



Nanoparticles in allergen immunotherapy

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Purpose of review

Allergen immunotherapy is the only recognized causal treatment for allergic disease that modulates the immune system toward a tolerogenic or desensitized state. Allergens or their derivative preparations are formulated with adjuvants of different origin and having diverse immunological functions, such as prolonged tissue release and specific immunomodulatory properties. In the last 2 decades, thanks to developments in the field of nanotechnology, more biosafe nanoscale materials have become available for use as pharmaceutical adjuvants in medical research.

Recent findings

Nanomaterials possess unique and versatile properties which can be employed to develop drug carriers with safer profiles, better stability in physiological conditions and immunomodulatory properties. Nanoparticles can have an adjuvant effect *per se* or also when they are packed in structures whose physical–chemical properties can be handled in a way that also influences its release dynamics. In particular, it has been suggested that nanoparticle preparations can be put in complexes or loaded with allergens or allergenic extracts, opening the way to innovative paradigms.

Summary

In this review, we analyze allergen/nanoparticle properties in terms of cytotoxicity, stability and immunogenic reaction in in-vitro and animal systems.

Keywords

adjuvant, allergen, allergen-specific immunotherapy, immunomodulation, nanoparticles

INTRODUCTION

Allergen immunotherapy (AIT) is currently the only causative treatment for allergic diseases in humans. Since 1911, when Noon published the first human trial [1], AIT has consisted of several repeated subcutaneous or sublingual administrations of increasing doses of the culprit allergenic source, with the final effect of inducing peripheral tolerance toward these allergens and, more importantly, inducing protective allergen-specific IgG antibodies. Despite the very encouraging advantages of AIT, there remain some major issues to resolve, particularly in reference to standardization, limited efficacy, potential side effects, low patient adherence, high costs and long duration (3–5 years) [2[•]]. To address these pitfalls, several novel formulations have been suggested, such as the use of highly purified recombinant allergens or hypoallergenic derivatives, and/or coupling to immunostimulatory substances and adjuvants to reduce allergenicity and modulate immune response [2[•]]. In particular, adjuvants are constituents of vaccines which induce stronger, quicker and longer lasting immune responses, but they also modulate immune response toward an antiallergic response [3[•]].

Recently, the use of nanoparticles (defined as particles of matter between 1 and 100 nm in diameter) in AIT has been suggested by several groups as nanomedicine shows great potential in pharmaceutical development. In particular, nanoparticles have a defined size, are easy to produce, can be functionalized, and possess targeting ability, making these molecules capable of being tailored and, thus, potentially more efficacious for novel AIT vaccines. Particulate materials may possess different properties, being biocompatible but also biodegradable, or nonbiodegradable, and what happens to them inside organisms is still a matter of discussion. Nanomedicines share physicochemical characteristics with pathogens, having dimensions which are

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KEY POINTS

- The physicochemical properties of nanomaterials and immune response.
- Nanomaterials containing or exposing allergenic molecules are capable of immunomodulation.
- Nanomaterials are capable of enhancing innate and adaptive immune response for both prophylactic and therapeutic allergen immune-therapy.

fraction of the cell diameter, significant liquid-solid interfaces, and patterned surfaces that make them capable of provoking strong immune responses [4].

Nanoparticles can act at different levels:

- (1) Protection from enzymatic degradation: nanoparticles can protect an encapsulated antigen by shielding the allergen from the proteolytic enzymes in the body. This can be particularly relevant for oral immunotherapy where vaccine formulations have to tackle the conditions of the gastrointestinal tract. Furthermore, masking the allergen-specific activation of effector cells such as basophils and mast cells may also be a strategy for making formulations hypoallergenic.
- (2) Depot effect: the depot effect is still considered one of the most important mechanisms for inducing immune tolerance. So far, it has been recognized that the persistence and prolonged release of an allergen can increase its exposure to immune cells, leading to immunomodulation.
- (3) Immunomodulation: the aim of AIT is to induce allergen-specific immune tolerance; therefore, the ability of nanoparticles to target antigen presenting cells and transduce signals capable of inducing tolerogenic responses are a powerful further expansion of potential nanomaterial platforms for curing allergic diseases (complexed or in combination therapy).

In this review, we describe some of the nanoparticle/allergen complexes tested in in-vitro and in-vivo studies reporting immunological data aimed at the formulation of antiallergic vaccines. Table 1 summarizes all the nanoparticles/allergen formulations described within the article.

