

Progress in the development of specific immunotherapies for house dust mite allergies

Expert Rev. Vaccines Early online, 1–11 (2014)

Philippe Moingeon

Stallergenes SA, Research and
Pharmaceutical Development, 6 rue
Alexis de Tocqueville, 92160 Antony,
France
Tel.: + 33 155 592 535
Fax: + 33 155 592 102
pmoingeon@stallergenes.com

Allergen-specific immunotherapy is used to treat patients exposed and co-sensitized to the two common house dust mites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Based on seroepidemiological studies and a detailed characterization of mite allergens, an optimal immunotherapeutic product should associate extracts from the two *Dermatophagoides* species, and include both bodies and fecal particles. Both subcutaneous and sublingual immunotherapies performed with aqueous mite extracts are safe and efficacious in children and adults with mite-induced rhinitis and/or asthma. Double-blind placebo-controlled studies are conducted to further document the efficacy of immunotherapeutic products, with promising results that were obtained already with sublingual tablets. Current developments of second-generation products relying upon recombinant allergens and peptides are reviewed.

KEYWORDS: allergen • *Dermatophagoides* • house dust mite • immunotherapy • vaccine

Epidemiology of house dust mite allergies

Exposure to house dust mites

House dust mites (HDMs) represent a major cause of perennial allergic rhinitis and asthma, affecting more than 500 million people throughout the world [1–6]. Mites constitute a large taxonomic group, belonging to the class Acaroidea. At least 40,000 mite species have been identified and have been broadly divided into the pyroglyphid mites (commonly referred to as house dust mites) and nonpyroglyphid (storage) mites [2,3]. The most common species within the Pyroglyphidae family, involved in more than 90% of HDM allergies worldwide, are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Common storage mites in Northern and Central Europe include Tyroglyphidae, such as *Acarus siro* and *Tyrophagus putrescentiae*, as well as Glyciphagidae (*Lepidoglyphus destructor*) [2,3]. Although not considered as a storage mite, *Blomia tropicalis* leads to frequent mite sensitization in tropical and sub-tropical areas. This review focuses only on immunotherapies for patients allergic to *Dermatophagoides* HDM species.

The formal demonstration that *Dermatophagoides* species were the source of allergens

present in house dust was made in 1967 [7]. Adult *Dermatophagoides* mites are about 300–600 µm in size and have four pairs of legs (FIGURE 1), with some morphological differences between male and female. The life cycle of mites encompasses various stages, including egg, larva, nymph and adult [2]. HDMs feed on a variety of substances, including human scales particularly. HDMs proliferate in a warm and humid environment, even though the optimal growth conditions are slightly different for *D. pteronyssinus* (25°C, 75% humidity) and *D. farinae* (30°C, 65% humidity) [3]. Nonetheless, since the *D. pteronyssinus* and *D. farinae* mite species have comparable requirements for growth, they usually cohabit in Europe, North America and Asia, with some country or regional quantitative differences reported in their relative distribution [3–6]. For example, *D. pteronyssinus* is prominent in the UK, Australia, New Zealand, Singapore and humid areas in general. In contrast, *D. farinae* predominates in Italy, Spain, South Korea and in continental areas [3].

There is no real seasonality in exposure to HDMs, considered as a prototypic perennial allergen, although there are more mite allergens in the environment during the early fall and less during winter time [8]. The magnitude of mite

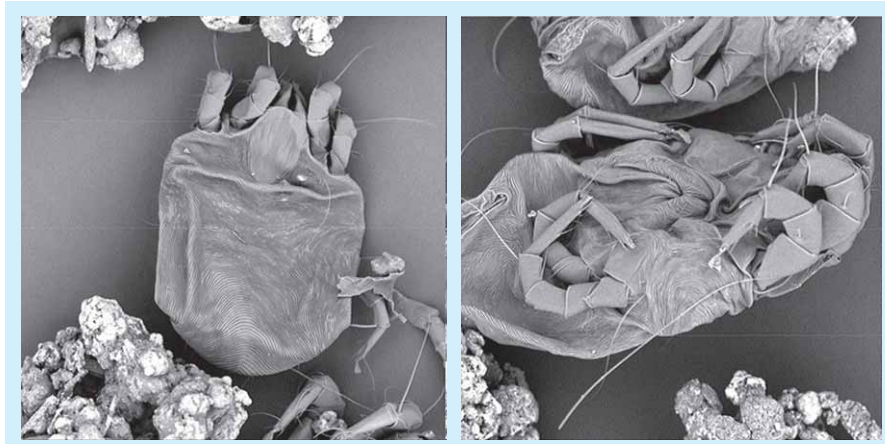


Figure 1. Microscopic view of *Dermatophagoides farinae*. (A) Dorsal view showing the striated cuticle. (B) Ventral view showing the four pairs of legs. *Dermatophagoides pteronyssinus* has a comparable morphology in females, whereas males have thicker anterior legs, when compared to *D. farinae*.

exposure is commonly determined by ELISA quantification of group 1 allergens (combining the major Der p 1 and Der f 1 allergens, see below) in dust samples. The amount of group 1 allergens reported in housing varies from 1 to 35 $\mu\text{g/g}$ of floor dust and from 3 to 50 $\mu\text{g/g}$ of mattress dust [8–12]. Thus, collectively, HDM of the *Dermatophagoides* genus are extremely common worldwide and are the major causes of type I (IgE-mediated) allergies, with most patients co-exposed to the two species.

