanocomplexes have been developed for the targeted delivery of plasmid DNA (pDNA) and also as carriers for RNA [122, 123].

Chitosan nanoparticles incorporating soluble antigens as diphtheria and tetanus toxoids and plasmid DNA induced high and long-lasting IgG immune responses, demonstrating the effectiveness of these devices for intranasal vaccination [93, 124]. This significant systemic and mucosal immune response enhancement after nasally administered chitosan nanoparticles has been demonstrated also for influenza, pertussis, and diphtheria vaccines [115, 124]. However, until now, the mechanism by which the antigen is delivered to the nasal mucosa and the role of the physicochemical characteristics of the particles in this processes have not been well determined, and there is some contradictory theories [124].

For oral vaccination studies, Roy and coworkers have demonstrated the effectiveness of orally delivered chitosan-DNA nanoparticles in inducing protective immunity in the peanut allergy mouse model [125]. More specifically, chitosan nanoparticles loaded with the gene for Ara h2 (main peanut allergen) allow to obtain a significant reduction in the levels of serum IgE, plasma histamine, and vascular leakage and the induction of specific mucosal IgA antibodies.

Other studies show a significant amelioration of ovalbumin-induced food allergy symptoms when the TGF-beta expressing DNA vector is orally administered after its encapsulation in chitosan nanoparticles, compared to the previously reported protein-based strategies [126].

However, in spite of all the described advantageous properties of chitosan, this polymer has a major limitation: its low solubility at physiological pH and therefore loses of its ability to enhance drug permeability and absorption [127, 128].

To improve this drawback, several derivatives of chitosan have been studied, such as trimethyl chitosan (TMC), that shows high solubility, bioadhesive properties, and ability to enhance permeability over a wide pH range [129, 130]. In fact, a recent study shows that trimethylated chitosan nanoparticles obtained by using a new mild method induced stronger humoral and mucosal immune responses compared to generate by chitosan conventional nanoparticles [131].

Other interesting alternative is the use of chitosan together with other biodegradable polyelectrolyte polymers with opposite charge to obtain combined nanoparticles suitable for mucosal vaccinations. A typical example of this second polymer can be sodium alginate, another biodegradable and biocompatible polysaccharide. For instance, it has been demonstrated that alginate-chitosan-coated nanoparticles are an effective system for subcutaneous and oral vaccination with the recombinant hepatitis B surface antigen [132–134]. Another study shows that these nanoparticles can prevent a burst release of loaded ovalbumin and improve its stability in simulated intestinal fluid at 37°C [132].

5.2. Nondegradable Polymeric Nanoparticles. Nondegradable nanoparticles of different materials such as latex, gold, silica, or polystyrene are being evaluated as antigen carriers for induction of immunity [70, 77, 135]. It is considered that by using these particles, the antigen can be presented to the immune system for extended periods of time, and thus improve the immunogenicity, probably due to the persistence of the nanoparticles in the tissues [70, 77].

On the other hand, these polymers have several technological advantages [64]. For example, polystyrene nanoparticles can be made with several functional groups on their surface to achieve effective conjugation with a variety of antigens. Furthermore, when the antigen is covalently coupled to the particle, it induces higher cellular and humoral responses than in the cases where the antigen is absorbed [136].

For latex particles, some examples in the literature have demonstrated that this polymer was presented 1000–10,000-fold more efficiently by MHC-Class I molecules than soluble antigens [137] or antigens presented via MHC-Class II molecules [138]. Gold nanoparticles also appear to enhance the effect of DNA vaccination by improving delivery onto cellular interiors [139]. However, these results are obtained using electroporation, which may not be applicable in humans due to cell mortality. In the absence of this technique, so by passive diffusion, the immunological effects were not so interesting [140]. Other recent studies show that the use of gold nanoparticles along with alum can enhance the immune response against PfMSP-119 and PvMSP-119 [141].

However, in general terms, it was shown that nondegradable particles were much less effective at cross-presenting antigens than degradable ones [142, 143]. Also, to use this type of nanoparticles for vaccination purposes is necessary to consider aspects of toxicity and particles aggregation in the tissues, which requires follow-up studies of *in vivo* clearance and the determination of possible adverse effects resulting from its use [77].

6. Conclusion

In summary, the use of nanotechnology platforms, although widespread in recent years for vaccination purposes [98, 106], is emerging in the field of allergen immunotherapy [103–105]. Given the promising results obtained so far, polymeric nanoparticles can be of interest to develop new therapeutic strategies able to improve both clinical efficacy and safety of allergen vaccines.

On the other hand, our knowledge of the nanoparticle interaction with the immune system has been increased in recent years, but it still remains insufficient. Thus, further studies related to the immunomodulatory effects of the polymeric nanoparticles are required to improve our understanding, and therefore our capability, to design better specific and effective allergen vaccines.

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