POLYMERIC NANOPARTICLES

Poly lactic-co-glycolic acid nanoparticles

Poly (lactic-co-glycolic acid) (PLGA) have been extensively investigated because of their biocompatibility

and biodegradability, and they have been approved by both the US Food and Drug Administration (FDA) and European Medicine Agency for use in vaccines, drug delivery and tissue engineering. The advantage of polymeric nanoparticles as drug delivery systems is based on their unique properties that allow for controlled drug release, increased blood circulation time, and protection of the drug from biodegradation and rapid clearance [5]. PLGA nanoparticles were synthesized to load purified allergenic molecules, such as the major birch allergen (Bet v 1) [6,7], the recombinant profilin from *Caryota mitis* (rCmP) [8], the *Chenopodium* allergen rChe a 3 [9,10], showing that different allergen formulations successfully modified the ongoing Th2 response after a few shots *in vivo*. The use of PLGA as a vehicle for the intranasal administration of OLE₁₀₉₋₁₃₀ peptide (a major Ole e 1 T-cell epitope) in a mouse model of sensitization was also investigated, showing that pretreatment with the OLE₁₀₉₋₁₃₀-PLGA complex was effective at preventing subsequent allergic sensitization to Ole e 1 [11,12]. PLGA particles were also tested in oral antigen delivery with beta-lactoglobulin (BLG)-derived peptides, demonstrating that PLGA nanoparticles facilitated the capacity of BLG peptides to suppress the allergic response to whole whey protein [13]. Moreover, coencapsulation of PLGA with CpG (unmethylated cytosine-phosphate-guanine) nucleotides and one of the major house dust mite (HDM) allergens (Der p 2) [14] or the *Bee venom* phospholipase A2 [15] stimulated a potent antigen-specific immune response with the induction of a strong Th1 response.

Another class of polymers belonging to this family of particles is the poly(γ -glutamic acid) (γ -PGA), a high molecular weight polypeptide composed of γ -linked glutamic acid units and α -carboxylate side chains. Monocyte-derived dendritic cells from grass allergic patients, stimulated with a mixture of γ -PGA and *Phleum pratense* extract, augmented allergen-specific IL-10 production and the proliferation of autologous CD4⁺ memory T cells [16].

New allergen-complexes with poly(D,L-lactide-co-glycolide) have been used to entrap nOle e 1 allergen and then inject it intraperitoneally into mice, eliciting high levels of specific IgG2a antibodies and low levels of both total IgE and specific IgG1 antibodies, demonstrating that this product induced a Th1-like immune response [17].

Poly(anhydride) nanoparticles

Poly(anhydride) nanoparticles are biodegradable materials with carboxylic acid as their main final metabolite; they have fewer acidic groups and a

Table 1. Allergens nanocomplexes

Allergen source	Components of the nanoparticle	Type of complex	References
<i>Betula verrucosa</i>	PLGA + Bet v 1	Particle + purified allergen	[7]
<i>Caryota mitis</i>	PLGA + <i>Caryota</i> profilin	Particle + purified allergen	[8]
<i>Chenopodium album</i>	PLGA + rChe a 3 allergen	Particle + recombinant allergen	[9]
<i>Olea europaea</i>	PLGA + OLE ₁₀₉₋₁₃₀	Particle + allergenic derived peptide	[11,12]
Cow's milk	PLGA + BLG-peptides	Particle + peptides	[13]
<i>Dermatophagoides pteronyssinus</i>	PLGA + Der p 2 + CpG	Particle + purified allergen + TLR-9 agonist	[14]
Bee venom	PLGA + PLA2 + CpG	Particle + purified allergen + TLR-9 agonist	[15]
Grass	γ -PGA + <i>Phleum pratense</i> extract	Particle + total extract	[16]
<i>O. europaea</i>	PLG and nOle e 1	Particle + purified allergen	[17]
Peanut	Poly(anhydride) nanoparticles + roasted peanut extract	Particle + extract	[23]
Grass	Gantrez AN 119 + Lolium extract	Particle + total extract	[24]
Cashew nut	Poly(anhydride) nanoparticles + cashew nut allergens (CNE-NP)	Particle + extract	[25]
Peanut	Mannosamine-modified nanoparticles + roasted peanut extract	Particle + extract	[27]
Grass	CBP + Phl p 5	Particle + purified allergen	[29]
Cat	CBP + Fel d 1	Particle + purified allergen	[30]
<i>D. farinae</i>	Chitosan + Der f 2 ₄₇₋₆₇	Particle + allergenic derived peptide	[33]
Peanut	Chitosan + Ara h 2	Particle + cDNA expressing Ara h 2	[34]
<i>D. pteronyssinus</i>	Chitosan + Der p 1	Particle + cDNA expressing Der p 1	[35]
<i>D. pteronyssinus</i>	Chitosan + Der p 1 + ubiquitin	Particle + cDNA coexpressing Der p 1 + ubiquitin	[36]
<i>D. pteronyssinus</i>	Chitosan + Der p 2	Particle + cDNA expressing Der p 2	[37]
<i>P. judaica</i>	PHEA + Par j 1/Par j 2	Particle + engineered purified allergens	[38]
<i>P. judaica</i>	SLN + Par j 2	Particle + purified allergen	[40]
Japanese cedar	OML + Cry j 1	Particle + purified allergen	[41]
Cockroach	Liposome + <i>Periplaneta americana</i> extract	Particle + total extract	[44]
Cockroach	Liposome + Per a 9	Particle + purified allergen	[44]
Peanut	Liposome + Ara h2 + CD33	Particle + purified allergen + CD33	[47]
Cat	Q β -VLP + Fel d 1	Particle + recombinant monomeric allergen	[51]
Major mugwort	VNP + Art v 1	Particles expressing surface-exposed or shielded allergens	[53]
Peanut	CuMVtt-Ara R, CuMVtt-Ara h 1, CuMVtt-Ara h 2	Particle + extract; particle + allergen	[59 ^{***}]
HDM	VLP + Der p 2	Particle + recombinant allergen	[61 ^{**}]
HDM	eBP + Der p 2	Particle + recombinant allergen	[62 ^{**}]
<i>C. album</i>	Chimeric HBc VLPs (HBcAg fused with Che a 3-derived peptide)	Particle + segment of hypoallergenic allergen-derived peptide	[63 [*]]
Peach	Mannose dendrons + Prup3-T-cell peptides (D ₁ ManPrup3 and D ₄ ManPrup3)	Mannose dendron structure + peptide	[66]
Olive	Mannosylated dendrimers conjugated with OE ₁₀₉₋₁₃₀ peptide from Ole e 1	Mannosylated dendrimers + peptide	[67 [*]]
HDM	HMSNs + Der f2	Particle + recombinant allergen	[70]