Sensitization to HDMs

Sensitization occurs through the airways, following exposure to allergens from mite bodies and fecal particles adsorbed onto dust particles. Mite allergens were also reported in breast milk, although it is not clear whether this leads to sensitization or tolerance in young infants [13]. A threshold level of 1–2 μg mite allergens/g of house dust has been proposed as a risk factor for sensitization, whereas 10 $\mu\text{g/g}$ was suggested to be a threshold level for acute asthma in sensitized suspects. Two micrograms of Der p 1 corresponds to fecal particles accumulated by about 100 mites within a week. Surprisingly, the relationship between HDM allergen levels and mite sensitization is not fully linear, with maximum sensitization observed at intermediate (i.e., 1.4–10 $\mu\text{g/g}$ dust) levels of group 1 exposure [14].

It is now broadly admitted that co-exposure to the two *Dermatophagoides* species leads to co-sensitization, with a contribution of the two species to the induction of allergic symptoms. More than 90% of *Dermatophagoides*-sensitized patients have IgEs to allergens from both *D. pteronyssinus* and *D. farinae* species, as determined by *in vivo* skin prick testing and/or *in vitro* IgE reactivity [3–6,15]. Due to their taxonomic proximity, *D. pteronyssinus* and *D. farinae* express homologous allergens exhibiting a significant IgE cross-reactivity (see below). However, several seroepidemiological studies conducted by the author and others have confirmed that most patients, if not all, also have IgE to allergens or derived epitopes which are specific for each of the two species [6,15–17]. The analysis of CD4+

T cell responses to mite allergens in sensitized patients also suggests an involvement of both epitopes common to the two mite species and species-specific T cell epitopes [18,19]. It is noteworthy that patients are heterogeneous in their T cell responses, with evidence of Th2, and also mixed Th1/Th2 or Th2/Th17 patterns of sensitization, although it is still unclear how those patterns relate to clinical responses during immunotherapy [20].

HDM allergens can elicit conventional Th2 responses by contributing B and T cell epitopes that promote allergen-specific IgE production as well as secretion of IL-4, IL-5 and IL-13 cytokines by CD4+ T cells. Such a cytokine milieu leads to the recruitment and activation of proinflammatory cells (i.e., basophils, eosinophils, mast cells) in respiratory mucosae [21,22]. The physiopathology of mite-induced allergies was recently found to be even more complex, with evidence that specific mite allergens can directly activate multiple innate immune pathways following interaction with Toll-like receptors [21–24]. Specifically, the capacity of mite allergen extracts to activate Toll-like receptor 4 in airway epithelial cells appears central in driving allergic inflammation, following production of thymic stromal lymphopoietin, granulocyte-macrophage colony stimulating factor, IL-25, IL-33 and subsequent activation of mucosal dendritic cells [24]. In addition, other non-allergenic molecules such as lipopolysaccharides (associated with *Bartonella* bacteria endosymbiotic to *Dermatophagoides* species), β glucans and chitin can also engage pathogen recognition receptors and elicit proinflammatory responses at the level of epithelial or innate immune cells [24–26].

HDM allergens

Exposure and sensitization conditions to HDM have been investigated at a molecular level. As of today, 23 distinct groups of allergens have been described in mite species, of which 21 are found in *Dermatophagoides* species (TABLE 1) [27–30]. The WHO/International Union of Immunological Societies nomenclature refers to those allergens using the first three letters of the genus, then the first letter of the species (i.e., Der p and Der f), followed by the group number reflecting the order in which the allergen was described [30]. Among these, group 1–3, 6–8, 10, 11, 14, 15, 18 and 21 allergens are found as homologous molecules in both *D. pteronyssinus* and *D. farinae* species. Group 4, 5, 9, 20 and 23 allergens are present in *D. pteronyssinus*, but are not yet described in *D. farinae* or not officially included in the International Union of Immunological Societies nomenclature [30]. In contrast, group 13, 16, 17 and 22 allergens are present in *D. farinae*, without any equivalent molecule yet reported in *D. pteronyssinus*. From this, it is evident that the allergen repertoires are not fully identical between the two species [27–30].

Table 1. Allergens identified in *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* species.

