

EXPERT
REVIEWS

Immunotherapy of type-1 allergies with virus-like particles and CpG-motifs

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Immunotherapy of type-I-allergies is regarded as the most efficient treatment option besides allergen avoidance. Different forms of allergen preparations are used as well as different routes of application. Virus-like particles represent a potent vaccine platform with proven immunogenicity and clinical efficacy. The addition of toll-like receptor ligands and/or depot-forming adjuvants further enhances immune cell activation. This article will focus on the function of virus-like particles loaded with DNA rich in CpG-motifs and discuss clinical experience in treatment of allergic rhinitis. Evidence will be presented that clinically effective treatment can be obtained even in the absence of allergens. Results encourage further investigation of virus-like particles and CpG-motifs in immunotherapy, either as a stand alone product, or as adjuvants for allergen-specific immunotherapy.

KEYWORDS: allergen • asthma • CpG • immunotherapy • rhinitis • toll-like receptor • virus-like particle

Allergic diseases of the upper airways are among the most common immunological disorders [1–3]. According to the initiative of the WHO ‘Allergic Rhinitis and its Impact on Asthma’, allergic rhinitis is defined as an IgE-mediated inflammatory reaction of the nasal mucosa after allergen exposition [2]. Allergen-specific immunotherapy (SIT) is accepted as the only causative treatment option besides allergen avoidance [4–7].

In general, SIT is defined as the serial application of allergen extracts using the sublingual or subcutaneous route with initially increasing allergen dose, eventually followed by a continuation phase [4–7]. Successful SIT leads to reduction of symptoms and reduced use of symptomatic medication, improvement of quality of life and several other improvements in the overall situation of the patient [4–7]. Moreover, clinical studies showed long-term effects that lasted far beyond the treatment phase [8,9], and for subcutaneous immunotherapy, it was shown that the development of new sensitizations and the progression to allergic asthma can be avoided [10,11]. However, SIT has disadvantages that need to be improved. Therapy-related adverse events should be reduced [12] as well as the duration of therapy in order to enhance treatment

compliance and adherence. Also, better treatment options should be developed for polysensitized patients [13]. Different therapeutic options have been discussed in this regard [14,15]. Relatively far developed are therapies with recombinant major allergens and hypoallergenic variants [16], peptide immunotherapy [17–19], intralymphatic immunotherapy [20] and epicutaneous immunotherapy [21]. A current dogma of immunotherapy is that the relevant allergens must be incorporated in a successful SIT. However, recent study data could overcome this dogma [22,23].

Virus-like particles (VLPs) are self-assembled nanoparticles that do not contain viral RNA or DNA and are therefore not able to replicate. VLPs are usually derived from capsid protein(s) of viruses or bacteriophages and provide a new and interesting vaccine platform for immunotherapy in inhalation allergies [24,25]. Vaccines against HBV and HPV are based on VLP and have been proven to be well tolerated, immunogenic and effective for the prophylactic vaccination against infection [24–26]. Moreover, VLPs of the bacteriophage Qbeta were investigated preclinically and clinically as carriers for antigen display for various vaccines against different neoplastic and other chronic diseases, as well as for infectious diseases [24].

As virus-like particles of the bacteriophage Qbeta (Qb-VLPs) consist of bacteriophage capsid proteins and have the same size and form as the original bacteriophage, which is designed by evolution to carry RNA. Qb-VLPs naturally pack bacterial RNA during the self-assembly process in *Escherichia coli*. The protein shell stabilizes these nucleic acids and protects them from degradation by enzymes. It is possible to disassemble these Qb-VLPs and reassemble them in the presence of oligodeoxynucleotides that are rich in CG motifs. Tumor antigens may be coupled to the surface of such VLPs, which results in the induction of specific CD8⁺ and CD4⁺ T cells in melanoma patients [27]. CpGs were also successfully used as adjuvants in the context of prophylactic vaccinations [26]. The VLP technology in combination with the use of CpG motifs could help to treat complex diseases such as malignant tumors, autoimmune disorders and allergies [25]. We describe the currently available clinical experience with bacteriophage VLPs that are filled with CpGs for the treatment of allergic diseases.

