



Bee Venom Immunotherapy: Current Status and Future Directions

Abida Zahirović¹ · Jernej Luzar¹ · Peter Molek¹ · Nika Kruljec¹ · Mojca Lunder¹

© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Bee venom immunotherapy is the main treatment option for bee sting allergy. Its major limitations are the high percentage of allergic side effects and long duration, which are driving the development of novel therapeutic modalities. Three general approaches have been evaluated including the use of hypoallergenic allergen derivatives, adjunctive therapy, and alternative delivery routes. This article reviews preclinical and clinical evidence on the therapeutic potential of these new therapies. Among hypoallergenic derivatives, hybrid allergens showed a markedly reduced IgE reactivity in mouse models. Whether they will offer therapeutic benefit over extract, it is still not known since clinical trials have not been carried out yet. T cell epitope peptides have proven effective in small clinical trials. Major histocompatibility complex class II restriction was circumvented by using long overlapping or promiscuous T cell epitope peptides. However, the T cell-mediated late-phase adverse events have been reported with both short and longer peptides. Application of mimotopes could potentially overcome both T cell- and IgE-mediated adverse events. During this evolution of vaccine, there has been a gain in safety. The efficacy was further improved with the use of Toll-like receptor-activating adjuvants and delivery systems. In murine models, the association of allergen Api m 1 with cytosine-guanosine rich oligonucleotides stimulated strong T-helper type-1 response, whereas its encapsulation into microbubbles protected mice against allergen challenge. An intralymphatic administration of low-dose vaccine has shown the potential to decrease treatment from 5 years to only 12 weeks. Bigger clinical trials are needed to follow up on these results.

Keywords Bee venom immunotherapy · Epitope-based peptide · Hypoallergenic derivative · Recombinant allergen

Abbreviations

BVIT	Bee venom immunotherapy
CpG	Cytosine-guanosine rich oligonucleotides
HBV	Honeybee venom
HLA	Human leukocyte antigen
Ig	Immunoglobulin
IL	Interleukin
IFN	Interferon
LSP	Long synthetic peptide
MHC	Major histocompatibility complex
PBMC	Peripheral blood mononuclear cell
PLGA-MS	Poly lactide-co-glycolide acid microspheres
PolyI:C	Polyriboinosinic polyribocytidylic acid
SCIT	Subcutaneous immunotherapy
SLIT	Sublingual immunotherapy
T _H 1	Type 1 helper T cell

TLR	Toll-like receptor
Treg	Regulatory T cell
3D structure	Three-dimensional structure

Introduction

Honeybee venom allergy is a potentially life-threatening allergic reaction following a bee sting. In Europe, insect stings cause 48.2% of all anaphylactic reactions in adults [1]. Bee venom immunotherapy (BVIT) is the only available treatment that can protect allergic patients from subsequent sting reactions. The conventional immunotherapy consists of repeated administration of honeybee venom (HBV) extracts over 3–5 years. It has been proven effective in 77–84% of patients [2–6]. The efficacy of BVIT is described with the term tolerance. It refers to clinical unresponsiveness to bee venom allergens that persists after BVIT is stopped. Several studies and meta-analyses have found that the risk of systemic sting reactions is 5–15% in the 5 to 10 years after discontinuation of immunotherapy. In food allergy, the induced tolerance is not equivalent to natural tolerance because it may wane with time, whereas the natural tolerance is generally thought to be

✉ Abida Zahirović
a.zahirovic@hotmail.com

¹ Faculty of Pharmacy, Department of Pharmaceutical Biology,
University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

permanent. Also, it has been found that the underlying mechanisms of these conditions are distinct. Because of these differences, the term-sustained unresponsiveness has been proposed in food allergic patients as a more adequate description of the clinical non-reactivity lasting for 1–6 months after successful immunotherapy. The only acceptable outcome for bee venom allergic patients is complete clinical tolerance. Anything less than that would potentially place the patient at risk for serious allergic reactions upon next bee sting.

To characterize the efficacy of BVIT, several immunologic changes have been used in correlation with the severity of symptoms upon allergen challenge. The successful treatment is associated with reduced basophil responsiveness [7], the rise of allergen-specific immunoglobulin G4 (IgG4), and gradual fall of IgE [8]. The essential step in BVIT is the induction of peripheral T cell tolerance with the generation of interleukin (IL)-10-secreting regulatory T cells (Treg) [9, 10]. IL-10 suppresses immune responses to venom allergens through various effects including immune deviation from allergen-specific T_H2 towards T_H1 response [11, 12], thus leading to a class-switch from IgE to IgG4 in B cells [13, 14]. More recently, BVIT was found to induce IL-10-secreting regulatory B cells as well [15]. It is still unknown which of these immunologic parameters is eventually responsible for the therapeutic effects of BVIT.

Immunotherapy with HBV extracts faces several important limitations. Due to the use of native allergens, its major disadvantage is a high risk of systemic allergic side effects which are reported in 14 to 20% of patients undergoing treatment [4]. Bee venom extracts are available for subcutaneous BVIT as nonpurified and purified aqueous preparations or as purified aluminum hydroxide adsorbed (depot) preparations. Purified aqueous preparations have been found to cause smaller local reactions compared with nonpurified aqueous preparations, whereas depot preparations seem to be associated with fewer local side effects in comparison with aqueous extracts. Another constraint to the broader use of BVIT is inconvenient therapy schedule requiring medically supervised injections every 4 to 8 weeks during 3–5 years [16]. BVIT typically consists of an up-dosing phase and a maintenance phase. Currently, four different treatment schedules are used in up-dosing phase. In a conventional schedule, the maintenance dose is reached in several weeks to months. In cases that require rapid development of tolerance, the rush or ultra-rush methods may be applied, where maintenance dose is reached in a few days or hours, respectively. Cluster schedule is a modified rush protocol, in which several injections are applied at 15- to 30-min intervals during the first visits and maintenance dose is reached in about 6 weeks. All schedules are equally effective, whereas the conventional regime appears to be best tolerated. Antihistaminic agents are used in combination with these

methods to increase the safety. Maintenance dose is applied at 4–8-week intervals during 3–5 years to ensure a sustained effect. The third drawback of BVIT is related to the high percentage of treatment failures; 15.8 to 17% of patients relapse after stopping BVIT [17, 18]. This might be associated with differences in patient sensitization profile, venom composition, and/or allergen dose, as was the case with Api m 10, which has been found underrepresented in licensed HBV extracts and turned out to be the reason for treatment failure in patients predominantly sensitized to this allergen [19, 20]. To address these drawbacks, many research efforts have been undertaken. Here we review alternative immunotherapeutic strategies for HBV allergy that have been developed to achieve safer, more convenient, and more effective treatment. Some of the main advantages and disadvantages of these methods are summarized in Table 1. The field has moved along three non-mutually exclusive directions: (i) replacement of native HBV extracts with hypoallergenic allergen preparations; (ii) adjunctive therapies; and (iii) alternative administration routes (Fig. 1).

Replacement of Native HBV Extracts with Hypoallergenic Allergen Preparations

To improve BVIT, first and foremost aim is to reduce the allergenic activity of HBV. The rationale for hypoallergenic preparations is based on the findings that the treatment efficacy is dose-dependent—the higher the dose, the greater the efficacy of the treatment. By manipulating allergen structure and IgE binding, allergenicity can be reduced and will possibly allow administration of higher doses of the allergen without a risk for adverse reactions. In that respect, several strategies to substitute highly allergenic HBV with safer allergen preparations have been developed.

Chemically and Heat-Treated HBV Extract

Early methods for reduction of allergenicity of HBV extract were based on chemical treatment with aldehydes, which mask or destroy IgE epitopes by acting as protein cross-linkers, thus generating allergen polymers termed allergoids [39, 40]. In the later study, heat denaturation of HBV allergens was used for the same purpose [41]. However, for production of pharmaceutically acceptable product, denatured allergens and high-molecular-weight allergoids cannot be easily manufactured in a reproducible manner. The degree of chemical derivatization of allergens is dependent on the availability of free amino-groups in the individual proteins and therefore the extent of the reduction of IgE reactivity can vary [42]. Moreover, due to high molecular weight, allergoids cannot cross biologic

Table 1 Summary of the main advantages and disadvantages of alternative approaches to bee venom immunotherapy

Approach	Study (year)	Study subject	Advantages	Disadvantages	Ref
Allergoids	Mistrello et al. (1996)	Mice	Reduced allergenicity	Poor control over product quality: reproducibility issues The extent of reduction of IgE reactivity varies Reduced immunogenicity	[21]
Recombinant hypoallergenic allergen derivatives	Akdis et al. (1998)	PBMCs	Strongly reduced allergenicity	Variations regarding allergenicity and immunogenicity	[22]
	Buhot et al. (2004)	Mice	Retained immunogenicity	Costly recombinant production, the possibility of bacterial contamination	[23]
		Mice	Contains complete T cell epitope repertoire		[24]
	Kussebi et al. (2005)	Mice			[25]
	Karamloo et al. (2005)				
T cell epitopes	Carballido et al. (1993)	PBMCs	Lack of B cell epitopes in peptides: avoidance of IgE cross-linking	MHC restriction	[26]
	Muller et al. (1998)	Humans	No need for dose escalation	Late-phase allergic reactions	[27]
	Fellrath et al. (2003)	PBMCs	No need for adjuvants		[28]
	Texier et al. (2000)	Humans	Short course of IT		[29]
	Tarzi et al. (2006)				[30]
Minotopes fused to a carrier	Zahirovic et al. (2019)	PBMCs	Induction of epitope-specific antibody response Nonallergenic No capacity to induce late-phase allergic reactions Retained or enhanced immunogenicity when bound to a carrier Cheap and easy to produce, highly stable in storage Enhanced immunogenicity Shortened treatment	Non-immunogenic: delivery system is necessary Identification of protective epitopes is paramount for efficacy At the early stage of development	[31]
Allergens- T_H1 adjuvants	Johansen et al. (2005)	Mice	Enhanced immunogenicity Shortened treatment	Safety concerns: an increased risk of autoimmune diseases Relatively high toxicity of parenterally applied CpG at the doses required to stimulate immune system Problems with stability, bioavailability, and cost Manufactural difficulties may appear: Issues on protein stability during microencapsulation, storage and release (low allergen encapsulation efficiency, denaturation, chemical reaction of allergen with polymer) Problematic dose optimization Low residence time of microbubbles in circulation Ultrasound destruction may cause microvasculature rupture and hemolysis Expertise and studies restrained to a limited number of centers	[32]
Microparticulate delivery systems (PLGA-MS, microbubbles, microemulsions)	Trindade et al. (2012)	Mice	Enhanced stability—protection of allergens from degradation by natural proteases and increase of their bioavailability		[33]
	Corthesy et al. (2016)	Mice	Protection of tissues from deleterious effects caused by the direct contact with the BV toxins.		[34]
	Kiselmann et al. (2018)	Humans	Augmented immunogenicity by stimulation of specific antigen-presenting cells and targeted delivery		[35]
	Schuppe et al. (2018)				[36]
Sublingual immunotherapy	Severino et al. (2008)	Humans (large local reactions)	Convenient for patient (applicable in the comfort of one's home) Beneficial for children with fear of injection Improved safety Short-course treatment Enhanced immunogenicity Low allergen doses required	Lengthy administration course Tablets must be taken every day: problems with patient compliance Higher doses are used Larger human studies needed Larger clinical studies warranted Confined to specialist centers	[37]
Intralymphatic immunotherapy	Martinez-Gomez et al. (2009)	Mice			[38]

membranes and thus are unable to express therapeutic effect via mucosal routes. They also displayed low immunogenicity in some studies probably because of the disruption of certain T cell epitopes in allergens by aldehyde treatment [43, 44]. To overcome these limitations, carbamylated allergoids were developed. Potassium cyanate used for carbamylation acts by converting the charged ϵ -amino group of the lysine moieties into uncharged ureido groups. These changes within IgE epitopes on allergens result in products with decreased allergenic activity [45, 46]. In this way, the original monomeric structure of allergens is retained, and the immunogenicity is preserved [47]. Monomeric allergoid from melittin-free HBV (patented by Lofarma S.p.A) showed significantly reduced IgE-binding activity and was able to induce HBV-specific IgG in mice [21]. It was proven to be as efficient as unmodified venom in preventing allergic reactions in patients upon sting challenge while causing fewer side effects.