Allergen groups	Biological function/activity	MW (kDa)	Presence reported in mite species		Sequence homology between the two species	Prevalence of IgE recognition	Location within mites
1	Cysteine protease	24–27	Dp	Df	81%, 24 isoforms for Der p 1 and 10 isoforms for Der f 1	60–80%	Feces
2	Nieman Pick C2 homolog	15	Dp	Df	88%, 15 isoforms for Der p 2 and 17 isoforms for Der f 2	≥70%	Body
3	Trypsin	25–30	Dp	Df	81%	10–15%	Body (Df)
4	α-amylase	56–63	Dp		?	10–25%	Feces
5	Unknown	14	Dp		?	10–40%	Feces
6	Chymotrypsin	25	Dp	Df	75%	41–65%	
7	Unknown	26–31	Dp	Df	86%	20–40%	Feces
8	Glutathione-S-transferase	25–27	Dp	Df	?	10–40%	Feces
9	Collagenolytic serine protease	24–29	Dp		?	?	Feces
10	Tropomyosin	33–37	Dp	Df	98%	5–10%	
11	Paramyosin	98–103	Dp	Df	97%	65–80%	
13	Fatty acid binding protein	15		Df	?	<25%	Feces
14	Apolipoprotein	177	Dp	Df	>80%	20–60%	Body
15	Chitinase	98–105	Dp	Df	90%	15–30%	
16	Gelsolin/villin	53–55		Df	?	35–47%	
17	Calcium-binding protein	53		Df	?	35%	
18	Chitin-binding protein	49–60	Dp	Df	88%	30–40%	
20	Arginine kinase	40	Dp		?	5–20%	Body
21	Unknown	14	Dp	Df	?	15–30%	Feces
22	Unknown	67		Df	?	?	Feces
23	Peritrophin-like protein domain	14	Dp		?	30–60%	Feces

Allergen groups 12 and 19 were reported in *Blomia*, but not in *Dermatophagoides* mite species.

Df: *Dermatophagoides farinae*; Dp: *Dermatophagoides pteronyssinus*; MW: Molecular weight.

From [30]/(WHO/International Union of Immunological Societies site providing the official list of registered allergens and their isoforms). Prevalence of IgE recognition was obtained from [4,15,30,35,36] and [PM, UNPUBLISHED DATA].

HDM allergens are found within both mite bodies (particularly within the digestive tract) and fecal particles [27–29]. Molecular analyses of purified bodies and feces have confirmed that they contain distinct sets of allergens (TABLE 1) [31]. For example, bodies contain more group 2, 3 (at least for *D. farinae*), 14 and 20 allergens, whereas fecal particles are rich in group 1,

4, 5, 7, 8, 9, 13, 21 and 23 allergens. Some of the allergens, such as of groups 3, 5, 7, 10, 11 and 14, are present in low quantities, possibly because they are unstable or sensitive to the hydrolytic enzymes abundant in the mites' gut [27,32]. In contrast, group 1, 2, 13 and 20 allergens are among the most abundant proteins found in *D. pteronyssinus* extracts [33].

Collectively, groups 1 and 2 are estimated to represent 1–2% of proteins found in an aqueous HDM extract. The analysis of IgE reactivity to purified or recombinant mite allergens has revealed that 80–90% of HDM allergic patients are sensitized to Der p 1, Der p 2, Der f 1 and Der f 2, and thus these are called major allergens [5,6,15,18]. IgEs against such major allergens commonly represent more than 80% of mite-specific IgEs in patients' sera. In addition, the author and others have demonstrated that many (i.e., 60–80%) patients also have IgE, albeit at low titers, directed to minor allergens such as of groups 3, 4, 5, 6, 7, 9, 11, 14 and 23 (TABLE 1) [4,5,15,18,34–36].

The allergenicity of mite allergens has been, to some extent, related to their biological activity. For example, Der p 1 and Der f 1 major allergens mediate a cysteine protease activity, thought to cleave intercellular epithelial tight junctions, thereby facilitating allergen exposure to submucosal immune cells [37,38]. The proteolytic activity of group 1 mite allergens appears as well to directly activate the immune cells in favor of Th2 responses, following cleavage of CD23 on B lymphocytes and CD25 on T cells [37]. Other mite allergens have a biological function likely contributing to their allergenicity. For example, group 2 allergen is analogous to the myeloid differentiation-2 adaptor molecule involved in TLR4 activation, group 3, 6 and 9 allergens mediate a serine protease activity, and group 7, 13, 14 and 21 allergens are lipid-binding proteins [39–41].

Each of the Der p 1, Der f 1, Der p 2 and Der f 2 allergens is expressed in mites as multiple variants/isoforms differing in their amino acid sequence by 1–13 amino acids (TABLE 1) [30]. These differences are known to impact IgE and T cell recognition [16,17,42]. The existence of natural variants is less documented for minor allergens, but is likely to be similar. The variability in major allergen sequences comes mostly from gene polymorphisms [42], whereas variations affecting group 3, 7 and 14 minor allergens relate not only to amino acid sequence differences but also to post-translational modifications [27,28]. The primary sequence of group 10 (tropomyosin) is evolutionarily conserved, not only among *Dermatophagoides* species (TABLE 1) but also between HDM, shrimp and snail. As a consequence, the group 10 mite allergen has been incriminated in IgE cross-reactivity with food allergens [43–45]. Some rare cases of IgE neosensitization to food allergens have been reported during HDM immunotherapy performed via the subcutaneous, but not the sublingual route [18,44,45].