Immunology of type 1 allergic reactions

Allergic diseases are caused by dysregulations of the immune response to environmental antigens. Generally, the immune response to an allergen is controlled by T lymphocytes, and the dysregulation includes an excessive development of IL-4, IL-5 and IL-13, thus a Th2-dominated cytokine pattern [28]. These immunological mechanisms are necessary for the understanding of the mode of action of VLPs and CpG motifs in allergic diseases.

Allergies can be improved by long-term administration of large quantities of the responsible allergen. A change of the misguided allergic immune response toward a normal immunity is discussed to be the underlying mechanism of this improvement [28].

The physiological immune response differs from the allergic in a high number of specific Treg cells

The allergic immune response is generated by activation of specific Th2 lymphocytes, which produce, in a positive feedback loop, high levels of IL-4, IL-5 and IL-13. IL-4 induces B lymphocytes to produce IgE and IL-5, promotes the eosinophilic response and also activates eosinophils [28]. With increasing duration of the allergic disease, Th1 cells also get involved in disease progression by production of IFN- γ and TNF- α . Th1 cells are directly involved in the destruction of the tissue in the inflamed organ [29]. In addition, IL-17-producing Th17 cells significantly contribute to the chronic inflammatory response [28]. They result from chronic activation of epithelial cells by TGF- β and IL-6 and are able to activate allergic effector cells. Th17 cells produce IL-17 and also large amounts of IL-21 and IL-22.

It is known that both the immune response to allergens and the immune response following SIT induce T cells that produce large amounts of IL-10 and/or TGF- β [30]. These two regulatory cytokines suppress proliferation and cytokine production of effector T cells. Similar to other T cell subsets, these T cells

develop from naive precursors to become an independent, Treg population [28]. They are therefore referred to as Treg. They are allergen-specific T cells, which are characterized by the production of immunoregulatory cytokines and a weak proliferation, and need a continuous activation by dendritic antigen-presenting cells (APCs) and contact to the antigen for their regulatory effects [28]. Accordingly, they express a large extent of various activation markers such as the IL-2 receptor CD25 [28].

In the early stages of development, they are also typically characterized by the transcription factor forkhead box protein 3 (Foxp3) [30]. Treg expressing Foxp3 needs high amounts of antigen and TGF- β for their development from naive precursors. In determining the number of peripheral Treg after antigenic stimulation of blood lymphocytes from allergic patients and healthy subjects, it was shown that the healthy immune response was characterized mainly by high numbers of allergen-specific Treg in relation to Th1 and Th2 cells, and by the formation of specific tolerance [29]. The generation of a high number of peripheral Treg and thus the induction of immunological tolerance to an allergen is the real goal of SIT. Treg cells, however, are mostly defined by their function and thus represent no uniform population [28].

Successful SIT is based on the development of Treg & induction of specific tolerance

Activation of naive T cells by dendritic cells (DCs) can lead to the development of Th1 and Th2, as well as to Th17 or Treg cells. A sufficiently high number of Treg cells in relation to effector T cells are necessary in order to maintain a balanced immune response [28]. All functional T cell subpopulations – also Treg – arise from the same naive precursors under the influence of various cytokines and develop into independent subpopulations [30]. The development of various types of functional T cells is controlled by cytokines and the transcription factors induced by them. Th1 cells with the transcription factor T-bet are induced by IL-12. Th2 cells with the transcription factor GATA-3 are induced by IL-4, and the development of the transcription factor Foxp3 in Treg requires TGF- β .

The formation of Th17 cells and the transcription factor ROR requires IL-6 together with TGF- β [29]. The corresponding transcription factors are relatively reliable markers of the individual T cell subpopulations [28].

So far, it is assumed that in subcutaneous and sublingual immunotherapies, the use of high amounts of antigen leads to an induction of allergen-specific Tregs and to the development of specific immune tolerance [28]. For the induction of tolerance and specific Treg, only the binding site for MHC class II and the T cell receptor for the antigen is necessary. Therefore, immunological activation and tolerance by SIT can be solely produced with a peptide mixture of immunogenic T cell epitopes [29]. Identical to a healthy immune response, successful SIT returns the allergic immune response to a normal – Treg balanced – immune response. This results in allergen-specific tolerance and a high number of IL-10-secreting Treg cells [30,31].