CuMVtt, cucumber mosaic virus including tetanus toxin epitopes; HBcAg, hepatitis B core antigen; HDM, house dust mite; HMSN, hollow mesoporous silica nanoparticle; PHEA, poly(hydroxyethyl)aspartamide; PLA2, phospholipase A2; PLG, poly(D,L-lactide-co-glycolide); PLGA, poly lactic-co-glycolic acid; TLR, Toll-like receptor; VLP, virus-like particle; γ -PGA, poly(L-glutamic acid).

lower acidity content than the PLGA system, and show no mutagenicity or cytotoxicity [18]. Among these, the copolymer of methyl vinyl ether and maleic anhydride (commercialized as Gantrez® AN 119 from Ashland, Inc. USA) is a poly(anhydride) that facilitates the efficient and easy preparation of nanoparticles under mild conditions, using a solvent displacement method. Moreover, poly(anhydride) nanoparticles have been recently identified as innate immunity inducers, activating the complement system [19] and Toll-like receptors [20]. Recently, the use of poly(anhydride) nanoparticles based on Gantrez AN have been proposed for AIT [21]. These nanoparticles have demonstrated their potential with different allergens, including peanut proteins [22,23], *Lolium perenne* [24] and cashew nut proteins [25], due to their ability to induce protective immune responses associated with increased Th1 and T-regulatory cell (Treg) profiles. In addition, these nanoparticles possess bioadhesive properties within the gut mucosa, a key factor for inducing strong mucosal immune responses after oral administration [26].

Poly(methyl vinyl ether-co-maleic anhydride) is a polyanhydride with great potential in biomedical applications [19]. Roasted peanut extract was conjugated in a polymer formed by the covalent binding of mannosamine to the copolymer of methyl vinyl ether and maleic anhydride. In an in-vivo murine model of anaphylaxis, nanoparticle-treatment was associated with both less serious anaphylaxis symptoms and higher survival rates than control, confirming the protective effect of this formulation against the challenge [23,27].

Polysaccharide polymers

Polysaccharides are easily-produced polymers derived from natural sources. In this respect, poly(D-glucosamine) (known as chitosan) is a very abundant polysaccharide in nature derived from shrimp and other crustacean shells. Chitosan is composed of randomly distributed β -[1-4]-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), and its usage has already been approved in the United States and Europe in bandages and other hemostatic agents for human use. Chitosan also possesses several advantageous properties, such as low production costs, biocompatibility, biodegradability and nontoxicity. A relevant feature of this class of molecules was introduced by Weinberger *et al.* [28] who showed that the coupling of carbohydrates to a protein can mask B-cell epitopes, making the protein 'hypoallergenic'. Carbohydrate-based particles covalently coupled to the timothy grass pollen allergen Phl p 5 (a major grass allergen) [29], rFel d 1 (a major cat

allergen) [30–32] and a peptide of the Der f 2 allergen (Der f 2_{47–67}) [33] exhibited desirable adjuvant properties with reduced allergic inflammation features.

Chitosan particles coupled with a cDNA expressing a dominant peanut allergen (pCMVArach2) [34], and chitosan nanoparticles containing plasmid DNA encoding for two of the major HDM allergens, pDer p 1 [35,36] and pDer p 2 [37], have been proposed as a novel form of vaccination showing immunomodulatory activities.

A copolymer based on poly(hydroxyethyl)aspartamide, functionalized in the side chains with butyryl and succinyl moieties, has been used to form nanoaggregates with an engineered derivative of the major *Parietaria judaica* allergens (Par j 1 and Par j 2) to produce a polymeric nanoaggregate of the allergens. The experimental data demonstrated that this copolymer retained its biological activity, suggesting its potential employment in a vaccine against *Parietaria* allergy [38].