Implications for designing the right product for immunotherapy

Our understanding of exposure and sensitization conditions at a molecular level can be used to design the most appropriate product for allergen immunotherapy (AIT) of mite-allergic patients. For example, evidence that such patients are sensitized to allergens originating from both bodies and fecal particles implies that the two should be combined for immunotherapy. Furthermore, given the homology and IgE cross-reactivity between allergens from *Dermatophagoides* species, the question arises as to whether a single mite species extract is sufficient for desensitizing

HDM-allergic patients, or whether mixed *D. pteronyssinus* and *D. farinae* extracts should be used. IgE sensitization to allergens from a single species, that is, *D. pteronyssinus* or *D. farinae* only, is not common, but can be documented in 2–10% of HDM-allergic patients [3,6,18]. Cross-inhibition studies have shown that *D. pteronyssinus* or *D. farinae* extracts do not inhibit the binding of IgEs from mite-allergic patients in a similar way, and thus, do not contain the same allergen repertoires. As stated above, the latter observation is fully consistent with our current knowledge of the allergen content of mite extracts which has emerged from extensive molecular characterizations (TABLE 1).

In addition, when comparing single allergens found in both *D. pteronyssinus* and *D. farinae*, despite a significant homology between the two species (commonly in the range of 80–85% amino acid sequence homology), some differences exist, resulting in species-specific B and T cell epitopes [6,16–19]. As a consequence, ≥80% of AIT courses applied to HDM-allergic patients in Europe and the USA involve a mixture of *D. pteronyssinus* and *D. farinae* extracts, whereas single extracts are only used in ≤20% of the treatments. It is noteworthy that in support of this practice, in a murine asthma model with animals sensitized to extracts from the two HDM species, immunotherapy with a mixture of both *D. pteronyssinus* and *D. farinae* extracts was consistently more efficacious than single extracts to decrease airway hyperresponsiveness and downregulate mite-specific Th2 responses [46].

Current status of HDM immunotherapy AIT as an etiologic treatment for HDM allergies

Allergen avoidance is considered of little use for a perennial allergy associated with HDM exposure. Pharmacologic drugs such as antihistaminics or corticosteroids can be used to treat symptoms. However, immunotherapy is the only treatment addressing the immunological cause underlying allergic inflammation and as such, able to provide sustained efficacy for several years, even after treatment cessation. AIT is a clinically documented therapy used since 1911 to treat patients with type I (IgE-mediated) respiratory allergies to pollens from either grasses, trees or weeds, as well as to cat dander, molds or common HDMs [47–51]. International guidelines recommend using AIT to treat patients with severe upper and mild to moderate lower respiratory allergies, most particularly when they are poorly controlled by symptomatic treatments or are unwilling to use pharmacologic treatments [47–51]. The rationale behind this approach is to administer aqueous allergen extracts prepared from natural sources in a reiterated manner, over 3–5 years, in a different context from natural exposure (i.e., with an adjuvant, or using the subcutaneous or sublingual route). AIT aims to re-educate the immune system to re-establish tolerance in an allergen-specific manner in allergic patients, following re-orientation of inappropriate Th2 responses toward regulatory immune responses [52,53].

Despite a century-long use of this form of therapeutic vaccination, significant progress was mostly made in the last decade, with an evolution from a largely empirical approach to a scientifically founded therapy, fulfilling the exigence of evidence-based

medicine. Specifically, the latter encompasses the following advances:

- The availability of pharmaceutical-grade standardized products for both diagnostic and immunotherapy [33,54,55].
- The emergence of the sublingual route as an alternative to the subcutaneous route: It is now broadly accepted that high-dose sublingual immunotherapy (SLIT) regimens have an efficacy comparable to subcutaneous immunotherapy (SCIT), with a much better safety profile, that is, leading to only rare systemic reactions and extremely rare anaphylactic shocks due to the limited exposure, if any, of the allergens to blood immune cells [48,53,56,57]. As of today, SLIT represents approximately 50% of the total AIT administered in Europe (>80% in Southern Europe). Sublingual tablets for grass and ragweed pollen allergies have been recently approved in the USA and are being registered as well in Japan for HDM allergies.
- A better understanding of the mechanisms of action: SLIT and SCIT rely upon comparable immune mechanisms, including a downregulation of allergen-specific CD4⁺ Th2 responses, with a shift toward Th1 (immunodeviation) and T regulatory (immunosuppression) responses [52,53]. The associated changes in the cytokine milieu due to repolarization of allergen-specific CD4⁺ T cells leads to a decrease in the recruitment and activation of proinflammatory cells (basophils, mast cells, eosinophils) in target mucosae, as well as a downregulation of allergen-specific IgE with a concomitant induction of allergen-specific IgG (IgG4) and IgA. Some of these high-affinity antibodies induced during AIT may exhibit a blocking activity (i.e., manifests as a capacity to inhibit mast cell or basophil activation, as well as allergen presentation to T cells) following competition with IgEs for binding to allergens [58].