In the course of SIT, IL-10 is also produced by B cells, DCs and monocytes/macrophages, making these cells involved in the maintenance of the tolerant state [28].

In addition to the induction of Treg that suppress Th2 cells, the induction of allergen-neutralizing IgG responses is very likely to be of major importance [32].

This was assumed after serological results showed an increase of allergen-specific IgG antibodies, especially of the IgG4 subtype after successful SIT. This was often found in combination with a decrease in specific IgE. Thus, the concept of 'blocking' antibodies had been developed assuming a competition between IgG and IgE antibodies binding to the allergen and Fc receptor on mast cells and basophils.

The binding strength & quantity of allergens determine the cytokine profile of T cells

Soluble antigen/allergen has to be uptaken, processed by APC and be presented as peptides bound to MHC class II molecules (HLA-DR, DQ, DP) to specific T cells via the T cell receptor. Additional binding molecules and receptors (accessory and costimulatory molecules) help to amplify and effectively bind APC and T cell in cases of weak antigen binding or small amounts of antigen [28].

A high number of interactions between antigen-specific T cells and APCs via the MHC-II molecules-bound antigen peptide and T cell receptor are necessary in order to correctly activate a T cell. A suboptimal interaction between APC and T cell due to small amounts of antigen leads to only partial T cell activation and development of a IL-4/IL-5-dominated Th2 cytokine pattern [33].

Obviously, much higher concentrations of allergen are necessary to develop a Th1 cytokine pattern and IL-10-producing Treg. The strength of the individual antigen detection by MHC II molecules with T cell receptors plays an important role. Indeed, it appears that the binding of allergen T cell peptides is significantly lower in allergic patients than in nonallergic individuals, what may be offset by higher amounts of antigen. Overall, these considerations indicate that high amounts of allergen, as are administered in long-term SIT, preferably induce a normal nonallergic immune response with IL-10/TGF- and IgG4/IgA antibody formation [29]. Allergic and nonallergic individuals recognize basically the same immunogenic determinants (epitopes) in an allergen [30].

Immune responses by VLPs & CpG motifs

At the beginning of a VLP-induced immune response, the antigens are taken up by APCs. Here, the optimal size, shape, surface structure, hydrophobic or hydrophilic properties and receptor interactions can promote the uptake [34].

The size of nanoparticles like VLPs is of particular importance for the uptake into the lymphatic system [35–37]. Within APCs, the antigen is processed, the proteins are degraded and peptides presented on the surface of the APC in conjunction with MHC class II molecules to stimulate CD4 T cells. Due to the biophysical properties of the VLPs, alternative presentation of antigenic peptides by MHC class I molecules and induction of a CD8 T cell response can be initiated [38,39].

DCs can be divided into different populations: myeloid DCs secrete mainly IL-12. Plasmacytoid DCs (pDCs) produce IFN- α [40–42].

Toll-like receptor (TLR) ligands such as CpG motifs strongly activate antigen-presenting DCs by binding to the TLR receptor [42,43]. For CpG motifs, it is of particular importance that in humans, only pDCs and no other DC type express TLR9, the receptor for CpG motifs.

CpG motifs

The human immune system can be activated by various pathogen-associated molecular patterns of viruses and other microorganisms. These include unmethylated CG-rich deoxy-nucleotide motifs, which are frequent in bacterial genomes, but rare in the genome of vertebrates. Unmethylated CpG motifs within CpGs or longer DNA molecules bind to the TLR9 receptor and thereby activate intracellular signaling pathways that are responsible for the secretion of cytokines and chemokines, and the upregulation of surface receptors [44]. CpG-induced innate immune responses are crucial in protection against microorganisms. CpG oligonucleotides are potent potential drugs as they enhance adaptive immune responses against malignant cells and may control inflammatory processes such as in allergies and asthma [45].