Liposomes

Liposomes are spherical vesicles composed of at least one lipid bilayer capable of entrapping aqueous solution inside themselves. They are highly biocompatible and biodegradable, and for this reason they have been used in approaches with several allergenic components. Allergens can be either attached to the outer surface of the liposome or encapsulated within the lumen of the lipid bilayer [39]. Several liposome/allergenic complexes have been reported in the literature, such as with *P. judaica* Par j 2 allergen [40], *Japanese cedar* pollen [41], HDM [42], cats [43] and cockroaches [44]. Furthermore, liposome-entrapped vaccines made of a single major allergen alone, or with a single refined major allergen linked with Tregitopes (Treg epitopes), are effective at reducing allergen-mediated respiratory tissue inflammation and remodeling [45]. A clinical trial performed with liposomes entrapped with the HDM Der p 1 and Der p 2 allergens showed reduced clinical and medication scores, reduced skin test response and bronchial challenge in asthmatic patients [46]. Furthermore, liposomal nanoparticles bearing an allergen and a high-affinity glycan ligand of the CD33 inhibitory receptor profoundly suppressed IgE-mediated activation of mast cells, prevented anaphylaxis in Tg mice with mast cells expressing human CD33, and desensitized mice to subsequent allergen challenge for several days [47].

Virus-like particles

Virus-like particles (VLPs) are derived from viral capsid proteins made by the spontaneous

self-assembly of recombinant viral coat/envelope proteins in the absence of viral genetic material, making them noninfectious agents. VLPs can adopt the conformation of native viruses, and they have been shown to be efficient for hepatitis B virus and human papilloma virus human vaccines [48]. They have been characterized as highly immunogenic and able to promote potent neutralizing antibody responses [49[□]].

The approach of inducing allergen-dependent immunomodulation has been pursued by a few research groups by means of the surface attachment of full-length allergens or B-cell epitopes, as shown for Der p 1 [50], Fel d 1 [51,52] and Art v 1 [53]. Despite the fact that this latter strategy could imply the induction of an adverse IgE-mediated reaction, allergen/VLP seems to show a reduced ability to induce mast cell degranulation (Fel d 1 and Art v1) [51,53]. Intranasal prophylactic vaccination with shielded Art v 1/VLP prevented the development of mugwort pollen-associated allergic sensitization. The engineered VLP was nonanaphylactogenic and non-IgE inducing but, when intranasal administered, was able to induce tolerance [53]. A different strategy was performed in in-vivo studies using fragments (T and B-cell epitopes) of allergens derived from the Der p 1 [54,55] and Asp f 2 [56] allergens, demonstrating an allergen-specific T-cell response.

Moloney murine leukemia virus (MoMLV)-based VLPs were engineered to present immunodominant T-cell epitopes in the context of HLA class II molecules and to express costimulatory elements (CD80, CD86 or CD58) [57]. MoMLV-VLPs, fused with major mugwort pollen allergen Art v 1 epitope (aa 25–36), could strongly activate Art v 1-specific T cells in in-vitro assays. The elimination of costimulatory molecule expression triggered Art v 1-specific T-cell anergy. The same particles expressing CD58 stimulated the differentiation of allergen-specific Tr1 cells secreting IL-10/IFN- γ [57].

An innovative approach for the treatment of cat allergy is represented by the strategy of fusing a Tetanus toxin-derived Th-cell epitope (aa 830–843) to the cucumber mosaic virus protein coat [58]. This strategy was applied for the Fel d 1 [58], Ara h 1 and Ara h 2 [59[□]] allergens.

Recently, using the SpyCatcher/SpyTag conjugation system [60], a VLP displaying multicopies of the major HDM allergen Der p 2 was generated, showing that the Der p 2/VLP was unable to trigger the degranulation of RBL-SX38 cells loaded with human specific IgE. Furthermore, in a mouse model of prophylactic vaccination, the Der p 2/VLP prevented development of HDM-induced allergic inflammation characterized by IFN- γ secretion

and a massive induction of blocking IgG-antibodies [61[□]].

Gomord *et al.* used a model of an enveloped bioparticle that presents trimers of the Der p 2 allergen at its surface (BP-Der p 2) which have been assembled in *Nicotiana benthamiana*. This hybrid bioparticle was used to demonstrate the capacity of plants to produce synthetic supramolecular allergen bioparticles, and the immunomodulatory potential of naturally-assembled synthetic allergen bioparticles. This new expression platform was used not only for the production of soluble natural-like allergens, but also to develop an immunostimulatory allergen presentation system that triggers a strong Th1/Treg-biased response with reduced allergenicity [62[□]].

Sani *et al.* designed chimeric VLPs composed of the hepatitis B core (HBc) antigen genetically fused to *Chenopodium album* polcalcin (Che a 3). Results from IgE reactivity and basophil activation tests showed that chimeric HBc VLPs lack IgE-binding capacity and basophil degranulation activity. Furthermore, chimeric HBc VLPs produced a strong polcalcin-specific Th1 cell response consisting predominantly of IgG2a [63[□]].