Whereas HDM can cause respiratory allergies (including rhinitis and asthma) as well as atopic dermatitis, the efficacy of immunotherapy in the latter condition is currently unproven, even if encouraging efficacy data have been obtained with SLIT in mice with mite-induced eczema [59]. In this context, the author focuses below on the application of AIT to respiratory allergies associated with *Dermatophagoides* mite species, which accounts for 45–50% of all desensitization treatments in Europe and in the USA [46,51].

Products for HDM immunotherapy

Natural extracts

As of today, only the products based on aqueous HDM extracts prepared from cultured mites are available to the patients [33]. Mites have been historically grown using human scales, fish or dog food, dried daphnia, cereals and mold cultures [60]. As of today, mites are cultured using pharmaceutical-grade components, without any protein of human or animal origin [33]. For example, a mixture of yeast extract, wheat germ and synthetic amino acids recapitulating the composition of the human stratum corneum allows efficient growth of both

D. pteronyssinus and *D. farinae* mite species. After 3 months of culture under controlled temperature and humidity conditions, cultures containing bodies, fecal particles and spent culture medium are harvested to prepare the allergen extracts. Such cultured mites are identical in terms of allergen content to the ones obtained using human scales [33]. Classically for SCIT, allergen extracts are combined (in Europe but not in the USA) with aluminum hydroxide or calcium phosphate as an adjuvant. SLIT is consistently performed with the mite extract administered for 2 min under the tongue, without any adjuvant. Some modified extracts (i.e., allergoids) in which proteins are polymerized with glutaraldehyde are also used to treat patients. These allergoids are hypoallergenic, that is, they react poorly with IgE due to steric hindrance associated with allergen polymerization. The efficacy of these allergoid mite extracts remains to be established in large clinical studies.

Recent studies drew attention on the issue of allergen extract consistency. Most particularly, significant variability was observed for *D. pteronyssinus* and *D. farinae* extracts made available by various manufacturers for *in vivo* diagnosis, both in terms of protein content and concentration in major and minor allergens [61,62]. Obviously, ensuring the quality and consistency of natural extracts intended for human use is critical to guarantee the safety and efficacy of AIT. Keeping this into consideration, well-characterized and standardized allergen extracts are produced, for which the allergenic activity is tested and adjusted using ELISA inhibition assays, in comparison to reference *D. pteronyssinus* and *D. farinae* extracts [54]. For HDM extracts used for diagnostic and/or immunotherapeutic purposes, acceptance criteria are established for total allergenic activity and major allergen content. These parameters are subsequently monitored as a quality check on all manufacturing intermediates, including source materials, drug substances and drug products [55].

Recombinant allergens & peptides

As an alternative to natural extracts, which consist into complex mixtures of proteins, there is also some interest in developing AIT products based on purified (synthetic or recombinant) molecules [63–65]. Schematically, two types of molecular products are considered for HDM immunotherapy, including recombinant mite allergens and peptides (or fragments) derived from such allergens [63–65]. It is noteworthy that although these candidate products have the advantage of being molecularly defined and are easy to produce in a consistent manner, they usually only cover the major group 1 and 2 allergens, which may represent a limitation to treat patients sensitized as well to minor mite allergens. Interestingly, a recent study suggested that treatment with peptides corresponding to selected epitopes from a given allergen may result in suppression of immune responses to other (linked) epitopes within the same molecule [66]. It remains, however, to be proven whether such a potential bystander immunological effect is associated with clinical benefit, most particularly in the long term.

Table 2. Status of immunotherapies for respiratory allergies to house dust mites.