Three different types of CpG oligonucleotides have been described: the A-, B- and C-type CpG oligonucleotides. Type A CpG oligonucleotides contain CpG motifs in a palindromic sequence surrounded by guanosine. The nucleotides are typically connected by phosphodiester bridges and are therefore rapidly degraded *in vivo* by DNases. The type B only contains phosphorothioate bridges. Type C includes both phosphorothioate bridges but also CpG motifs and palindromes linked phosphodiester bonds. The activity profiles of different CpG oligonucleotide types are different: Type A induces mainly IFN- α in pDCs, whereas type B CpGs are strong activators of B lymphocytes and virtually do not induce IFN- α . Type C has both properties to some degree.

In humans, TLR9 is expressed in pDCs and B lymphocytes and stimulates production of Th1 cytokines, chemokines and the class switch to IgG [46]. As a result of CpG administration, the patient's immune system may react in different ways [22]: with a local Th1 immune response suppressing Th2-mediated allergic reactions; with IFN- α production also potentially reversing a Th2 response in human PBMCs *in vitro* [47]; with a downregulation of IgE synthesis; with a response of effector cells such as mast cells, basophils, eosinophils as a result of TLR9 stimulation in a human model [48].

In nasal biopsies, TLR9 is found to be expressed in mast cells, neutrophils, eosinophils and basophils [48] and in keratinocytes [49].

Rationale for VLPs as immunotherapeutic agents

The human immune system has evolved very effective mechanisms for responding to highly repetitive structures such as found in pathogenic microorganisms. Therefore, viruses and

Table 1. Actual clinical experiences with the use of virus-like particles and CpG motifs for treatment of allergic rhinitis.

Vaccine type	Treatment groups	Patients	Study design	Ref.
Q β VLP coupled to a synthetical Der p 1 sequence (Q β -Der p1)	i.m. 50 μ g Q β -Der p1 (n = 6), s.c. 50 μ g Q β -Der p1 (n = 6), i.m. 10 μ g Q β -Der p1 (n = 6), s.c. 10 μ g Q β -Der p1 (n = 6)	24 healthy controls	Randomized	[66]
AIC	AIC (n = 14), Placebo (n = 11)	25 patients allergic to ragweed (Ambrosia)	Randomized, double blind, placebo controlled	[65]
Type A CpG-ODN packed in Q β VLP (Q β G10) + house dust mite allergen extract	Q β G10 + house dust mite allergen (n = 20)	20 patients allergic to house dust mites	Open, monocentric	[67]
Q β VLP filled with Type A-CpG-ODN (Q β G10)	0.5 mg Q β G10 (n = 99), 1 mg Q β G10 (n = 103), Placebo (n = 97)	299 patients Allergic to house dust mites	Randomized, double blind, placebo controlled	[23]

AIC: Amb a 1 Antigen conjugated to CpG-ODN; HDM: House dust mite.
Adapted with permission from [22].

viral proteins are potent activators of the human immune system. Furthermore, the repetitive array of antigen epitopes can also stimulate the production of antibodies in B cells without the need for helper T cells. Therefore, VLPs represent the so-called T cell-independent antigens [50].

The repetitive characteristics of viral capsids are also observed on VLPs. They form spontaneously during the expression of certain capsid proteins of the virus. In contrast to complete viruses, VLPs do not contain any genomic information of the virus and are therefore not infectious. Typically, they are well tolerated and highly immunogenic. The antigenic determinants of the VLP are recognized by B cell receptors, and the particles are efficiently taken up by APCs presenting the VLP epitopes as T helper cell epitopes to MHC molecules.

VLPs & CpG motifs

VLPs are supramolecular particles having a diameter between 25 and 100 nm [51]. A key feature of VLPs is their ability to spontaneously assemble into particles with the shape and size of viruses. Some VLPs, such as those derived from bacteriophage Q β do this spontaneously in the presence of negatively charged macromolecules, such as RNA or DNA fragments. Thus, these nucleic acids can be packaged into VLPs. This could be shown for Q β G10 – a Q β -VLP that contains oligonucleotides with CpG motifs [24,51].