Glycodendrimers

The term ‘glycodendrimer’ describes a well defined structure with a central and multifunctional core unit and peripheral reactive groups that constitute the bioactive saccharide binding sites [64]. Recent studies have demonstrated that glycodendrimers can enhance allergen uptake and presentation, modulating the immunological response [65]. The immunomodulatory effects of glycodendropeptides were studied in complexes containing Pru p 3 T-cell peptides in a mouse model of SLIT [66], showing a significant decrease in the level of Pru p 3-specific IgE and IgG1 and decreased proliferation of Pru p 3-specific CD4 splenocytes [66]. Furthermore, a dendrimeric scaffold conjugated to the T-cell immunodominant epitope of Ole e 1 (OLE_{109–130}) was studied on peripheral blood mononuclear cell from olive pollen-allergic patients, showing significant induction of IL10⁺ Treg proliferation, but not of Th₂ lymphocytes [67[□]].

Hollow mesoporous silica nanoparticles

Hollow mesoporous silica nanoparticles (HMSNs) are inorganic nanocarriers for antigen delivery with large surface areas, tunable pore sizes and well defined surface properties [68]. They have been approved by the FDA as a new biocompatible material [69]. HMSNs were loaded with Der f 2, and the

immunological characterization of the Der f 2/HMSN complexes was evaluated in a mouse model of allergic inflammation, showing that Der f 2-HMSNs could efficiently downregulate Th2 immune response and prevent allergic inflammation at an efficacy higher than Der f 2 adsorbed on Al(OH)₃ [70].

CONCLUSION

Since the commercialization of the first liposomes used for drug delivery, tremendous progress has been made in understanding interactions between nanomedicines and biological systems. The use of nanotechnology-based formulation represents a very promising new field with a continuous of novel discoveries and description of features in the field of nanomedicine. Nanovaccines have been widely experimented as prophylaxis of important diseases in bacterial, viral, cancers (primary and metastatic) and parasitic infections [71–73]. The future challenge will be the increase of our knowledge about the interaction of these formulations with the immune systems and the postdrug delivery which probably represent the most relevant challenge task for the future of nanomedicine in AIT [74^{••},75^{••}].

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Conflicts of interest

The authors have no conflicts of interest.

REFERENCES AND RECOMMENDED READING

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

- of special interest
- of outstanding interest

1. Noon L. Prophylactic inoculations against hay fever. *Lancet* 1911; 177:1572–1573.
 2. Komlasi ZI, Kovacs N, Sokolowska M, *et al.* Highlights of novel vaccination strategies in allergen immunotherapy. *Immunol Allergy Clin North Am* 2020; 40:15–24.
- This article reviews in a wide manner some of the major open issues about allergen-specific immunotherapy.
3. Schijns V, Fernandez-Tejada A, Barjaktarovic Z, *et al.* Modulation of immune responses using adjuvants to facilitate therapeutic vaccination. *Immunol Rev* 2020; 296:169–190.
- Therapeutic and prophylactic vaccination offers great promise as an intervention for a diversity of infectious and noninfectious conditions. The use of safe and effective vaccine adjuvants to promote protective immunity and/or reduce deleterious immune responses is of paramount importance for the development of innovative approaches.