Immunotherapeutic products	Status	Meta-analysis/comments	Ref.
Natural HDM extracts			
– Single <i>Dermatophagoides pteronyssinus</i> extract – Single <i>Dermatophagoides farinae</i> extract – Mixtures of <i>D. pteronyssinus</i> and <i>D. farinae</i> extracts – Modified extracts (allergoids) +/- Aluminum hydroxide or calcium phosphate	Commercially available	• Calamita <i>et al.</i> (SLIT): n = 25 clinical studies in patients with allergic asthma, n = 1706 patients. Sublingual immunotherapy leads to a significant reduction of asthma severity	[73]
		• Penagos <i>et al.</i> (SLIT): n = 9 clinical studies in children with allergic asthma, n = 441 patients. A significant relief from symptoms (p = 0.02) and reduction in medication use (p = 0.007) were observed following SLIT	[74]
		• Compalati <i>et al.</i> (SLIT): n = 8 clinical studies in patients with allergic rhinitis, n = 382 patients. Reduction of symptoms and rescue medication (p = 0.04) n = 9 clinical studies in patients with allergic asthma, n = 476 patients. Significant relief from symptoms and reduction in rescue medication (p = 0.02)	[75]
		• Lin <i>et al.</i> (SLIT): n = 63 clinical studies, n = 5131 patients. Moderate evidence of improvement in rhinitis and conjunctivitis symptoms, with 9 out of 36 studies demonstrating greater than 40% improvement versus comparator Strong evidence of improvement of allergic asthma symptoms, with 8 out of 13 clinical studies demonstrating symptom improvement greater than 40% versus comparator, as well as a decrease in medication use	[76]
		• Abramson <i>et al.</i> (SCIT): DBPC trials in adults and children with allergic asthma. Subcutaneous immunotherapy is efficacious in relieving the symptoms and decreasing the use of rescue medication	[77]
		• Calderon <i>et al.</i> (SCIT and SLIT): n = 44 clinical studies in patients with allergic rhinitis and asthma Several SCIT and SLIT studies report a significant improvement in allergic rhinitis symptoms, although the dosing and immunization schemes are heterogeneous SCIT is efficacious in adults and in children with allergic asthma in relieving symptoms, decreasing rescue medication use, and improving bronchial hyperresponsiveness. SLIT is also beneficial in asthmatic patients, although the effect size reported is often moderate and varies greatly from one study to another This meta-analysis concluded that there is a lack of consensus on basic treatment parameters such as dose and duration. Consequently, more rigorous, DBPC randomized trials are needed	[78]
		Products in development	
Sublingual tablets	Clinical, Phase III	• A DBPC study confirmed the efficacy and safety of sublingual tablets (combining both <i>D. pteronyssinus</i> and <i>D. farinae</i> extracts) in a cohort of 509 patients with HDM-associated allergic rhinitis. Decrease of up to 20.2% (p = 0.0066) in the mean average adjusted symptom scores in the active group versus placebo Onset of action was observed after 4 months of treatment, and efficacy was maintained during the treatment-free follow-up year • A DBPC study in 604 patients of age ≥14 years with HDM-associated allergic rhinitis and mild to moderate asthma reduced the daily inhaled corticosteroids needed to control asthma in the active group versus placebo (p = 0.004)	[81,82]

CHO: Chinese hamster ovary; DBPC: Double-blind placebo-controlled; HDM: House dust mite; SCIT: Subcutaneous immunotherapy; SLIT: Sublingual immunotherapy.

Table 2. Status of immunotherapies for respiratory allergies to house dust mites (cont.).

Immunotherapeutic products	Status	Meta-analysis/comments	Ref.
Products in development			
Group 1 and 2 peptides	Clinical, Phase II	A mixture of short peptides corresponding to T cell epitopes present in HDM major allergens was administered in a DBPC study to 172 patients with HDM-associated allergic rhinitis and asthma, with the aim to elicit specific Treg. Patients were exposed regularly to HDM challenges in a chamber. Four intradermal administrations of mite peptides over 12 weeks improved significantly the symptoms of allergic rhinitis 1 year after starting treatment ($p = 0.02$ vs placebo)	[83]
Der p 1 and Der p 2 peptides associated with carrier proteins	Clinical, Phase I/ Preclinical	Mite allergen-derived peptides lacking IgE epitopes were linked to carrier proteins (e.g., Hepatitis B preS antigen, or capsid protein from rhinovirus or Qβ phage) in order to elicit « blocking » IgEs. Evidence of strong IgG responses to the mite peptide was obtained in 24 healthy volunteers receiving the vaccine via the subcutaneous or intramuscular route	[84]
Recombinant Der p 1 and Der p 2 allergens	Preclinical	Der p 1 and Der p 2 allergens have been produced in <i>Escherichia coli</i>, yeast, CHO cells and plant-based expression systems. These proteins are recognized by patients' IgE and activate basophils from mite-allergic patients. Limited evidence of efficacy in murine models	[67–72]

CHO: Chinese hamster ovary; DBPC: Double-blind placebo-controlled; HDM: House dust mite; SCIT: Subcutaneous immunotherapy; SLIT: Sublingual immunotherapy.

Various expression systems have been used to express recombinant HDM allergens in a natural conformation, including *Escherichia coli*, yeast (*Saccharomyces cerevisiae* and *Pichia pastoris*), Chinese hamster ovary cells, rice and tobacco plants [67–71]. Collectively, these studies confirm that it is possible to produce with good yields recombinant Der p 1 or Der p 2 molecules capable of binding patients' IgE in a comparable manner to purified natural allergens, suggesting that recombinant mite allergens are properly folded. Nonetheless, whereas Der p 2 can easily be produced in a natural-like conformation [71], some specific issues are associated with the expression of recombinant Der p 1 (or Der f 1) molecules. The latter include the presence of a propeptide, which is cleaved off from group 1 allergens to produce the mature form, thus generating potential molecular heterogeneity regarding the NH₂-terminal amino acid sequence and the cysteine protease activity of the molecule, creating potential stability problems for the allergen itself, or for other allergens potentially combined in the vaccine [69]. As an alternative, an approach based on fusion proteins associating from head to tail various forms of Der p 1 and Der p 2 molecules has been attempted [70]. However, the allergens expressed under those conditions do not exhibit natural conformations, likely due to constraints imposed within the chimera, leading to inappropriate intra- and inter-molecular cysteine pairing and subsequent aggregation of the molecules. As of today, AIT based on recombinant mite allergens is still at a preclinical stage, with preliminary results obtained using *in vitro* systems and murine models [67,68,71].