Recombinant Hypoallergenic Derivatives of HBV Allergens

To achieve better safety of BVIT, several attempts have concentrated on the concept of a molecular approach to allergy immunotherapy, which is based on recombinant allergens and their hypoallergenic derivatives (Fig. 1). An important advantage of this therapy is that it allows the application of individual, well-characterized allergen molecules with known biological activity [48]. The vaccine is thus devoid of irrelevant components and can be tailored to the patient's sensitization profile [49, 50]. The evidence from clinical trials in inhalational allergies suggests that recombinant allergens can replace natural allergen extracts [50, 51]. In the case of HBV allergy, several major allergens have been expressed in recombinant form [52–54], using the expression systems capable of performing posttranslational modifications for allergens

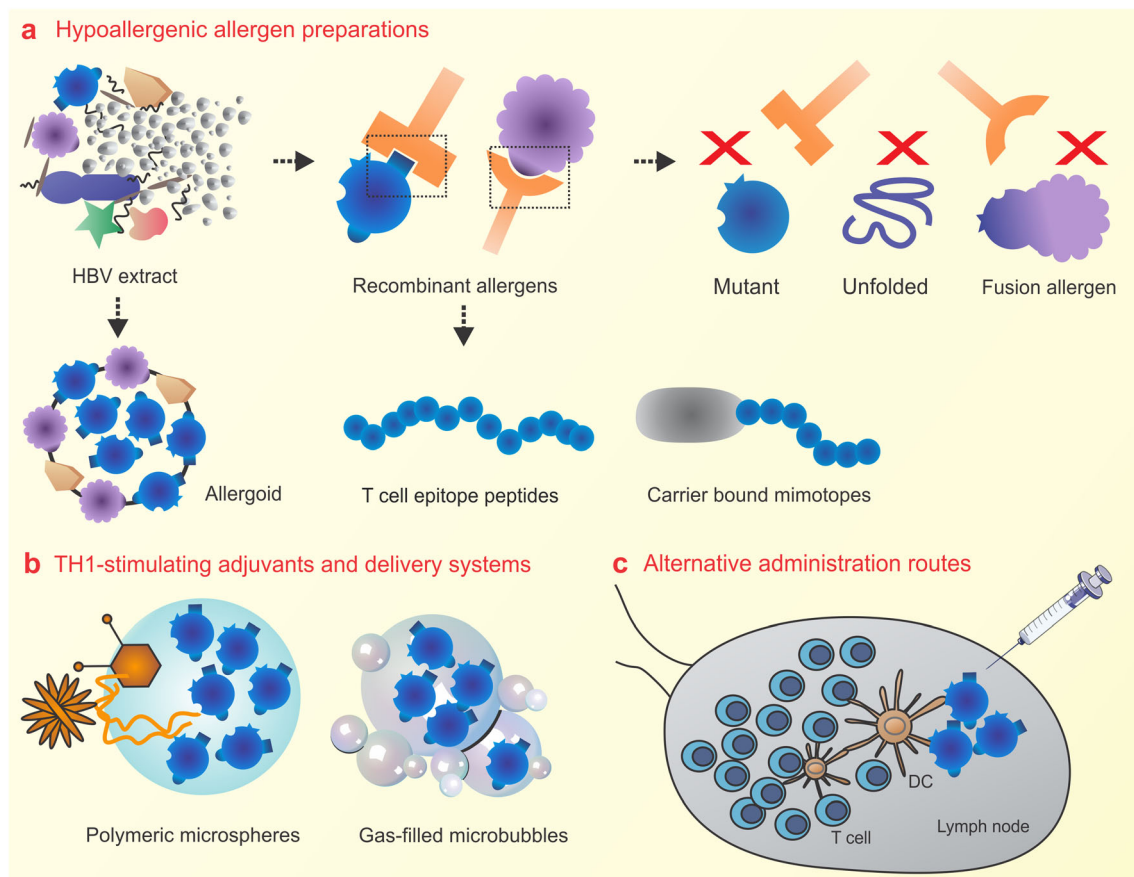
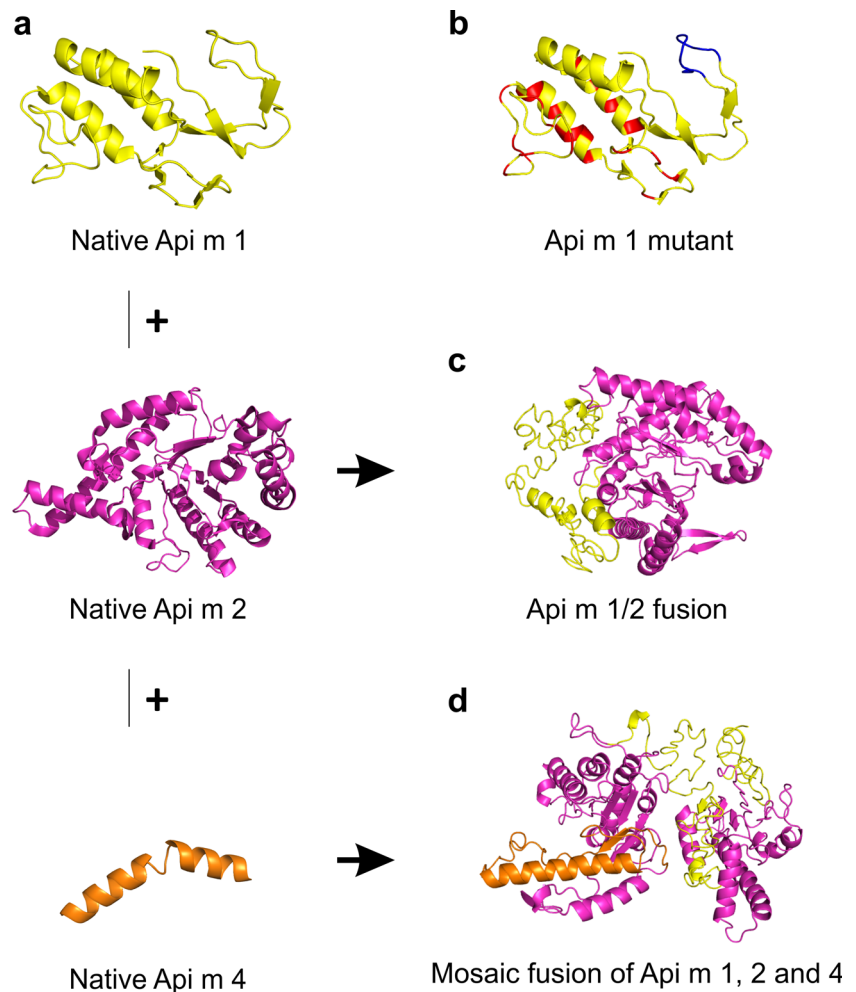


Fig. 1 Development of alternative immunotherapies for bee venom allergy. The research is focusing on three main areas. **a** Design of hypoallergenic allergen components, whose rationale is to greatly reduce the risk of IgE cross-linking and thus of anaphylactic reactions by destroying IgE epitopes (dashed rectangles) using either chemical treatment or recombinant techniques such as fragmentation, mutation, or hybridization. **b** Implementation of adjunctive therapies in the form of novel adjuvants and delivery systems to direct allergen-specific

responses towards protective T_H1 -type immunity and/or to achieve targeted delivery and controlled release. **c** Utilization of alternative routes to reduce systemic unwanted reactions and promote modulation of immune responses towards tolerization by targeting delivery to the immune cells involved in tolerance induction. All the measures are aiming at reducing allergenicity and improving the efficacy of current honeybee venom (HBV) extract-based immunotherapy

Fig. 2 Three-dimensional structures of recombinant HBV allergens and hypoallergenic variants. **a** Native Api m 1 (PDB ID: 1POC) [58]. **b** Api m 1 mutant (mutation sites are colored in red, deletion in blue). **c** Homology model of fusion protein containing Api m 1 and Api m 2 (PDB ID: 1FCQ) [59]. **d** Homology model of mosaic fusion protein consisting of rearranged fragments of Api m 1, Api m 2, and Api m 4 (PDB ID: 1MLT) [60]. Tertiary structures of Api m 1/2 fusion and mosaic fusion of Api m 1, 2, and 4 were predicted by I-TASSER [61, 62]. The figure was prepared with the PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC 8