4. Nel AE, Madler L, Velegol D, *et al.* Understanding biophysicochemical interactions at the nano-bio interface. *Nat Mater* 2009; 8:543–557.
5. Ding D, Zhu Q. Recent advances of PLGA micro/nanoparticles for the delivery of biomacromolecular therapeutics. *Mater Sci Eng C Mater Biol Appl* 2018; 92:1041–1060.
6. Scholl I, Kopp T, Bohle B, Jensen-Jarolim E. Biodegradable PLGA particles for improved systemic and mucosal treatment of Type I allergy. *Immunol Allergy Clin North Am* 2006; 26:349–364. ix.
7. Scholl I, Weissenböck A, Forster-Waldl E, *et al.* Allergen-loaded biodegradable poly(D,L-lactic-co-glycolic) acid nanoparticles down-regulate an ongoing Th2 response in the BALB/c mouse model. *Clin Exp Allergy* 2004; 34:315–321.
8. Xiao X, Zeng X, Zhang X, *et al.* Effects of *Caryota mitis* profilin-loaded PLGA nanoparticles in a murine model of allergic asthma. *Int J Nanomedicine* 2013; 8:4553–4562.
9. Hajavi J, Hashemi M, Sankian M. Evaluation of size and dose effects of rChe a 3 allergen loaded PLGA nanoparticles on modulation of Th2 immune responses by sublingual immunotherapy in mouse model of rhinitis allergic. *Int J Pharm* 2019; 563:282–292.
10. Salari F, Varasteh AR, Vahedi F, *et al.* Down-regulation of Th2 immune responses by sublingual administration of poly(lactic-co-glycolic) acid (PLGA)-encapsulated allergen in BALB/c mice. *Int Immunopharmacol* 2015; 29:672–678.
11. Marazuela EG, Prado N, Moro E, *et al.* Intranasal vaccination with poly(lactide-co-glycolide) microparticles containing a peptide T of Ole e 1 prevents mice against sensitization. *Clin Exp Allergy* 2008; 38:520–528.
12. Marazuela EG, Rodríguez R, Fernández-García H, *et al.* Intranasal immunization with a dominant T-cell epitope peptide of a major allergen of olive pollen prevents mice from sensitization to the whole allergen. *Mol Immunol* 2008; 45:438–445.
13. Kostadinova AI, Middelburg J, Ciulla M, *et al.* PLGA nanoparticles loaded with beta-lactoglobulin-derived peptides modulate mucosal immunity and may facilitate cow's milk allergy prevention. *Eur J Pharmacol* 2018; 818:211–220.
14. Joshi VB, Adamcakova-Dodd A, Jing X, *et al.* Development of a poly(lactic-co-glycolic acid) particle vaccine to protect against house dust mite induced allergy. *AAPS J* 2014; 16:975–985.
15. Martínez Gómez JM, Fischer S, Csaba N, *et al.* A protective allergy vaccine based on CpG- and protamine-containing PLGA microparticles. *Pharm Res* 2007; 24:1927–1935.
16. Broos S, Lundberg K, Akagi T, *et al.* Immunomodulatory nanoparticles as adjuvants and allergen-delivery system to human dendritic cells: implications for specific immunotherapy. *Vaccine* 2010; 28:5075–5085.
17. Batanero E, Barral P, Villalba M, Rodríguez R. Encapsulation of Ole e 1 in biodegradable microparticles induces Th1 response in mice: a potential vaccine for allergy. *J Control Release* 2003; 92:395–398.
18. Basu A, Domb AJ. Recent advances in polyanhydride based biomaterials. *Adv Mater* 2018; 30:e1706815.
19. Camacho AI, Da Costa Martins R, Tamayo I, *et al.* Poly(methyl vinyl ether-co-maleic anhydride) nanoparticles as innate immune system activators. *Vaccine* 2011; 29:7130–7135.
20. Tamayo I, Irache JM, Mansilla C, *et al.* Poly(anhydride) nanoparticles act as active Th1 adjuvants through Toll-like receptor exploitation. *Clin Vaccine Immunol* 2010; 17:1356–1362.
21. Gómez S, Gamazo C, Roman BS, *et al.* Gantrez AN nanoparticles as an adjuvant for oral immunotherapy with allergens. *Vaccine* 2007; 25:5263–5271.
22. Gamazo C, García-Azpiroz M, Souza Rebouças J, *et al.* Oral immunotherapy using polymeric nanoparticles loaded with peanut proteins in a murine model of fatal anaphylaxis. *Immunotherapy* 2017; 9:1205–1217.
23. Brotons-Canto A, Gamazo C, Martín-Arbella N, *et al.* Evaluation of nanoparticles as oral vehicles for immunotherapy against experimental peanut allergy. *Int J Biol Macromol* 2018; 110:328–335.
24. Gómez S, Gamazo C, San Roman B, *et al.* A novel nanoparticulate adjuvant for immunotherapy with *Lolium perenne*. *J Immunol Methods* 2009; 348:1–8.
25. Pereira MA, Rebouças JS, Ferraz-Carvalho RS, *et al.* Poly(anhydride) nanoparticles containing cashew nut proteins can induce a strong Th1 and Treg immune response after oral administration. *Eur J Pharm Biopharm* 2018; 127:51–60.
26. Arbos P, Campanero MA, Arango MA, *et al.* Influence of the surface characteristics of PVM/MA nanoparticles on their bioadhesive properties. *J Control Release* 2003; 89:19–30.
27. Brotons-Canto A, Gamazo C, Martín-Arbella N, *et al.* Mannosylated nanoparticles for oral immunotherapy in a murine model of peanut allergy. *J Pharm Sci* 2019; 108:2421–2429.
28. Weinberger EE, Himly M, Myschik J, *et al.* Generation of hypoallergenic neoglycoconjugates for dendritic cell targeted vaccination: a novel tool for specific immunotherapy. *J Control Release* 2013; 165:101–109.
29. Gronlund H, Vrtala S, Wiedermann U, *et al.* Carbohydrate-based particles: a new adjuvant for allergen-specific immunotherapy. *Immunology* 2002; 107:523–529.
30. Andersson TN, Ekman GJ, Gronlund H, *et al.* A novel adjuvant-allergen complex, CBP-rFel d 1, induces up-regulation of CD86 expression and enhances cytokine release by human dendritic cells in vitro. *Immunology* 2004; 113:253–259.