VLPs have the key immunological properties of vaccines and are immunostimulatory such as viruses. Thus, VLPs offer several advantages:

The lack of genetic information for viral replication or integration increases safety; they can be produced economically with standard good manufacturing practice; and their size and shape facilitate fast and strong humoral immune responses; VLPs can transport any antigens on their surface. Antigens can be bound either by genetic fusion with the VLP protein (chimera) or by chemical binding to the surface of the VLP

(coupled epitopes); immunostimulators can be packaged into the VLPs; adjuvants with depot properties can mimic kinetics of viral infections [25].

In recent years, CpG oligodeoxynucleotides have been used successfully in various clinical indications [45,52–57]. Type B CpG oligonucleotides have been successfully used as adjuvants in vaccines against hepatitis B and influenza [26,58,59]. They have also been used as adjuvants to enhance the T cell response in a number of tumors including malignant lymphomas [60], melanoma [61], basal cell carcinoma [62] and glioblastoma [63]. Type C CpG oligonucleotides have been tested in the treatment of hepatitis C [64].

Clinical experience with the use of VLPs & CpG motifs in the treatment of allergic rhinitis

Data from an early study with CpG oligonucleotides with a ragweed allergen conjugate were initially promising [65]. Experiences from four clinical trials were published in allergic rhinitis (TABLE 1).

Kündig *et al.* conducted a randomized Phase I study with Q β VLPs coupled to a synthetic 16 amino acid sequence of the house dust mite allergen Der p1 (Q β Der p1). The substance was administered three-times to 24 healthy subjects [66]. Antibody titers were measured over a period of 18 months. Side effects were only mild local reactions. A fast increase in specific IgM and IgG (particularly IgG1 and IgG3) to Der p1 peptides and VLPs could be found. IgE antibodies were not measurably increased. No differences were observed between intramuscular or subcutaneous administration [66]. The study showed that the administered vaccine was safe and well tolerated. In another study, Creticos *et al.* tested an Amb a 1 antigen conjugated to CpG ODN in 25 ragweed allergic patients [65]. In this randomized, double-blind, placebo-controlled Phase II study, adult patients were treated six-times at weekly intervals. However, the primary endpoint (vascular permeability of the

nasal mucosa measured as albumin in nasal lavage fluid) was not significantly affected. Symptoms of allergic rhinitis, however, were improved as well as the quality of life scores (Quality of life) were significantly increased even in the first ragweed pollen season after treatment. This improvement was maintained during the next pollen season (2 years after vaccination). No serious adverse effects were seen during the therapy. The measured allergen-specific IgE antibodies were decreased in the ragweed season.

Senti *et al.* investigated safety, tolerability and clinical effectiveness of a treatment with type A CpGs administered packaged into Q β -VLPs (QbG10) [67]. The vaccine was given together with a standard extract of house dust mite allergen. Twenty house dust mite allergic patients were enrolled in an open monocentric study. Immunotherapy began with injections of pure dust mite extract according to a clustered dose escalation protocol. This was followed by injections of a mixture of allergen and QbG10, which were administered six-times at intervals of 1–2 weeks. Clinical endpoints of this study included a conjunctival provocation test, clinical symptoms of asthma and allergic rhinitis quality-of-life measures, prick test results and determination of antibody titers. The results showed a high level of safety and good tolerability for the combination of house dust mite allergen and QbG10. There were no therapy-related adverse events. Symptoms of allergic rhinitis and allergic asthma were significantly reduced, and allergen-specific IgG antibodies increased. IgE antibodies decreased after a slight initial increase. The authors suggested that CpGs reduced allergic symptoms by activation of a Th1 immune response and specific IgG [67].

Klimek *et al.* published a randomized, double-blind, placebo-controlled Phase II multicenter study in which they used the identical VLP filled with type A CpG G10 (CYT003-QbG10) [23].

CYT003-QbG10 is a VLP filled with an oligonucleotide, called 'G10'. This VLP spontaneously assembles from coat proteins of the bacteriophage Q β during recombinant expression. The oligonucleotide G10 bears unmethylated CpG motives that are known to be recognized by TLR9. G10 is added during reassembly of the VLP, leading to its encapsulation inside the particle. These CYT003-QbG10 VLPs consist of 180 Q β monomers with the same symmetry as the native Q β capsid and a size of about 30 nm. The oligonucleotide G10 content of the reassembled particle is approximately 20% of the total mass, corresponding to approximately 60 oligonucleotide molecules per CYT003-QbG10 VLP.