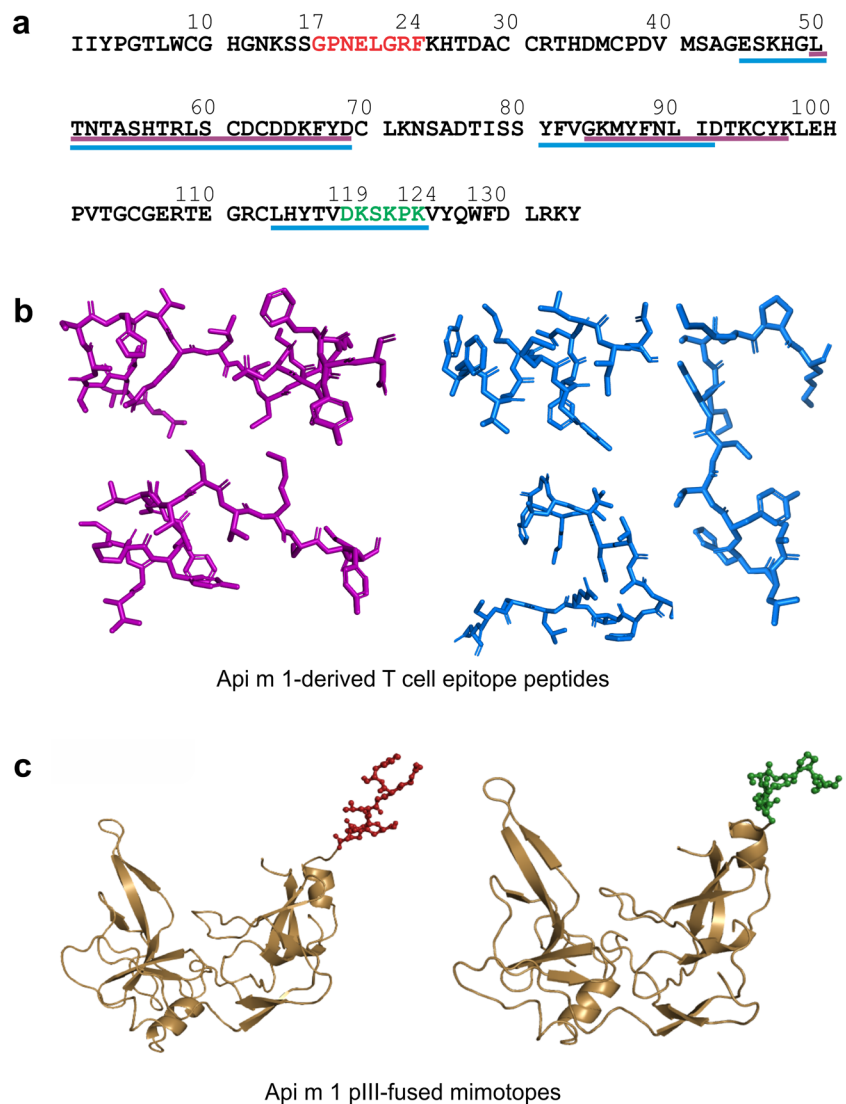


with carbohydrate epitopes [55]. Among 12 allergens present in HBV, Api m 1 (phospholipase A2) is identified as the major disease-causing agent recognized by specific IgE of up to 90% of HBV-allergic patients [56, 57]. Api m 1 is, therefore, most commonly chosen as a candidate for molecule-based immunotherapeutic approaches (Fig. 2a). However, recombinant Api m 1 possesses a comparable allergenic activity to that of natural allergen; hence, it would probably elicit similar allergic side effects during immunotherapy [63]. Hypoallergenic derivatives of Api m 1 were therefore produced and examined (Fig. 2b–d).

In a study by Akdis et al. [22], the recombinant hypoallergenic variants of Api m 1 were generated through disruption of its three-dimensional (3D) structure by preventing correct folding. This was based on the finding that the recognition of Api m 1 by IgE depends on conformation of the allergen [64]. In cultures of peripheral blood mononuclear cells (PBMC) from HBV-allergic patients, the unfolded Api m 1 variants exhibited immunomodulatory effects by stimulating the production of T_H1-dominant cytokines and IgG4 as opposed to the correctly folded variants which induced T_H2-type cytokines and IgE secretion.

In contrast to the previous approach, site-directed mutagenesis is aimed at changing the epitopes of the allergen without influencing its 3D structure. In order to obtain safe hypoallergenic recombinant molecules, an understanding of IgE-reactive sites is necessary because retention of IgE reactivity can lead to allergic reactions. This is particularly relevant for bee venom allergens given the high incidence of adverse allergic reactions with potentially fatal consequences. Buhot et al. [23] constructed a number of Api m 1 mutants by introducing 24 single amino acid substitutions and deletion between residues 106 and 115 in an exposed surface area (Fig. 2b). The knowledge of the 3D structure and the location of T cell epitope regions was applied to avoid local structural alteration and destruction of T cell epitopes [26, 29, 65, 66]. All mutated molecules were evaluated for IgE binding, and thus, the contribution of individual amino acid positions to IgE binding was determined. It was found that only the Api m 1 mutant comprising of all the mutations and a deletion exhibited a strongly reduced reactivity with patients' IgE. However, IgG antibodies elicited upon immunization of mice with this mutated protein did not recognize native allergen, possibly due to significant modification of allergen topology which

Fig. 3 Peptide immunotherapy for HBV allergy. **a** Api m 1 primary structure. T cell epitopes are underlined (magenta and blue); IgE epitopes are colored (red and green). **b** Molecular representation of Api m 1–derived T cell epitope peptides. **c** Molecular representation of Api m 1 mimotopes (dark red and dark green) fused to bacteriophage M13 minor coat protein pIII carrier (PDB ID 1G3P) [72]. The figure was prepared with the PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC



led to dynamic and flexible tertiary structure [67]. In addition to the difficulties with obtaining the molecule that is functional upon mutation, a large number of mutants that needs to be produced and tested to determine the best candidate render this approach cumbersome and expensive.

In the case of multiple sensitivities, the costs of recombinant vaccine would be even higher because it is likely that a cocktail of allergen molecules would be required. Since HBV-allergic patients are typically sensitized to several different allergens in HBV [68], the treatment with only one major allergen might be insufficient [69]. This issue was addressed by Kussebi et al. [24] who produced hybrid Api m (1/2) fusion protein containing a complete sequence of two clinically important HBV allergens, Api m 1 and Api m 2 (hyaluronidase) (Fig. 2c). Due to the destruction of conformational B cell epitopes of each individual allergen in the fusion molecule, Api m (1/2) protein showed abolished IgE reactivity and significant decrease in skin test reactivity. On the other hand, the

capacity of Api m (1/2) to induce T cell responses was preserved. Pretreatment of mice with Api m (1/2) fusion protein suppressed the production of specific IgE and other antibody isotypes upon stimulation with native Api m 1 [24]. Similar effects were observed with another engineered fusion protein from HBV allergens consisting of rearranged fragments of Api m 1, Api m 2, and Api m 4 (melittin) (Fig. 2d). This mosaic protein showed 100 to 1000 times reduced skin test reactivity in allergic patients [25]. Hybrid allergens have appeal for patients who are sensitive to more than one allergen from bee venom. Given that only one molecule has to be produced and processed instead of several recombinant allergens, fusion vaccine can reduce manufacturing costs. Moreover, the hybridization may result in improved immunogenicity [70].

Even though the clinical efficacy of recombinant hypoallergenic variants of HBV allergens has not been assessed yet, studies on inhalation allergens have shown that the immunization with the whole allergen stimulates IgGs which are

directed against all parts of allergen molecule, and, as such, may confer different biological effects (may enhance or inhibit allergic reaction) [71]. As a potential solution, the use of only a minimal allergen component (immunodominant antigenic determinant, i.e., epitope) has been proposed.

Epitope-Based Peptides

Epitope-specific treatment offers a possibility for the development of the next generation of allergy vaccines containing solely the necessary T and B cell epitopes or their mimetics (mimotopes), thus eliminating undesirable side effects induced by irrelevant determinants. Compared with whole allergen, epitope vaccines have many advantages, including increased safety, rational engineering of epitopes for increased potency, and the ability to focus the immune response on conserved epitopes.

Api m 1-Derived T Cell Epitope-Bearing Peptides

T cell epitope-bearing peptides are derived from allergen primary sequence (Fig. 3a). When applied in immunotherapy, they primarily target the T cell arm of the immune response and thus bypass IgE-mediated side effects [73]. Induction of T cell tolerance is found to be their major mechanism of action [74]. In initial studies, a restricted set of short T cell epitope peptides was used (Fig. 3b). Muller et al. [27] immunized five HBV-allergic patients with a mixture of three Api m 1-derived T cell reactive peptides [26] over a period of 2 months. The treatment was safe and led to the T cell hyporesponsiveness and inhibition of T_H1 and T_H2 cytokine production in PBMCs from treated patients. After provocation with subcutaneous injection of native Api m 1, no allergic reactions were observed in treated patients. However, upon sting challenge, mild systemic skin allergic symptoms appeared in two patients. The reason for incomplete protection in these two patients might be a sensitization to other HBV allergens, besides Api m 1, which was found later on in the study. This trial was the first in-human study which validated the concept of T cell epitope peptides and started elucidating the mechanism of peptide-induced immune tolerance, thus laying the ground for peptide immunotherapy in allergic diseases. Altered peptide ligands are another type of short T cell epitope peptides which were considered for therapy of HBV allergy. They are obtained by substituting certain amino acid residues found in contact of the peptide with T cell receptor. Peptides modified in such a manner may influence T cell proliferation and cytokine production through the delivery of antagonist or partial agonist signal [75]. Immunization of mice with altered peptide ligand of immunodominant T cell epitope of Api m 1 (amino acid 81–92 in which phenylalanine at position 82 is replaced with alanine) led to reduced IL-4 production in Api m 1-specific T cells. This effect was ascribed to the lower binding

affinity of the altered ligand to major histocompatibility complex (MHC) class II molecules compared with the native peptide [76]. Aforementioned studies suggest that the short immunodominant T cell epitope peptides of Api m 1 might be able to suppress immune response against the entire allergen in allergic patients. However, in clinical practice, due to a great amount of heterogeneity of human leukocyte antigen (HLA) genotype found within the population, the treatment with short peptides will require predetermination of T cell responses of the individual patient. This represents a major burden in the conception of such vaccine.

As an alternative, the peptides covering a wide range of T cell epitopes were considered to circumvent MHC class II restriction of human T lymphocytes. Kämmerer et al. [65] examined the use of long synthetic peptides (LSPs) encompassing the entire sequence of Api m 1 with overlaps that ensure the presence of all possible T cell epitopes. In proliferation experiments on PBMCs isolated from HBV-allergic patients, long 40–60-mer peptides were more tolerogenic than the short 15-mer peptides bearing immunodominant T cell epitopes [65, 77]. Importantly, LSPs exhibited low IgE binding activity despite their size probably because of the lack of original three-dimensional structure. In a murine model of HBV allergy, intraperitoneal administration of three Api m 1-derived LSPs induced deviation of established T_H2 immune response towards T_H1 type and efficiently protected the mice against anaphylaxis after allergen challenge. This was accompanied by the inhibition of specific IgE and enhanced production of IgG2a (equivalent to human IgG4) [78]. Intranasal administration of these peptides to mice induced systemic tolerance in both prophylactic and therapeutic settings [79]. Fellrath et al. [28] investigated further these Api m 1-derived LSPs in humans, in a randomized, double-blind placebo-controlled phase I trial on 16 HBV-allergic patients. Immunotherapy lasted 70 days and consisted of a 1-day dose escalating protocol followed by five subsequent maintenance doses of each of the LSPs. In contrast to the placebo, active treatment induced an enhanced production of IL-10 and immune deviation towards T_H1 -type response in PBMC cultures from treated patients. Allergen-specific IgG4, and not IgE, was increased throughout the study period. The ability of LSPs to induce allergen-specific IgG may provide a clinical benefit to short T cell epitope peptide vaccines. However, approximately 3 h after the peptide injection, two patients developed systemic allergic reactions (hand pruritus and a flash of the upper trunk). The delayed character of these reactions was suggestive of late-phase side effects and might be related to the IgE-independent stimulation of allergen-specific T cells. As a second strategy for overcoming MHC II restriction, Tarzi et al. [30] evaluated Api m 1-derived promiscuous T cell epitope peptides with binding affinity for commonly expressed HLA-DR molecules [29, 80] in 12 subjects with mild HBV allergy. The peptides caused decreased skin

sensitivity to HBV along with a suppressed proliferation of effector T cells, reduced secretion of IL-13 and IFN- γ , and increased IL-10 production. Although this approach has shown promise, its applicability in clinical practice is limited because it would be laborious and time-consuming to perform direct binding assay (HLA-typing), particularly when several disease-causing allergens need to be covered.