31. Neimert-Andersson T, Thunberg S, Swedin L, *et al.* Carbohydrate-based particles reduce allergic inflammation in a mouse model for cat allergy. *Allergy* 2008; 63:518–526.
 32. Thunberg S, Neimert-Andersson T, Cheng Q, *et al.* Prolonged antigen-exposure with carbohydrate particle based vaccination prevents allergic immune responses in sensitized mice. *Allergy* 2009; 64:919–926.
 33. Liu Z, Guo H, Wu Y, *et al.* Local nasal immunotherapy: efficacy of *Dermaphagoides farinae*-chitosan vaccine in murine asthma. *Int Arch Allergy Immunol* 2009; 150:221–228.
 34. Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan – DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nat Med* 1999; 5:387–391.
 35. Chew JL, Wolfowicz CB, Mao HQ, *et al.* Chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen, Der p 1 for oral vaccination in mice. *Vaccine* 2003; 21:2720–2729.
 36. Ou J, Shi W, Xu Y, Tao Z. Intranasal immunization with DNA vaccine coexpressing Der p 1 and ubiquitin in an allergic rhinitis mouse model. *Ann Allergy Asthma Immunol* 2014; 113:658–665.e1.
 37. Li GP, Liu ZG, Liao B, Zhong NS. Induction of Th1-type immune response by chitosan nanoparticles containing plasmid DNA encoding house dust mite allergen Der p 2 for oral vaccination in mice. *Cell Mol Immunol* 2009; 6:45–50.
 38. Licciardi M, Montana G, Bondi ML, *et al.* An allergen-polymeric nanoaggregate as a new tool for allergy vaccination. *Int J Pharm* 2014; 465:275–283.
 39. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, *et al.* Liposome: classification, preparation, and applications. *Nanoscale Res Lett* 2013; 8:102.
 40. Bondi ML, Montana G, Craparo EF, *et al.* Lipid nanoparticles as delivery vehicles for the *Parietaria judaica* major allergen Par j 2. *Int J Nanomedicine* 2011; 6:2953–2962.
 41. Ishii M, Koyama A, Iseki H, *et al.* Antiallergic potential of oligomannose-coated liposome-entrapped Cry j 1 as immunotherapy for *Japanese cedar* pollinosis in mice. *Int Immunopharmacol* 2010; 10:1041–1046.
 42. Chaisri U, Tungtrongchitr A, Indrawattana N, *et al.* Immunotherapeutic efficacy of liposome-encapsulated refined allergen vaccines against *Dermatophagoides pteronyssinus* allergy. *PLoS One* 2017; 12:e0188627.
 43. Tasanlyananda N, Chaisri U, Tungtrongchitr A, *et al.* Mouse model of cat allergic rhinitis and intranasal liposome-adjuvanted refined Fel d 1 vaccine. *PLoS One* 2016; 11:e0150463.
 44. Meechan P, Tungtrongchitr A, Chaisri U, *et al.* Intranasal, liposome-adjuvanted cockroach allergy vaccines made of refined major allergen and whole-body extract of *Periplaneta americana*. *Int Arch Allergy Immunol* 2013; 161:351–362.
 45. Prangtaworn P, Chaisri U, Seesuwat W, *et al.* Tregitope-linked refined allergen vaccines for immunotherapy in cockroach allergy. *Sci Rep* 2018; 8:15480.
 46. Basomba A, Tabar AI, de Rojas DH, *et al.* Allergen vaccination with a liposome-encapsulated extract of *Dermatophagoides pteronyssinus*: a randomized, double-blind, placebo-controlled trial in asthmatic patients. *J Allergy Clin Immunol* 2002; 109:943–948.
 47. Duan S, Koziol-White CJ, Jester WF, *et al.* CD33 recruitment inhibits IgE-mediated anaphylaxis and desensitizes mast cells to allergen. *J Clin Invest* 2019; 129:1387–1401.
 48. Mohsen MO, Zha L, Cabral-Miranda G, Bachmann MF. Major findings and recent advances in virus-like particle (VLP)-based vaccines. *Semin Immunol* 2017; 34:123–132.
 49. Kratzer B, Hofer S, Zabel M, Pickl WF. All the small things: how virus-like particles and liposomes modulate allergic immune responses. *Eur J Immunol* 2020; 50:17–32.
- This article describes the various technical advances in virus-like particle (VLP)-research and liposome-research context by referring and critically discussing the relevant studies performed to treat allergic diseases.
50. Kundig TM, Senti G, Schnetzler G, *et al.* Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J Allergy Clin Immunol* 2006; 117:1470–1476.
 51. Engeroff P, Caviezel F, Storni F, *et al.* Allergens displayed on virus-like particles are highly immunogenic but fail to activate human mast cells. *Allergy* 2018; 73:341–349.
 52. Schmitz N, Dietmeier K, Bauer M, *et al.* Displaying Fel d 1 on virus-like particles prevents reactivity despite greatly enhanced immunogenicity: a novel therapy for cat allergy. *J Exp Med* 2009; 206:1941–1955.
 53. Kratzer B, Kohler C, Hofer S, *et al.* Prevention of allergy by virus-like nanoparticles (VNP) delivering shielded versions of major allergens in a humanized murine allergy model. *Allergy* 2019; 74:246–260.
 54. Hirschberg S, Layton GT, Harris SJ, *et al.* CD4(+) T cells induced by virus-like particles expressing a major T cell epitope down-regulate IL-5 production in an ongoing immune response to Der p 1 independently of IFN- γ production. *Int Immunol* 1999; 11:1927–1934.
 55. Harris SJ, Roth JF, Savage N, *et al.* Prediction of murine MHC class I epitopes in a major house dust mite allergen and induction of T1-type CD8+ T cell responses. *Int Immunol* 1997; 9:273–280.