However, in contrast to the previous study, there was no allergen and no allergen components given together with the vaccine. A total of 299 patients with house dust mite allergy were enrolled and received 6 weekly injections of the study drug or placebo. The core study took 9 weeks for each patient. Clinical endpoints were the symptom and medication score, quality-of-life scores and a conjunctival provocation test. The treatment was considered safe and well tolerated.

The average combined symptom and medication score according to the World Allergy Organization recommendations

was used as the primary endpoint to assess the patient's clinical status pre- and post-treatment, and this Symptom and medication scores improved significantly in the group treated with high-dose verum therapy. In detail [23], at baseline, all three treatment groups revealed similar median scores between 0.60 and 0.63. Possible score range was between 0 and 3. After the treatment phase with 0.5 mg CYT003-QbG10 or 1 mg CYT003-QbG10 or placebo, the median of the average combined symptom (ACS) and medication scores was reduced compared with pretreatment values. The ACS had fallen in the CYT003-QbG10 high-dose treatment group from 0.60 points pretreatment to 0.31 points post-treatment (-48%). The effect was less pronounced in the low-dose group with 0.61 points pretreatment and 0.45 points post-treatment (-26%) [23]. In the placebo group, only a moderate decrease of median ACS from 0.63 points pretreatment to 0.52 points post-treatment (-17%) was observed. The difference between placebo and the 1 mg treatment group reached statistical significance at study end ($p = 0.04$) but not pre- to posttreatment comparisons within the three treatment arms [23].

The authors concluded that a monotherapy with VLPs filled CpGs can affect symptoms of allergic rhinitis positively, even if no allergen is coadministered. The exact mechanism of the underlying immunological effect, however, is not yet known. From various animal experiments and *in vitro* studies, it has been suggested that the induction of a strong Th1 immune response and production of IFN- γ as described above attenuates the allergic Th2 immune response. Furthermore, a direct effect on mast cells was assumed since these also express TLR9 [68–70]. CpGs could also affect the allergic immune response via a change in the activity of the indoleamine 2,3 dioxygenase as a potent enzyme in the context of T cell regulation [71]. In mice, the upregulation of indoleamine 2,3 dioxygenase in specific DCs via type 1 interferon receptors following CpG stimulation resulted in a T cell suppression [72]. This mechanism could explain some of the anti-inflammatory effects of CpGs in allergic diseases.

The idea of the application of CpG motifs in the treatment of allergies was inspired by the so-called 'hygiene hypothesis'. According to this hypothesis, the risk of developing atopic diseases increases with reduced microbial exposure in childhood [73].

This hypothesis is supported by epidemiological studies showing a lower incidence of asthma in children who grow up on a farm, in children with several older siblings or children who grow up in nurseries from early infancy [74–76]. Here, the positive effect of microbial exposure is likely to induce an 'allergy protective' Th1 immune response, eventually a shift from a Th2 response toward a Th1 response [32]. Furthermore, Treg probably plays a significant role in this regard [72]. Interestingly, it was described that for CpGs, they are able to activate ICOS ligands on pDCs, an important costimulatory molecule for the induction of Treg [77].

CpGs that are packaged into VLPs are thus not only protected from enzymatic digestion. Particles of the size of viruses

are efficiently transported into the draining lymph nodes. There, they are efficiently phagocytosed by APC. By packing the CpGs into VLPs, they can be delivered more efficiently to TLR9 receptors localized in phagolysosomes [37].

However, the ‘hygiene hypothesis’ also has its pitfalls and is not generally accepted to be the only reason for the increasing numbers of allergic patients worldwide [78].