Altogether, the aforementioned clinical trials gave us some insights into the immune mechanisms underlying therapy with Api m 1–derived T cell epitope peptides. However, due to the small number of enrolled patients, no level of evidence can be ascribed to any type of the peptides examined. Moreover, it is noteworthy that described studies are of an older date (late 1990s or early 2000s) and there are no new clinical trials scheduled.

Api m 1 Mimotopes

According to aforementioned studies in patients with bee venom allergy as well as the studies pertaining to cat allergy [81, 82] and the most recent clinical trial in birch pollen allergy [83], late-phase side effects appeared to be a common problem in the clinical application of T cell epitope peptides, most likely due to the activation of allergen-specific T cells [28]. Mimotopes are short peptides that mimic B cell epitopes of allergen and hold the promise to overcome both IgE- and T cell-mediated side effects during immunotherapy [84]. Mimotopes are nonallergenic because they are too short to engage receptor-bound IgE on effector cells. On the other hand, they do not contain allergen-derived T cell epitopes and thus could not trigger late-phase T cell-mediated side effects. Since mimotopes are epitope-specific, they are more likely to induce protective IgG antibodies compared with the whole allergen [71]. In a recent study, we reported the identification of mimotopes of Api m 1 by screening phage display random peptide libraries against anti-Api m 1 IgG antibodies [31]. Thirty-six out of 46 positive phage clones exhibited high and specific binding for target antibodies and competed with Api m 1 for the same paratopes, thereby confirming to be mimetic of Api m 1 IgG epitopes. The relevance of the selected mimotopes in HBV-allergic patients was subsequently established by demonstrating their specific binding to IgE from patients' sera. Apart from their use in immunotherapy, mimotopes can be employed for the identification of epitopes and thus help us in understanding which structural features of allergens are responsible for their allergenic potential. Using selected mimotope sequences in combination with computational tools, we mapped the corresponding epitopes of Api m 1 between amino acids 17–24 and 119–124 (Fig. 3a, red and green). Since mimotopes are very poor immunogens on their own [85], in order to be considered for active immunization,

they necessitate the assistance of a carrier or delivery system (Fig. 3c).

Overall, the epitope-focused approach offers prospects for effective, safe, and specific treatment of bee venom allergy but requires further characterization of major allergens' epitopes. While a number of T cell epitopes have been identified, there is scant information regarding the IgE epitopes of bee venom allergens. Candidate epitopes must be properly designed and engineered by taking into account genetic instability and heterogeneity of different patients to cover all protective epitopes in treated population.

Adjunctive Therapies

While molecular vaccines have a better safety profile when compared with whole HBV extracts, they are often associated with lower immunogenicity and stability. For that reason, recent efforts are aiming at enhancing vaccine efficacy by application of specific delivery systems [86] and/or T_H1-stimulating adjuvants [87] (Fig. 1).

Allergen-Delivery Systems

Microparticulate delivery systems are used in allergy immunotherapy to achieve sustained release and selective targeting of specific immune cells [88]. They exhibit these beneficial effects by promoting the uptake of allergen in antigen-presenting cells such as dendritic cells and macrophages and/or by carrying immunomodulatory substances such as Toll-like receptor (TLR) agonists [86].

Due to their role in immune responses, skin dendritic cells have become attractive targets for allergy vaccines [89]. Epicutaneous delivery of an allergen via a transdermal patch or specially developed delivery system allows direct targeting of these antigen-presenting cells. Microemulsions are especially useful for that purpose thanks to their ability to increase skin hydration and disrupt the lipid barrier of the stratum corneum, whereas thermodynamical stability and the ability to solubilize both hydrophilic and lipophilic molecules make them suitable for the formulation of a protein containing drug delivery system [90]. Additionally, symptoms of contact dermatitis which frequently occur after application of dermal patches can be avoided by using microemulsion. Kiselmann et al. [35] incorporated Api m 1 into a stable microemulsion and explored its usage as a delivery system for epicutaneous immunotherapy. Epidermal penetration assessment showed that Api m 1 was released over time into deeper skin layers and reached Langerhans cells or even dendritic cells presumably by the formation of an epidermal drug depot. In a study by Schuppe et al., the developed microemulsion displayed immunomodulatory and therapeutic effects in a

mouse model [36]. The main disadvantage associated with microemulsion method is the necessity for the high concentrations of surfactant and/or cosurfactant to stabilize the droplets of microemulsion, which may cause an irritation. Microbubbles are used in clinical practice as an imaging contrast agent with a very good safety profile [91]. Ultrasound-mediated microbubble destruction has recently been proposed as a novel way of drug delivery. It includes the application of drug-carrying microbubbles to the specific area of interest and then bursting the microbubbles by ultrasound to achieve site-specific delivery of bioactive material. Bioley et al. [92] tested this delivery method on a murine model of HBV allergy. A prophylactic administration of microbubble-associated denatured Api m 1 protected mice against allergen challenge. The treatment led to elevated levels of specific IgG and IgA, decreased production of T_H2 type cytokines and increased frequencies of Treg cells. Subsequently, administration of microbubble-encapsulated denatured Api m 1 to mice with established allergy yielded similar immunological changes and protected all challenged animals [34]. Microbubbles have good safety record as a contrast agent. However, when used as delivery vehicles, ultrasound used for their destruction may cause rupture of microvasculature and hemolysis. Today, the studies and the expertise involving administration of microbubbles are still restrained to a limited number of centers. Since microbubbles stay in circulation for only a short time, the optimal dosage and timing of microbubbles administration are the questions that remain open.

Trindade et al. [33] produced and evaluated poly lactide-co-glycolide acid microspheres (PLGA-MS) with encapsulated HBV to determine whether they can control the delivery of venom and thus possibly allow a reduction of a total number of injections during traditional BVIT. Among six different types of polymer microparticles, it was found that the 34 kDa-COOH PLGA-MS possess the most favorable release profile with a low initial burst of HBV followed by slow release over time. The strong interactions between positively charged HBV and negatively charged polymers are probably responsible for the delayed release. This formulation applied in vivo is therefore expected to constantly stimulate immune system. The problems that may arise in the manufacture of polymer microparticles are protein instability during microencapsulation, storage, and release as well as the denaturation of allergen, low encapsulation efficiency, and chemical reaction of allergen with polymer. It is also difficult to optimize the dose. In terms of immunogenicity, Gomez et al. [93] showed that PLGA-MS containing Api m 1 were able to provoke only a weak antibody response in mice. However, when Api m 1 was co-encapsulated with cytosine-guanosine rich oligonucleotides (CpG) within PLGA-MS,

it stimulated strong production of Api m 1-specific IgG2a. The effect was further augmented by complexation of the CpG with protamine. As this study shows, microparticles may not be adequately immunogenic on their own and therefore, an adjuvant (e.g., TLR agonist) is added to achieve more potent or targeted effect.

Such synergistic action can be accomplished with the application of microbes or their components as delivery systems because they can concurrently deliver the therapeutic molecules and stimulate an immune response [94]. Among different candidates, bacteriophages have shown good potential as carriers of allergen epitopes and mimotopes in animal models [95–97]. Studies have found that bacteriophages induce more focused IgG response against displayed epitope in comparison with ovalbumin [98, 99]. Nonetheless, their use in humans is restrained by strict regulation of genetically modified organisms [100, 101] and an increased risk of transferring antibiotic resistance to F pili positive intestinal *E. coli* [102]. Moreover, mimotopes are often displayed in multiple copies on phage particle and may thus cause IgE cross-linking and activation of effector cells during allergy immunotherapy. We, therefore, examined the application of a single minor coat protein pIII from M13 bacteriophage as a carrier of Api m 1 mimotopes described above [31]. A representative of pIII-fused mimotopes proved to be nonallergenic in basophil activation test. In PBMC cultures of allergic patients, which secreted predominantly type 2 cytokines on stimulation with HBV and Api m 1, pIII-fused mimotope increased the production of type 1 cytokine (IFN- γ). This may imply a shift from T_H2 to T_H1 immune response and suggests that characterized mimotopes may serve as candidates for further immunotherapy development.

Co-administration of Allergen with T_H1 -Stimulating Adjuvants

To further direct immune response towards long-lasting tolerance, adjuvants that can skew the immune response to allergen towards T_H1 -type (e.g., TLR agonists) have been employed in allergy vaccine instead of aluminum hydroxide with proven T_H2 -promoting effects [103]. In a mouse model of HBV allergy, immunomodulatory effects of Api m 1 conjugated with different TLR agonists were compared with aluminum hydroxide-adjuvanted allergen preparations [32]. The study found that TLR ligands differ in adjuvancy and T_H1 -promoting capacity. Polyriboinosinic polyribocytidylic acid (PolyI:C) and lipopolysaccharide were the most potent adjuvants. However, they stimulated both T_H1 and T_H2 -type immune response, whereas CpG motifs and peptidoglycan were weaker adjuvants but induced a stronger T_H1 -polarized antibody response. CpG motifs, PolyI:C, and 3M003 (imidazoquinoline, TLR-7 agonist) showed therapeutic potential by suppressing IgE production and preventing provoked

anaphylaxis in mice. Even though these formulations have not yet been tested in HBV-allergic patients, clinical trials in patients allergic to house dust mite and ragweed suggest that the injection of allergen conjugates with CpG motifs could inhibit the immune responses to sensitizing extracts and alleviate allergic symptoms [104, 105]. Regarding clinical application of CpG and poly(I:C), the safety concerns have been raised about the increased risk of autoimmune diseases. The problem of widespread stimulation of non-hematopoietic bystander cells by poly(I:C) could be circumvented by using minimal dose of poly(I:C) assembled on the surface of PLGA microspheres. This holds promise to improve both efficacy and safety of poly(I:C) as vaccine adjuvant. Similarly, the strategies used to limit the systemic toxicity of CpG include conjugation or coencapsulation of CpG with antigen.