 56. Svirshchevskaya EV, Alekseeva L, Marchenko A, *et al.* Immune response modulation by recombinant peptides expressed in virus-like particles. *Clin Exp Immunol* 2002; 127:199–205.
 57. Leb VM, Jahn-Schmid B, Kueng HJ, *et al.* Modulation of allergen-specific T-lymphocyte function by virus-like particles decorated with HLA class II molecules. *J Allergy Clin Immunol* 2009; 124:121–128.
 58. Zeltins A, West J, Zabel F, *et al.* Incorporation of tetanus-epitope into virus-like particles achieves vaccine responses even in older recipients in models of psoriasis, Alzheimer's and cat allergy. *NPJ Vaccines* 2017; 2:30.
 59. Storni F, Zeltins A, Balke I, *et al.* Vaccine against peanut allergy based on engineered virus-like particles displaying single major peanut allergens. *J Allergy Clin Immunol* 2020; 145:1240–1253.e3.
- In this study, the immunological behavior of VLPs derived from cucumber mosaic virus including tetanus toxin epitopes and single peanut allergens can induce protection against a complex allergen mixture.
60. Thrane S, Janitzek CM, Matondo S, *et al.* Bacterial superglue enables easy development of efficient virus-like particle based vaccines. *J Nanobiotechnology* 2016; 14:30.
 61. Soongrun T, Mongkorntanyatip K, Peepim T, *et al.* Virus-like particles displaying major house dust mite allergen Der p 2 for prophylactic allergen immunotherapy. *Allergy* 2020; 75:1232–1236.
- A novel approach against house dust mite (HDM) allergy by means of a virus-like particle displaying multicopies of the major HDM allergen Der p 2 using the SpyCatcher/SpyTag conjugation system is described.
62. Gomord V, Stordeur V, Fitchette AC, *et al.* Design, production and immunomodulatory potency of a novel allergen bioparticle. *PLoS One* 2020; 15:e0242867.
- This article describes artificial modular bioparticles composed of trimers of the Der p 2 allergen produced as recombinant and *in vivo* assembled in plant.
63. Sani MZ, Bargahi A, Momenzadeh N, *et al.* Genetically engineered fusion of allergen and viral-like particle induces a more effective allergen-specific immune response than a combination of them. *Appl Microbiol Biotechnol* 2021; 105:77–91.
- In this study, hepatitis B core (HBc) antigen was genetically fused to *Chenopodium album* polcalcin (Che a 3)-derived peptide and the chimeric HBc VLP immunologically characterized.
64. Turnbull WB, Stoddart JF. Design and synthesis of glycodendrimers. *J Biotechnol* 2002; 90:231–255.
 65. Le Guevel X, Perez Perrino M, Fernandez TD, *et al.* Multivalent glycosylation of fluorescent gold nanoclusters promotes increased human dendritic cell targeting via multiple endocytic pathways. *ACS Appl Mater Interfaces* 2015; 7:20945–20956.
 66. Rodriguez MJ, Ramos-Soriano J, Perkins JR, *et al.* Glycosylated nanostructures in sublingual immunotherapy induce long-lasting tolerance in LTP allergy mouse model. *Sci Rep* 2019; 9:4043.
 67. Benede S, Ramos-Soriano J, Palomares F, *et al.* Peptide glycodendrimers as potential vaccines for olive pollen allergy. *Mol Pharm* 2020; 17:827–836.
- This article proposes the use of mannosylated dendrimeric scaffolds conjugated to the T-cell immunodominant epitope of Ole e 1 (OE_{109–130}).
68. Sun R, Wang W, Wen Y, Zhang X. Recent advance on mesoporous silica nanoparticles-based controlled release system: intelligent switches open up new horizon. *Nanomaterials (Basel)* 2015; 5:2019–2053.
 69. Tang F, Li L, Chen D. Mesoporous silica nanoparticles: synthesis, biocompatibility and drug delivery. *Adv Mater* 2012; 24:1504–1534.
 70. Peng X, Liang Y, Yin Y, *et al.* Development of a hollow mesoporous silica nanoparticles vaccine to protect against house dust mite induced allergic inflammation. *Int J Pharm* 2018; 549:115–123.
 71. Collins KA, Snaith R, Cottingham MG, *et al.* Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. *Sci Rep* 2017; 7:46621.
 72. Xiang SD, Wilson KL, Goubier A, *et al.* Design of peptide-based nano-vaccines targeting leading antigens from gynecological cancers to induce HLA-A2.1 restricted CD8(+) T cell responses. *Front Immunol* 2018; 9:2968.
 73. Ding P, Jin Q, Chen X, *et al.* Nanovaccine confers dual protection against influenza A virus and porcine circovirus type 2. *Int J Nanomedicine* 2019; 14:7533–7548.
 74. Keikha R, Daliri K, Jebali A. The use of nanobiotechnology in immunology and vaccination. *Vaccines (Basel)* 2021; 9:74.
- This review describes different nanostructures that can be applied to form a new generation of vaccines, adjuvants and immune system drugs.
75. Lenders V, Koutsoumpou X, Sargsian A, Manshian BB. Biomedical nanomaterials for immunological applications: ongoing research and clinical trials. *Nanoscale Adv* 2020; 2:5046–5089.
- This is an extensive review covering the nature of biomedical nanomaterials for immunological application describing the chemical aspects and the biological interactions.