Other products in development include synthetic peptides, or allergen fragments coupled to carrier proteins (TABLE 2).

Peptides are designed to encompass either T cell epitopes, B cell epitopes or both. The rationale behind these distinct peptide-based approaches is either to elicit allergen-specific regulatory T lymphocytes or blocking IgG4 antibodies. Alternatively, peptide selection is also guided by safety concerns, to avoid IgE binding and cross-linking of corresponding Fc receptors on mast cells or basophils, or to preclude stimulation of mite-specific Th2 cells leading to late-phase reactions [72]. Whereas most of those candidate products based on peptides are still at a preclinical stage, one peptide mix and a conjugate between Der p 1 peptides and virus-like particles have been tested in humans (see below).

Overview of clinical studies conducted with HDM immunotherapeutic products

As of today, only the products based on natural aqueous extracts are available to treat patients. As mentioned above, these extracts were made in the past from either *D. pteronyssinus* or *D. farinae* only, but today the most common approach is to rely upon a mixture of the two (TABLE 2). To provide an overview of the data generated in numerous clinical studies conducted in the last 30 years, a summary of recent meta-analyses focusing on double-blind placebo-controlled (DBPC) studies is provided in TABLE 2 [73–78]. Specifically, HDM extracts administered either via the subcutaneous or the sublingual route were shown to be well tolerated by patients. Most particularly, these studies documented the excellent safety profile of SLIT, associated with frequent, but mild local reactions occurring at the administration site at the beginning of AIT. Systemic reactions were rare during mite-specific immunotherapy

via the sublingual route, with no fatal anaphylactic reaction reported [56,73–78]. This safety profile represents a significant advantage over SCIT, which may lead to some systemic reactions, as well as extremely rare, but potentially fatal anaphylactic shocks [57].

With respect to efficacy, AIT with natural HDM extracts relieved the symptoms, reduced the need for symptomatic treatments and improved the quality of life in patients with allergic rhinitis. These products were also shown to improve symptoms in asthmatic patients [73–78]. Only few DBPC studies have been conducted as well with depigmented mite extracts subsequently polymerized with glutaraldehyde prior to adsorption on aluminum hydroxide. Patients receiving SCIT with such modified mite extracts experienced improvement in their symptoms following bronchial provocation with the allergen [79,80]. Some of these meta-analyses concluded that additional DBPC studies should be performed in large cohorts of patients to further document the efficacy of HDM immunotherapeutic products [75,78]. Of note, two such DBPC Phase III studies recently conducted in patients with mite-induced allergic rhinitis and asthma, respectively, confirmed the safety and efficacy of sublingual tablets [81,82]. Generally speaking, additional dedicated studies are also needed in the future to demonstrate a disease-modifying effect of AIT in mite allergies, in the form of a long-term benefit maintained several years after treatment cessation.

Clinical studies are currently ongoing to evaluate the efficacy of synthetic peptides administered via the intradermal route, with some encouraging results already observed in a Phase II clinical study conducted in an environmental exposure chamber [83]. Interestingly, patients receiving four injections of short peptides encompassing T cell epitopes from major mite allergens had a significant improvement of their symptoms 1 year after starting the treatment [83]. In parallel, SCIT with a Der p 1 peptide linked to virus-like particles made from the capsid protein of the Q β phage elicited strong Der p 1-specific IgG responses in healthy volunteers [84].

Expert commentary

AIT has been successfully established in the last few years as a valid option to treat allergic rhinoconjunctivitis associated with grass, tree and weed pollens. Applying AIT to HDM sensitization, that is, the most frequent perennial allergen associated with rhinitis and asthma, is currently the next frontier. Whereas there is already strong evidence that AIT is safe and efficacious in patients with HDM-induced rhinitis and asthma, a challenge for the near future is to document its long-term efficacy in those two distinct pathologies, in the context of DBPC studies conducted in large cohorts of patients.

In this regard, it is critical to rely upon the right product, which can now be rationally designed on the basis of recent epidemiological data. For instance, it is well understood that the vast majority of patients are exposed and sensitized to allergens from the *D. pteronyssinus* and *D. farinae* species. The latter includes a frequent involvement of group 1 and group 2 major

allergens from the two mite species, as well as sensitization to minor allergens from both bodies and fecal particles [3–6,15,18]. As previously demonstrated for grass pollen allergic patients exposed and sensitized to allergens from multiple Pooideae species [85–87], mite-allergic patients appear to mount as well IgE and CD4⁺ T cell responses against both cross-reactive and also species-restricted epitopes from the allergens of *D. pteronyssinus* and *D. farinae*.

Altogether, these results support the use of a combination of extracts made from the two *Dermatophagoides* species, including bodies and feces, to manufacture a universal HDM vaccine that is capable of desensitizing patients in Europe, Asia, North and South America. As for grass pollen immunotherapy, such a mixture of allergens from two HDM species recapitulates optimally the broad spectrum of allergens, as well as derived B and T cell epitopes to which patients are sensitized, to allow a comprehensive re-orientation of the immune system [86–88].