Premedication with the Anti-IgE Antibody (Omalizumab)

As a third form of an adjunctive therapy, the use of anti-IgE antibody (omalizumab) in combination with BVIT has been studied to determine whether it can enable desensitization in subjects who have failed immunotherapy with conventional protocol. Apart from one unsuccessful case study [106], in a handful of published case reports, HBV-allergic patients who experienced serious systemic adverse reactions to BVIT, including patients with mastocytosis, were able to tolerate BVIT when premedicated with the anti-IgE antibody [107–110]. Omalizumab was used as pretreatment for a couple of weeks in some cases, while in others, it was an integral part of the combined therapy until the end of BVIT [109] or it was discontinued after some months and maintenance BVIT pursued without any problem [111]. Because anti-IgE does not seem to be equally effective in all patients, it is difficult to determine the dose of anti-IgE therapy that would give a satisfactory level of protection from adverse reactions for every patient. The optimal protocol in terms of therapy duration and dosage of omalizumab still needs to be established. Moreover, the treatment with anti-IgE antibody is still associated with considerable costs, which is likely to be prohibitive in many countries.

Exploration of Alternative Administration Routes

Administration route plays an important role in directing the immune response to immunotherapy towards proallergenic or tolerogenic type. The sublingual application is advantageous for allergen immunotherapy due to the presence of a high population of tolerogenic dendritic cells in mucosal tissues. A trial conducted by Severino et al. [37] assessed the efficacy of sublingually applied

HBV in 26 patients with large local reactions to a bee sting during 6 months. The treatment was well tolerated and led to more than a double reduction of the wheal size in 57% of patients in the active group compared with the placebo-treated control group. Since large local reactions are not an indication for BVIT, these results must be validated in patients with systemic reactions [112, 113].

In many countries, sublingual immunotherapy (SLIT) is already used in clinical practice for the treatment of inhalational allergies [114]. Generally, it has gained interest thanks to the favorable safety profile and a convenient home administration [115, 116]. However, although initially seemed that the SLIT would be a better option for patients than subcutaneous immunotherapy (SCIT), studies reported rather poor patient adherence to SLIT, which was found to be even lower than with SCIT [117]. The analysis of prescription data on SCIT and SLIT for inhalational allergies found an unsatisfactory adherence and persistence rates into the third year of treatment in up to 51% of adult patients and of 44.1% in children and adolescents [118]. Approximately 70% of them reported heavy time demands of the treatment as the main reason for non-compliance. Therefore, an attractive concept of intralymphatic allergen administration was recently proposed to reduce the dose and number of injections. In studies on inhalation allergies, intralymphatic immunotherapy was found to ameliorate patient symptoms with only 3 injections within 12 weeks compared with SCIT which required up to 100 injections over 3 to 5 years to induce the same effect [119]. This is explained by geographical concept of immunogenicity, which relies on findings that strong immune response can be generated by antigens that reach the secondary lymphoid organs, where the exposure to high numbers of T and B cells can occur, whereas antigens that bypass secondary lymphoid organs have a less chance to encounter specific T or B cells and thus are mostly ignored [120]. Martinez-Gomez et al. [38] tested this approach on a mouse model of HBV allergy and found that intralymphatic administration of 100-fold lower doses of Api m 1 elicited enhanced T cell responses and more than tenfold higher production of IgG2a compared with subcutaneous immunization. The treatment increased secretion of IL-2 and IFN- γ as well as IL-4 and IL-10 suggesting that, rather than polarized, an overall stronger response was induced. In the proof-of-concept clinical trial, seven of eight HBV-allergic patients treated with three low-dose intralymphatic injections of HBV were protected after sting challenge. Similar preliminary results were obtained in a multi-center trial with 66 HBV-allergic patients [119].

ILIT injections are generally painless, but intralymphatic administration is more complex than SCIT and thus restricted

to specialist centers. Since targeted nodes are relatively small (0.5–1.5 cm), complex medical equipment and higher technical precision are required. Despite the use of a fine needle and ultrasound guidance, it is sometimes difficult to penetrate the capsule surrounding the lymph node, which may lead to inadequate administration of allergen. These technical aspects should be taken into consideration when discussing ILIT. Furthermore, it is important that the injections are given at 4-week intervals, which has been highlighted in the recent study where 2-week injections showed no clinical improvement despite the indications of immunological tolerance. The reason for this is thought to be the short interval between injections that does not allow enough time for the development of antigen-specific immune responses [121].

Conclusion

Many new forms of immunotherapy for HBV allergy have been tested in preclinical settings with quite a success. The research approaches have evolved from initial chemical modification of allergen preparations aimed primarily at reducing their allergenic activity to recent innovations in adjuvants and delivery systems which together with application through alternative routes are aimed at enhancing vaccine efficacy. However, their clinical development has generally been slow. Api m 1–derived T cell epitope peptides are the only recombinant derivatives of HBV allergens that have been clinically tested thus far. They showed limited therapeutic efficacy in several phase I clinical studies, but the research has not been pursued further. The issues with MHC II restriction and late-phase T cell–mediated side effects make T cell epitope peptides, which originally appeared to be attractive, somewhat less appealing. Though at the early stage of research, mimotope technology creates an opportunity of targeted epitope-specific therapy without the risk of T cell–mediated adverse events. Among other clinically tested modalities, a combination of omalizumab with BVIT was successful in preventing severe side effects during BVIT in high-risk patients, whereas clinical trials exploring the intralymphatic application of HBV allergens showed encouraging preliminary results but need more data. Overall, the advances outlined in this review need more investigation, particularly in larger clinical trials, to fully ascertain their potential to improve BVIT. Based on the conducted studies presented herein, we can say that the knowledge-based design of epitope-focused vaccines offers many advantages over traditional vaccines. However, the efficacy of individual therapeutic modalities might be insufficient to achieve complete clinical tolerance. Thus, we anticipate that a combination vaccine, which consists of all protective epitopes coupled with T_H1-stimulating adjuvants or incorporated into delivery systems and administered directly to the specific immune cells might provide a solution to

overcome the limitations of individual methods possibly through synergistic action.

Challenges and Future Prospects

The design of allergy therapy that has high safety, efficacy, and the ability to induce immune tolerance in a short time has appeared to be more complex than anticipated at the beginning. This is evident by the fact that after 50 years of research in the field (from the first allergoids' studies in the 1970s until now), only sublingual immunotherapy and monophosphoryl lipid A-adjuvanted allergoid vaccines are licensed. The translation of results from successful proof-of-principle reports in animal models to well-executed human clinical trials has proved to be particularly difficult in most of the cases.

In the next years, the allergy research field will probably evolve in various directions. We now understand that the response to allergy vaccine is dose and individual dependent. The growing understanding of the molecular mechanisms of immune tolerance is expected to be translated into more optimized design of multiepitope vaccine. Future vaccine regimens will cope with the diversity of immune response in the target population. More epitope candidates and newly designed vaccines are expected. The field of bioinformatics has opened the possibilities for finding of epitopes *in silico* and will be used more extensively together with experimental methods to facilitate the design of epitope-focused vaccines. Moreover, an increasing knowledge of the molecular mechanisms of immunodominance is expected to be translated into the identification of epitopes with increased biological activity. In terms of vehicle, additional delivery systems and routes will probably be evaluated. Finally, the field of allergy diagnosis is undergoing a drastic improvement in the area of molecular testing with defined, mainly recombinant allergens which allow high-resolution diagnosis. With the ability to determine patients' IgE sensitization profiles to individual allergens and peculiar reactivity patterns to specific epitopes, it is conceivable that we may define the patients in which a given therapy will work as well as those who are at increased risk of side effects during immunotherapy and thus tailor the therapy to individual patient (personalized treatment).