The importance of the VLP-specific B cell response for the positive clinical effect is not yet sufficiently understood. Both in mouse models and in humans, VLPs are able to induce B cell responses without adjuvants [79,80]. On the other hand, potent T cell responses can be improved by adequate adjuvants [27]. Klimek *et al.* showed that VLPs filled with CpG motifs can improve allergic symptoms without relevant changes in IgE levels [23]. In many cases, it was shown that a successful subcutaneous immunotherapy is associated with an increase in allergen-specific IgG4 and IgG1 responses [81–83]. The role of IgG3 antibodies is not yet sufficiently understood. The interaction between VLPs and APCs or Treg should be better investigated. This might help answering the question whether a combination of allergens and VLPs can improve the treatment. In future, VLPs could also play a role in the prophylactic vaccination against allergic diseases.

Expert commentary

VLPs in general and CpGs as adjuvants were successfully used in the treatment of infectious diseases and malignancies. Based on their viral immunomodulatory properties, they were also used for treating allergic diseases – in particular for the treatment of allergic rhinitis. So far, not all immunological mechanisms have been fully understood, but VLPs filled with CpGs appear to promote the normalization of the immune response in allergic patients.

First clinical experiences with different compositions of VLP and/or CpG vaccines showed good clinical tolerance, and good immunological and clinical effects (symptoms, medication use, provocation tests). Large controlled studies are needed to collect more extensive clinical experience with this new technology. Certainly, VLPs in combination with CpG motifs have great potential in the treatment for those already diseased from allergies – but also in the prophylactic application of persons who are at higher risks to develop an allergy.

Five-year view

The immune system has evolved to rapidly and strongly respond to viral infections. A single encounter with a virus is sufficient to induce life long and robust immunological changes and immunity. This is in stark contrast to conventional allergen immunotherapy that requires numerous allergen injections over years to ameliorate allergy symptoms. We therefore strongly believe that ‘dressing-up of an allergen’ to look like a virus to the immune system, such as a VLP containing ODNs,

is a highly promising strategy to enhance allergen immunotherapy. Ideally, the allergen should be covalently coupled to the VLP, ensuring that both the allergen and the ODNs are targeted to the same APC. Just admixing VLPs containing ODNs to the allergen as an adjuvant has the advantage that the same VLPs can be used for the entire spectrum of allergens. While first clinical results are encouraging, it remains to be seen whether allergens only mixed with VLPs are as potent drugs as allergens that are covalently coupled. Finally, results using VLP-encapsulated ODNs without allergen are intriguing. Of course, omitting the allergen has tremendous advantage in terms of safety with respect to allergic side effects, and one vaccine would work for all allergies. On the other hand, omitting the allergen raises questions about the longevity of the immune response, which is a hallmark of antigen-specific adaptive immunity. Moreover, the question needs to be answered, whether a successful treatment with VLP-encapsulated ODNs without allergen requires a concurrent environmental exposure to the allergen? For example, would the treatment work for pollen allergies if it were administered in the winter?

However, the hygiene hypothesis, suggesting that contact with bacterial products in the first year of life protects from allergies in the long term, suggests that, at least early in life, ODN-induced immunological changes may be persistent as well.

In conclusion, we believe that VLPs have a bright future in allergen immunotherapy, but it remains to be seen whether the allergen needs to be covalently coupled to the VLPs, merely mixed together, or whether VLPs filled with ODNs could make future allergen immunotherapy ‘allergen-free’.

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Key issues

- Current allergen immunotherapy requires too many allergen administrations and has a risk of allergic side effects.
- CG-rich bacterial DNA (CpGs) represents a potent Th1-inducing adjuvant.
- Virus-like particles (VLPs) can be used to package, stabilize and target CpGs to lymphatic organs and dendritic cells.
- Allergens can readily be coupled to the surface of VLPs, making them look like a virus to the immune system.
- VLPs filled with CpGs can also be used as an adjuvant which is mixed together with allergens.
- VLPs filled with CpGs, even without allergens, have been found to ameliorate allergy symptoms.
- CpGs are potent immunomodulators with toll-like receptor 9 expressed in plasmacytoid DCs, B cells, mast cells, neutrophils, eosinophils and basophils.
- More research needs to compare the therapeutic potency of allergen-coupled VLPs, allergen admixed to VLPs and CpG-loaded VLPs without allergens.

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- of interest
- of considerable interest

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