Funding This work was financially supported by the Slovenian Research Agency through research program P4-0127.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Clark S, Camargo CA Jr (2007) Epidemiology of anaphylaxis. *Immunol Allergy Clin N Am* 27(2):145–163. <https://doi.org/10.1016/j.iac.2007.03.002>
- Watanabe AS, Fonseca LA, Galvao CE, Kalil J, Castro FF (2010) Specific immunotherapy using Hymenoptera venom: systematic review. *Sao Paulo Med J* 128(1):30–37. <https://doi.org/10.1590/S1516-31802010000100007>
- Hockenhull J, Elremeli M, Cherry MG, Mahon J, Lai M, Darroch J, Oyee J, Boland A, Dickson R, Dundar Y, Boyle R (2012) A systematic review of the clinical effectiveness and cost-effectiveness of Pharmed® for the treatment of bee and wasp venom allergy. *Health Technol Assess* 16(12). <https://doi.org/10.3310/hta16120>
- Dhami S, Zaman H, Varga EM, Sturm GJ, Muraro A, Akdis CA, Antolin-Amérigo D, Bilò MB, Bokanovic D, Calderon MA, Cichocka-Jaros E, Oude Elberink JNG, Gawlik R, Jakob T, Kosnik M, Lange J, Mingomataj E, Mitsias DI, Mosbech H, Ollert M, Pfaar O, Pitsios C, Pravettoni V, Roberts G, Rüeff F, Sin BA, Asaria M, Netuveli G, Sheikh A (2017) Allergen immunotherapy for insect venom allergy: a systematic review and meta-analysis. *Allergy* 72(3):342–365. <https://doi.org/10.1111/all.13077>
- Dhami S, Panesar SS, Roberts G, Muraro A, Worm M, Bilò MB, Cardona V, Dubois AE, DunnGalvin A, Eigenmann P, Fernandez-Rivas M, Halken S, Lack G, Niggemann B, Rueff F, Santos AF, Vlieg-Boerstra B, Zolkipli ZQ, Sheikh A (2014) Management of anaphylaxis: a systematic review. *Allergy* 69(2):168–175. <https://doi.org/10.1111/all.12318>
- Boyle RJ, Elremeli M, Hockenhull J, Cherry MG, Bulsara MK, Daniels M, Oude Elberink JNG (2012) Venom immunotherapy for preventing allergic reactions to insect stings. *Cochrane Database Syst Rev* 10. <https://doi.org/10.1002/14651858.CD008838.pub2>
- Eržen R, Košnik M, Šilar M, Korošec P (2012) Basophil response and the induction of a tolerance in venom immunotherapy: a long-term sting challenge study. *Allergy* 67(6):822–830. <https://doi.org/10.1111/j.1398-9995.2012.02817.x>
- Varga EM, Francis JN, Zach MS, Klunker S, Aberer W, Durham SR (2009) Time course of serum inhibitory activity for facilitated allergen-IgE binding during bee venom immunotherapy in children. *Clin Exp Allergy* 39(9):1353–1357. <https://doi.org/10.1111/j.1365-2222.2009.03303.x>
- Meiler F, Zumkehr J, Klunker S, Ruckert B, Akdis CA, Akdis M (2008) In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med* 205(12):2887–2898. <https://doi.org/10.1084/jem.20080193>
- Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K (1998) Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 102(1):98–106. <https://doi.org/10.1172/JCI2250>
- Jutel M, Pichler WJ, Skrbic D, Urwyler A, Dahinden C, Muller UR (1995) Bee venom immunotherapy results in decrease of IL-4 and IL-5 and increase of IFN-gamma secretion in specific allergen-stimulated T cell cultures. *J Immunol* 154(8):4187–4194
- Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J (1997) Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. *Eur J Immunol* 27(5):1131–1139. <https://doi.org/10.1002/eji.1830270513>
- Satoguina JS, Weyand E, Larbi J, Hoerauf A (2005) T regulatory-1 cells induce IgG4 production by B cells: role of IL-10. *J Immunol* 174(8):4718–4726
- Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M (2008) Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. *Allergy* 63(11):1455–1463. <https://doi.org/10.1111/j.1398-9995.2008.01774.x>
- Boonpiyathad T, Meyer N, Moniuszko M, Sokolowska M, Eljaszewicz A, Wirz OF, Tomasiak-Lozowska MM, Bodzenta-Lukaszyk A, Ruxrungtham K, van de Veen W (2017) High-dose bee venom exposure induces similar tolerogenic B-cell responses in allergic patients and healthy beekeepers. *Allergy* 72(3):407–415. <https://doi.org/10.1111/all.12966>
- Fiedler C, Mische U, Treudler R, Kiess W, Prenzel F (2017) Long-term follow-up of children after venom immunotherapy: low adherence to anaphylaxis guidelines. *Int Arch Allergy Immunol* 172(3):167–172. <https://doi.org/10.1159/000458707>
- Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G (2009) Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy* 64(4):543–548. <https://doi.org/10.1111/j.1398-9995.2008.01794.x>
- Lerch E, Muller UR (1998) Long-term protection after stopping venom immunotherapy: results of re-stings in 200 patients. *J Allergy Clin Immunol* 101(5):606–612. [https://doi.org/10.1016/S0091-6749\(98\)70167-8](https://doi.org/10.1016/S0091-6749(98)70167-8)
- Frick M, Fischer J, Helbling A, Rüeff F, Wiczorek D, Ollert M, Pfützner W, Müller S, Huss-Marp J, Dorn B, Biedermann T, Lidholm J, Ruecker G, Bantleon F, Mische M, Spillner E, Jakob T (2016) Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. *J Allergy Clin Immunol* 138(6):1663–1671.e1669. <https://doi.org/10.1016/j.jaci.2016.04.024>
- Blank S, Seismann H, Michel Y, McIntyre M, Cifuentes L, Braren I, Grunwald T, Darsow U, Ring J, Bredehorst R, Ollert M, Spillner E (2011) Api m 10, a genuine *A. mellifera* venom allergen, is clinically relevant but underrepresented in therapeutic extracts. *Allergy* 66(10):1322–1329. <https://doi.org/10.1111/j.1398-9995.2011.02667.x>
- Mistrello Giovanni, Roncarolo Daniela, Zanoni Dario, Falagiani Paolo, 2008 inventor; Lofarma SpA, assignee. Allergens and allergoids from bee venom. US patent US8637037B2. December 16, 2008
- Akdis CA, Blesken T, Wymann D, Akdis M, Blaser K (1998) Differential regulation of human T cell cytokine patterns and IgE and IgG4 responses by conformational antigen variants. *Eur J Immunol* 28(3):914–925. [https://doi.org/10.1002/\(SICI\)1521-4141\(199803\)28:03<914::AID-IMMU914>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1521-4141(199803)28:03<914::AID-IMMU914>3.0.CO;2-C)
- Buhot C, Chenal A, Sanson A, Pouvelle-Moratille S, Gelb MH, Ménez A, Gillet D, Maillère B (2004) Alteration of the tertiary structure of the major bee venom allergen Api m 1 by multiple mutations is concomitant with low IgE reactivity. *Protein Sci* 13(11):2970–2978. <https://doi.org/10.1110/ps.04885404>
- Kussebi F, Karamloo F, Rhyner C, Schmid-Grendelmeier P, Salagianni M, Mannhart C, Akdis M, Soldatova L, Markovic-Housley Z, Von Beust BR, Kundig T, Kemeny DM, Blaser K, Cramer R, Akdis CA (2005) A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. *J Allergy Clin Immunol* 115(2):323–329. <https://doi.org/10.1016/j.jaci.2004.11.041>
- Karamloo F, Schmid-Grendelmeier P, Kussebi F, Akdis M, Salagianni M, von Beust BR, Reimers A, Zumkehr J, Soldatova L, Housley-Markovic Z, Müller U, Kundig T, Kemeny DM, Spangfort MD, Blaser K, Akdis CA (2005) Prevention of allergy by a recombinant multi-allergen vaccine with reduced IgE binding and preserved T cell epitopes. *Eur J Immunol* 35(11):3268–3276. <https://doi.org/10.1002/eji.200425522>
- Carballido JM, Carballido-Perrig N, Kägi MK, Meloen RH, Wüthrich B, Heusser CH, Blaser K (1993) T cell epitope

- specificity in human allergic and nonallergic subjects to bee venom phospholipase A2. *J Immunol* 150(8):3582–3591
27. Muller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, Blaser K (1998) Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 101(6 Pt 1):747–754. [https://doi.org/10.1016/S0091-6749\(98\)70402-6](https://doi.org/10.1016/S0091-6749(98)70402-6)
 28. Fellrath J-M, Kettner A, Dufour N, Frigerio C, Schneeberger D, Leimgruber A, Corradin G, Spertini F (2003) Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a phase I trial. *J Allergy Clin Immunol* 111(4):854–861. <https://doi.org/10.1067/mai.2003.1337>
 29. Texier C, Pouvelle S, Busson M, Herve M, Charron D, Menez A, Maillere B (2000) HLA-DR restricted peptide candidates for bee venom immunotherapy. *J Immunol* 164(6):3177–3184. <https://doi.org/10.4049/jimmunol.164.6.3177>
 30. Tarzi M, Klunker S, Texier C, Verhoef A, Stapel SO, Akdis CA, Maillere B, Kay AB, Larche M (2006) Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clin Exp Allergy* 36(4):465–474. <https://doi.org/10.1111/j.1365-2222.2006.02469.x>
 31. Zahirovic A, Koren A, Kopac P, Strukelj B, Korosec P, Lunder M (2018) Identification of bee venom Api m 1 IgE epitopes and characterization of corresponding mimotopes. *J Allergy Clin Immunol* 143(2):791–794.e5. <https://doi.org/10.1016/j.jaci.2018.10.003>
 32. Johansen P, Senti G, Martinez Gomez JM, Storni T, Beust BR, Wuthrich B, Bot A, Kundig TM (2005) Toll-like receptor ligands as adjuvants in allergen-specific immunotherapy. *Clin Exp Allergy* 35(12):1591–1598. <https://doi.org/10.1111/j.1365-2222.2005.02384.x>
 33. Trindade RA, Kiyohara PK, de Araujo PS, Bueno da Costa MH (2012) PLGA microspheres containing bee venom proteins for preventive immunotherapy. *Int J Pharm* 423(1):124–133. <https://doi.org/10.1016/j.ijpharm.2011.02.027>
 34. Corthésy B, Lassus A, Terretaz J, Tranquart F, Bioley G (2016) Efficacy of a therapeutic treatment using gas-filled microbubble-associated phospholipase A2 in a mouse model of honeybee venom allergy. *Allergy* 71(7):957–966. <https://doi.org/10.1111/all.12859>
 35. Kiselmann C, Dobler D, Schmidts T, Eicher AC, Mobs C, Pfutzner W, Runkel F (2018) Development of a skin-friendly microemulsion for dermal allergen-specific immunotherapy. *Int J Pharm* 550(1–2):463–469. <https://doi.org/10.1016/j.ijpharm.2018.09.002>
 36. Schuppe M, Kiselmann C, Dobler D, Wacker A, Schmidt O, Runkel F, Schmidts T, Pfutzner W, Mobs C (2018) Epidermal tolerance induction in a preclinical animal model of Api m 1-sensitized mice. *Allergy* 73:187–188
 37. Severino MG, Cortellini G, Bonadonna P, Francescato E, Panzini I, Macchia D, Campi P, Spadolini I, Canonica WG, Passalacqua G (2008) Sublingual immunotherapy for large local reactions caused by honeybee sting: a double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 122(1):44–48. <https://doi.org/10.1016/j.jaci.2008.03.031>
 38. Martinez-Gomez JM, Johansen P, Erdmann I, Senti G, Cramer R, Kundig TM (2009) Intralymphatic injections as a new administration route for allergen-specific immunotherapy. *Int Arch Allergy Immunol* 150(1):59–65. <https://doi.org/10.1159/000210381>
 39. Marsh DG, Norman PS, Roebber M, Lichtenstein LM (1981) Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 68(6):449–459. [https://doi.org/10.1016/0091-6749\(81\)90199-8](https://doi.org/10.1016/0091-6749(81)90199-8)
 40. Norman PS, Lichtenstein LM, Marsh DG (1981) Studies on allergoids from naturally occurring allergens. IV. Efficacy and safety of long-term allergoid treatment of ragweed hay fever. *J Allergy Clin Immunol* 68(6):460–470. [https://doi.org/10.1016/0091-6749\(81\)90200-1](https://doi.org/10.1016/0091-6749(81)90200-1)
 41. Johansen P, Senti G, Gomez JMM, Wuthrich B, Bot A, Kundig TM (2005) Heat denaturation, a simple method to improve the immunotherapeutic potential of allergens. *Eur J Immunol* 35(12):3591–3598. <https://doi.org/10.1002/eji.200535076>
 42. Marsh DG, Lichtenstein LM, Campbell DH (1970) Studies on “allergoids” prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. *Immunology* 18(5):705–722
 43. Lund L, Henmar H, Wurtzen PA, Lund G, Hjortskov N, Larsen JN (2007) Comparison of allergenicity and immunogenicity of an intact allergen vaccine and commercially available allergoid products for birch pollen immunotherapy. *Clin Exp Allergy* 37(4):564–571. <https://doi.org/10.1111/j.1365-2222.2007.02687.x>
 44. Henmar H, Lund G, Lund L, Petersen A, Würtzen PA (2008) Allergenicity, immunogenicity and dose-relationship of three intact allergen vaccines and four allergoid vaccines for subcutaneous grass pollen immunotherapy. *Clin Exp Immunol* 153(3):316–323. <https://doi.org/10.1111/j.1365-2249.2008.03710.x>
 45. Mosges R, Ritter B, Kayoko G, Allekotte S (2010) Carbamylated monomeric allergoids as a therapeutic option for sublingual immunotherapy of dust mite- and grass pollen-induced allergic rhinoconjunctivitis: a systematic review of published trials with a meta-analysis of treatment using Lais(R) tablets. *Acta Dermatovenerol Alp Pannonica Adriat* 19(3):3–10
 46. Lombardi C, Gargioni S, Melchiorre A, Tiri A, Falagiani P, Canonica GW, Passalacqua G (2001) Safety of sublingual immunotherapy with monomeric allergoid in adults: multicenter post-marketing surveillance study. *Allergy* 56(10):989–992. <https://doi.org/10.1034/j.1398-9995.2001.00181.x>
 47. Mistrello G, Brenna O, Roncarolo D, Zanoni D, Gentili M, Falagiani P (1996) Monomeric chemically modified allergens: immunologic and physicochemical characterization. *Allergy* 51(1):8–15. <https://doi.org/10.1111/j.1398-9995.1996.tb04543.x>
 48. Tscheppe A, Breiteneder H (2017) Recombinant allergens in structural biology, diagnosis, and immunotherapy. *Int Arch Allergy Immunol* 172(4):187–202. <https://doi.org/10.1159/000464104>
 49. Valenta R, Linhart B, Swoboda I, Niederberger V (2011) Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens. *Allergy* 66(6):775–783. <https://doi.org/10.1111/j.1398-9995.2011.02565.x>
 50. Klimek L, Schendzielorz P, Pinol R, Pfaar O (2012) Specific subcutaneous immunotherapy with recombinant grass pollen allergens: first randomized dose-ranging safety study. *Clin Exp Allergy* 42(6):936–945. <https://doi.org/10.1111/j.1365-2222.2012.03971.x>
 51. Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R, Purohit A, Arvidsson M, Kavina A, Schroeder JW, Mothes N, Spitzauer S, Montagut A, Galvain S, Melac M, André C, Poulsen LK, Malling H-J (2008) Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 122(5):951–960. <https://doi.org/10.1016/j.jaci.2008.09.017>
 52. Spillner E, Blank S, Jakob T (2014) Hymenoptera allergens: from venom to “venome”. *Front Immunol* 5(77). <https://doi.org/10.3389/fimmu.2014.00077>

53. Muller UR (2002) Recombinant Hymenoptera venom allergens. *Allergy* 57(7):570–576. <https://doi.org/10.1034/j.1398-9995.2002.02157.x>
54. Bilo MB, Antonicelli L, Bonifazi F (2012) Honeybee venom immunotherapy: certainties and pitfalls. *Immunotherapy* 4(11):1153–1166. <https://doi.org/10.2217/imt.12.113>
55. Blank S, Michel Y, Seismann H, Plum M, Greunke K, Grunwald T, Bredehorst R, Ollert M, Braren I, Spillner E (2011) Evaluation of different glycoforms of honeybee venom major allergen phospholipase A2 (Api m 1) produced in insect cells. *Protein Pept Lett* 18(4):415–422. <https://doi.org/10.2174/092986611794653923>
56. Vachova M, Panzner P, Kopac P, Stojkovic UB, Korosec P (2018) Routine clinical utility of honeybee venom allergen components. *J Allergy Clin Immunol Pract*. <https://doi.org/10.1016/j.jaip.2018.08.012>
57. Sobotka AK, Franklin RM, Adkinson NF, Jr., Valentine M, Baer H, Lichtenstein LM (1976) Allergy to insect stings. II. Phospholipase A: the major allergen in honeybee venom. *J Allergy Clin Immunol* 57(1):29–40. [https://doi.org/10.1016/0091-6749\(76\)90076-2](https://doi.org/10.1016/0091-6749(76)90076-2)
58. Scott DL, Otwinowski Z, Gelb MH, Sigler PB (1990) Crystal structure of bee-venom phospholipase A2 in a complex with a transition-state analogue. *Science* 250(4987):1563–1566. <https://doi.org/10.1126/science.2274788>
59. Markovic-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Muller U, Schirmer T (2000) Crystal structure of hyaluronidase, a major allergen of bee venom. *Structure* 8(10):1025–1035. [https://doi.org/10.1016/S0969-2126\(00\)00511-6](https://doi.org/10.1016/S0969-2126(00)00511-6)
60. Terwilliger TC, Eisenberg D (1982) The structure of melittin. I Structure determination and partial refinement. *J Biol Chem* 257(11):6010–6015
61. Zhang Y (2008) I-TASSER server for protein 3D structure prediction. *BMC Bioinformatics* 9:40. <https://doi.org/10.1186/1471-2105-9-40>
62. Roy A, Kucukural A, Zhang Y (2010) I-TASSER: a unified platform for automated protein structure and function prediction. *Nat Protoc* 5(4):725–738. <https://doi.org/10.1038/nprot.2010.5>
63. Forster E, Dudler T, Gmachl M, Aberer W, Urbanek R, Suter M (1995) Natural and recombinant enzymatically active or inactive bee venom phospholipase A2 has the same potency to release histamine from basophils in patients with Hymenoptera allergy. *J Allergy Clin Immunol* 95(6):1229–1235. [https://doi.org/10.1016/S0091-6749\(95\)70080-3](https://doi.org/10.1016/S0091-6749(95)70080-3)
64. Schneider T, Lang AB, Carballido JM, Santamaria Babi LF, Dudler T, Kagi MK, Blaser K, Suter M (1994) Human monoclonal or polyclonal antibodies recognize predominantly discontinuous epitopes on bee venom phospholipase A2. *J Allergy Clin Immunol* 94:61–70. [https://doi.org/10.1016/0091-6749\(94\)90072-8](https://doi.org/10.1016/0091-6749(94)90072-8)
65. Kammerer R, Kettner A, Chvatchko Y, Dufour N, Tiercy JM, Corradin G, Spertini F (1997) Delineation of PLA(2) epitopes using short or long overlapping synthetic peptides: interest for specific immunotherapy. *Clin Exp Allergy* 27(9):1016–1026. <https://doi.org/10.1111/j.1365-2222.1997.tb01253.x>
66. Dhillon M, Roberts C, Nunn T, Kuo M (1992) Mapping human T cell epitopes on phospholipase A2: the major bee-venom allergen. *J Allergy Clin Immunol* 90(1):42–51. [https://doi.org/10.1016/S0091-6749\(96\)80009-6](https://doi.org/10.1016/S0091-6749(96)80009-6)
67. Carra JH, Welcher BC, Schokman RD, David CS, Bavari S (2003) Mutational effects on protein folding stability and antigenicity: the case of streptococcal pyrogenic exotoxin a. *Clin Immunol* 108(1):60–68. [https://doi.org/10.1016/S1521-6616\(03\)00058-5](https://doi.org/10.1016/S1521-6616(03)00058-5)
68. Köhler J, Blank S, Müller S, Bantleon F, Frick M, Huss-Marp J, Lidholm J, Spillner E, Jakob T (2014) Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J Allergy Clin Immunol* 133(5):1383–1389.e1386. <https://doi.org/10.1016/j.jaci.2013.10.060>
69. Hofmann SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T (2011) Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 127(1):265–267. <https://doi.org/10.1016/j.jaci.2010.06.042>
70. Linhart B, Mothes-Luksch N, Vrtala S, Kneidinger M, Valent P, Valenta R (2008) A hypoallergenic hybrid molecule with increased immunogenicity consisting of derivatives of the major grass pollen allergens, Phl p 2 and Phl p 6. *Biol Chem* 389(7):925–933. <https://doi.org/10.1515/BC.2008.105>
71. Denepoux S, Eibensteiner PB, Steinberger P, Vrtala S, Visco V, Weyer A, Kraft D, Banchereau J, Valenta R, Lebecque S (2000) Molecular characterization of human IgG monoclonal antibodies specific for the major birch pollen allergen Bet v 1. Anti-allergen IgG can enhance the anaphylactic reaction. *FEBS Lett* 465(1):39–46. [https://doi.org/10.1016/S0014-5793\(99\)01703-2](https://doi.org/10.1016/S0014-5793(99)01703-2)
72. Lubkowski J, Hennecke F, Pluckthun A, Wlodawer A (1998) The structural basis of phage display elucidated by the crystal structure of the N-terminal domains of g3p. *Nat Struct Mol Biol* 5(2):140–147
73. Prickett SR, Rolland JM, O'Hehir RE (2015) Immunoregulatory T cell epitope peptides: the new frontier in allergy therapy. *Clin Exp Allergy* 45(6):1015–1026. <https://doi.org/10.1111/cea.12554>
74. Patel D, Couroux P, Hickey P, Salapatek AM, Laidler P, Larche M, Hafner RP (2013) Fel d 1-derived peptide antigen desensitization shows a persistent treatment effect 1 year after the start of dosing: a randomized, placebo-controlled study. *J Allergy Clin Immunol* 131(1):103–109.e101–107. <https://doi.org/10.1016/j.jaci.2012.07.028>
75. Candia M, Kratzer B, Pickl WF (2016) On peptides and altered peptide ligands: from origin, mode of action and design to clinical application (immunotherapy). *Int Arch Allergy Immunol* 170(4):211–233. <https://doi.org/10.1159/000448756>
76. Faith A, Akdis CA, Akdis M, Joss A, Wymann D, Blaser K (1999) An altered peptide ligand specifically inhibits Th2 cytokine synthesis by abrogating TCR signaling. *J Immunol* 162(3):1836–1842
77. Kammerer R, Chvatchko Y, Kettner A, Dufour N, Corradin G, Spertini F (1997) Modulation of T-cell response to phospholipase A2 and phospholipase A2-derived peptides by conventional bee venom immunotherapy. *J Allergy Clin Immunol* 100(1):96–103. [https://doi.org/10.1016/S0091-6749\(97\)70200-8](https://doi.org/10.1016/S0091-6749(97)70200-8)
78. Von Garnier C, Astori M, Kettner A, Dufour N, Heusser C, Corradin G, Spertini F (2000) Allergen-derived long peptide immunotherapy down-regulates specific IgE response and protects from anaphylaxis. *Eur J Immunol* 30(6):1638–1645. [https://doi.org/10.1002/1521-4141\(200006\)30:6<1638::AID-IMMU1638>3.0.CO;2-R](https://doi.org/10.1002/1521-4141(200006)30:6<1638::AID-IMMU1638>3.0.CO;2-R)
79. Astori M, von Garnier C, Kettner A, Dufour N, Corradin G, Spertini F (2000) Inducing tolerance by intranasal Administration of Long Peptides in naive and primed CBA/J mice. *J Immunol* 165(6):3497–3505. <https://doi.org/10.4049/jimmunol.165.6.3497>
80. Texier C, Pouvelle-Moratille S, Busson M, Charron D, Menez A, Maillere B (2001) Complementarity and redundancy of the binding specificity of HLA-DRB1, -DRB3, -DRB4 and -DRB5 molecules. *Eur J Immunol* 31(6):1837–1846. [https://doi.org/10.1002/1521-4141\(200106\)31:6<1837::AID-IMMU1837>3.0.CO;2-H](https://doi.org/10.1002/1521-4141(200106)31:6<1837::AID-IMMU1837>3.0.CO;2-H)
81. Moldaver D, Larché M (2011) Immunotherapy with peptides. *Allergy* 66(6):784–791. <https://doi.org/10.1111/j.1398-9995.2011.02610.x>

82. Barry Kay A, Larché M (2004) Allergen immunotherapy with cat allergen peptides. *Semin Immunopathol* 25(3–4):391–399. <https://doi.org/10.1007/s00281-003-0146-y>
83. Spertini F, DellaCorte G, Kettner A, de Blay F, Jacobsen L, Jutel M, Worm M, Charlon V, Reymond C (2016) Efficacy of 2 months of allergen-specific immunotherapy with Bet v 1–derived contiguous overlapping peptides in patients with allergic rhinoconjunctivitis: results of a phase IIb study. *J Allergy Clin Immunol* 138(1):162–168. <https://doi.org/10.1016/j.jaci.2016.02.044>
84. Luzar J, Štrukelj B, Lunder M (2016) Phage display peptide libraries in molecular allergology: from epitope mapping to mimotope-based immunotherapy. *Allergy* 71(11):1526–1532. <https://doi.org/10.1111/all.12965>
85. Hancock DC, O'Reilly NJ (2005) Synthetic peptides as antigens for antibody production. In: R. B (ed) *immunochemical protocols*. Methods Mol Biol: Humana Press; 295:13–26
86. Di Felice G, Colombo P (2017) Nanoparticle-allergen complexes for allergen immunotherapy. *Int J Nanomedicine Volume 12*: 4493–4504. <https://doi.org/10.2147/ijn.s134630>
87. Chesné J, Schmidt-Weber CB, Esser von-Bieren J (2016) The use of adjuvants for enhancing allergen immunotherapy efficacy. *Immunol Allergy Clin N Am* 36(1):125–145. <https://doi.org/10.1016/j.iac.2015.08.009>
88. Scholl I, Boltz-Nitulescu G, Jensen-Jarolim E (2005) Review of novel particulate antigen delivery systems with special focus on treatment of type I allergy. *J Control Release* 104(1):1–27. <https://doi.org/10.1016/j.jconrel.2004.12.020>
89. Johansen P, von Moos S, Mohanan D, Kundig TM, Senti G (2012) New routes for allergen immunotherapy. *Hum Vaccin Immunother* 8(10):1525–1533. <https://doi.org/10.4161/hv.21948>
90. Lopes CM, Coelho PB, Oliveira R (2015) Novel delivery systems for anti-allergic agents: allergic disease and innovative treatments. *Curr Drug Deliv* 12(4):382–396. <https://doi.org/10.2174/1567201812666150421111222>
91. Kang ST, Yeh CK (2012) Ultrasound microbubble contrast agents for diagnostic and therapeutic applications: current status and future design. *Chang Gung Med J* 35(2):125–139
92. Bioley G, Lassus A, Terrettaz J, Tranquart F, Corthésy B (2016) Prophylactic immunization of mice with phospholipase A2-loaded gas-filled microbubbles is protective against Th2-mediated honeybee venom allergy. *Clin Exp Allergy* 46(1):153–162. <https://doi.org/10.1111/cea.12555>
93. Gómez JMM, Fischer S, Csaba N, Kundig TM, Merkle HP, Gander B, Johansen P (2007) A protective allergy vaccine based on CpG- and protamine-containing PLGA microparticles. *Pharm Res* 24(10):1927–1935. <https://doi.org/10.1007/s11095-007-9318-0>
94. Zahirovic A, Lunder M (2018) Microbial delivery vehicles for allergens and allergen-derived peptides in immunotherapy of allergic diseases. *Front Microbiol* 9:1449. <https://doi.org/10.3389/fmicb.2018.01449>
95. Sidhu SS (2000) Phage display in pharmaceutical biotechnology. *Curr Opin Biotechnol* 11(6):610–616. [https://doi.org/10.1016/S0958-1669\(00\)00152-X](https://doi.org/10.1016/S0958-1669(00)00152-X)
96. Luzar J, Molek P, Šilar M, Korošec P, Košnik M, Štrukelj B, Lunder M (2016) Identification and characterization of major cat allergen Fel d 1 mimotopes on filamentous phage carriers. *Mol Immunol* 71:176–183. <https://doi.org/10.1016/j.molimm.2016.02.004>
97. Henry KA, Arbabi-Ghahroudi M, Scott JK (2015) Beyond phage display: non-traditional applications of the filamentous bacteriophage as a vaccine carrier, therapeutic biologic, and bioconjugation scaffold. *Front Microbiol* 6:755. <https://doi.org/10.3389/fmicb.2015.00755>
98. Van Houten NE, Zwick MB, Menendez A, Scott JK (2006) Filamentous phage as an immunogenic carrier to elicit focused antibody responses against a synthetic peptide. *Vaccine* 24(19): 4188–4200. <https://doi.org/10.1016/j.vaccine.2006.01.001>
99. Van Houten NE, Henry KA, Smith GP, Scott JK (2010) Engineering filamentous phage carriers to improve focusing of antibody responses against peptides. *Vaccine* 28(10):2174–2185. <https://doi.org/10.1016/j.vaccine.2009.12.059>
100. Pelfrene E, Willebrand E, Cavaleiro Sanches A, Sebris Z, Cavaleri M (2016) Bacteriophage therapy: a regulatory perspective. *J Antimicrob Chemother* 71(8):2071–2074. <https://doi.org/10.1093/jac/dkw083>
101. Huys I, Pimay J-P, Lavigne R, Jennes S, De Vos D, Casteels M, Verbeken G (2013) Paving a regulatory pathway for phage therapy. *EMBO Rep* 14(11):951–954. <https://doi.org/10.1038/embor.2013.163>
102. Nilsson AS (2014) Phage therapy—constraints and possibilities. *Ups J Med Sci* 119(2):192–198. <https://doi.org/10.3109/03009734.2014.902878>
103. Jensen-Jarolim E (2015) Aluminium in allergies and allergen immunotherapy. *World Allergy Organ J* 8(1):7. <https://doi.org/10.1186/s40413-015-0060-5>
104. Senti G, Johansen P, Haug S, Bull C, Gottschaller C, Müller P, Pfister T, Maurer P, Bachmann MF, Graf N, Kundig TM (2009) Use of A-type CpG oligodeoxynucleotides as an adjuvant in allergen-specific immunotherapy in humans: a phase I/IIa clinical trial. *Clin Exp Allergy* 39(4):562–570. <https://doi.org/10.1111/j.1365-2222.2008.03191.x>
105. Klimek L, Bachmann MF, Senti G, Kundig TM (2014) Immunotherapy of type-1 allergies with virus-like particles and CpG-motifs. *Expert Rev Clin Immunol* 10(8):1059–1067. <https://doi.org/10.1586/1744666X.2014.924854>
106. Soriano Gomis V, Gonzalez Delgado P, Niveiro Hernandez E (2008) Failure of omalizumab treatment after recurrent systemic reactions to bee-venom immunotherapy. *J Investig Allergol Clin Immunol* 18(3):225–226
107. Ricciardi L (2016) Omalizumab: a useful tool for inducing tolerance to bee venom immunotherapy. *Int J Immunopathol Pharmacol* 29(4):726–728. <https://doi.org/10.1177/0394632016670920>
108. Kontou-Fili K, Filis CI (2009) Prolonged high-dose omalizumab is required to control reactions to venom immunotherapy in mastocytosis. *Allergy* 64(9):1384–1385. <https://doi.org/10.1111/j.1398-9995.2009.02045.x>
109. Galera C, Soohun N, Zankar N, Caimmi S, Gallen C, Demoly P (2009) Severe anaphylaxis to bee venom immunotherapy: efficacy of pretreatment and concurrent treatment with omalizumab. *J Investig Allergol Clin Immunol* 19(3):225–229
110. Da Silva EN, Randall KL (2013) Omalizumab mitigates anaphylaxis during ultrarush honey bee venom immunotherapy in monoclonal mast cell activation syndrome. *J Allergy Clin Immunol Pract* 1(6):687–688. <https://doi.org/10.1016/j.jaip.2013.07.004>
111. Stretz E, Oppel EM, Rawer HC, Chatelain R, Mastnik S, Przybilla B, Rueff F (2017) Overcoming severe adverse reactions to venom immunotherapy using anti-IgE antibodies in combination with a high maintenance dose. *Clin Exp Allergy* 47(12):1631–1639. <https://doi.org/10.1111/cea.12997>
112. Rueff F, Bilo MB, Jutel M, Mosbech H, Muller U, Przybilla B (2009) Sublingual immunotherapy with venom is not recommended for patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 123(1):272–273; **author reply 273**. <https://doi.org/10.1016/j.jaci.2008.10.028>
113. Patriarca G, Nucera E, Roncallo C, Aruanno A, Lombardo C, Decinti M, Pascolini L, Milani M, Buonomo A,

- Schiavino D (2009) Sublingual immunotherapy with venom for patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 124(2):385; **author reply 385-386**. <https://doi.org/10.1016/j.jaci.2009.03.025>
114. Calderon MA, Penagos M, Sheikh A, Canonica GW, Durham S (2011) Sublingual immunotherapy for treating allergic conjunctivitis. *Cochrane Database Syst Rev* 7:CD007685. <https://doi.org/10.1002/14651858.CD007685.pub2>
 115. Calderón MA, Simons FER, Malling HJ, Lockey RF, Moingeon P, Demoly P (2012) Sublingual allergen immunotherapy: mode of action and its relationship with the safety profile. *Allergy* 67(3):302–311. <https://doi.org/10.1111/j.1398-9995.2011.02761.x>
 116. Senna G, Caminati M, Canonica GW (2013) Safety and tolerability of sublingual immunotherapy in clinical trials and real life. *Curr Opin Allergy Clin Immunol* 13(6):656–662. <https://doi.org/10.1097/ACI.0000000000000007>
 117. Leader BA, Rotella M, Stillman L, DelGaudio JM, Patel ZM, Wise SK (2016) Immunotherapy compliance: comparison of subcutaneous versus sublingual immunotherapy. *Int Forum Allergy Rhinol* 6(5):460–464. <https://doi.org/10.1002/alr.21699>
 118. Eberle P, Schreder H, Shah-Hosseini K, Mosges R (2013) Medication persistence in children and young people on long-term, grass pollen-specific immunotherapy - measured by prescription renewal rates. *Allergologie* 36(1):9–18. <https://doi.org/10.5414/ALX01536>
 119. Senti G, Kundig TM (2015) Intralymphatic immunotherapy. *World Allergy Organ J* 8(1):9. <https://doi.org/10.1186/s40413-014-0047-7>
 120. Zinkernagel RM, Ehl S, Aichele P, Oehen S, Kundig T, Hengartner H (1997) Antigen localisation regulates immune responses in a dose- and time-dependent fashion: a geographical view of immune reactivity. *Immunol Rev* 156:199–209. <https://doi.org/10.1111/j.1600-065X.1997.tb00969.x>
 121. Witten M, Malling HJ, Blom L, Poulsen BC, Poulsen LK (2013) Is intralymphatic immunotherapy ready for clinical use in patients with grass pollen allergy? *J Allergy Clin Immunol* 132(5):1248–1252.e5. <https://doi.org/10.1016/j.jaci.2013.07.033>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.