Five-year view

Although AIT performed with natural mite extracts is commonly used in Europe and the USA, the use of such extracts will be extended to treat patients in Asia, Australia and South America. This will be facilitated by the availability of standardized and well-characterized extracts for both immunotherapy and diagnostic purposes. The convenience and the well-established safety profile of the sublingual route will be instrumental in broadening the use of AIT in mite-allergic patients worldwide. The growing interest in the sublingual route, commonly used to treat European patients as of today, will be confirmed with the registration of mite tablets as pharmaceutical specialties in Asia and North America. Long-term efficacy of mite AIT will be demonstrated in patients with rhinitis and/or asthma. Based on those successes in mite-associated respiratory allergies, some clinical studies will be launched as well to evaluate the benefit of AIT in atopic dermatitis. Recombinant mite allergens or peptides will not be broadly used for therapy, facing as a limitation the involvement of multiple allergens in most patients. Nonetheless, if their efficacy is confirmed in Phase III field studies, they could be used to treat those patients sensitized mostly to group 1 and group 2 major mite allergens. In contrast, panels of purified recombinant mite allergens will be broadly used for diagnostic purposes (as part of component-resolved diagnostic tests) to better characterize the patients' patterns of IgE sensitization. Some candidate biomarkers of efficacy, including both predictive and follow-up markers, will be identified, opening the way to the development of companion diagnostics supporting physicians in their decision to include and maintain a patient under AIT.

Financial & competing interests disclosure

The author is an employee of Stallergenes. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject

matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Products for immunotherapy of house dust mite allergic patients should recapitulate natural exposure and sensitization conditions at a molecular level, that is, they should combine allergen extracts from both *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, including bodies and feces from the two species.
- The quality and consistency of house dust mite extracts are critical to their safety and efficacy, and thus, only pharmaceutical-grade well-characterized extracts should be used for allergen immunotherapy.
- Patient heterogeneity should be better understood, regarding both type/severity/chronicity of symptoms and immune status.
- Dosing regimens and duration of mite-specific immunotherapy to reach sustained efficacy and a disease-modifying effect should be documented in both challenge chamber studies and long-term field studies.
- Clinical endpoints to measure clinical benefit, and clinical relevance (size of the effect anticipated from allergen immunotherapy) should be defined in collaboration with regulatory authorities.
- Biomarkers associated with clinical efficacy should be identified and developed as companion diagnostics.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- Bousquet J, Khaltaev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;63(Suppl 86): 8-160
- Van Bronswijk JE, Sinha RN. Pyroglyphid mites (Acari) and house dust mite allergy. *J Allergy* 1971;47:31-52
- Thomas WR. Geography of house dust mite allergens. *Asian Pac J Allergy Immunol* 2010;28:211-24
- Detailed analysis of the epidemiology of house dust mites exposure worldwide.**
- Hales BJ, Martin AC, Pearce LJ, et al. IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol* 2006;118:361-7
- Weghofer M, Thomas WR, Kronqvist M, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest* 2008;38:959-65
- Barber D, Arias J, Boquete M, et al. Analysis of mite allergic patients in a diverse territory by improved diagnostic tools. *Clin Exp Allergy* 2012;42:1129-38
- Voorhorst R, Spieksma F, Varekamp H, et al. The house dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house dust allergen. *J Allergy* 1967;39: 323-39
- Miyazawa H, Sakaguchi M, Inouye S, et al. Seasonal changes in mite allergen (Der pI and Der pII) concentrations in Japanese homes. *Ann Allergy Asthma Immunol* 1996;76:170-4
- Moscato G, Perfeti L, Galdi E, et al. Levels of house-dust-mite allergen in homes of nonallergic people in Pavia, Italy. *Allergy* 2000;55:873-8
- Ngelhart S, Bieber T, Exner M. House dust mite allergen levels in German day-care centers. *Int J Hyg Environ Health* 2002;205:453-7
- Sidenius KE, Hallas TE, Poulsen LK, Mosbech H. House dust mites and their allergens in Danish mattresses - results from a population based study. *Ann Agric Environ Med* 2002;9:33-9
- Raffan E, Lawrence H, Henderson T, et al. Prevalence of the group 1 *Dermatophagoides* allergens Der p 1 and Der f 1 in homes with no dogs, healthy dogs and *Dermatophagoides*-sensitized atopic dogs in Liverpool. *Vet Dermatol* 2005;16:253-60
- Macchiaverni P, Rekima A, Turfkruyer M, et al. Respiratory allergens in human milk: potential impact on susceptibility to allergic asthma. *Allergy* 2014;69:395-8
- Schram-Bijkerk D, Doekes G, Boeve M, et al. Nonlinear relations between house dust mite allergen levels and mite sensitization in farm and non-farm children. *Allergy* 2006;61:640-7
- Zock JP, Heinrich J, Jarvis D, et al. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006;118:682-90
- Smith AM, Benjamin DC, Hozic N, et al. The molecular basis of antigenic cross reactivity between group 2 mite allergens. *J Allergy Clin Immunol* 2001;107:977-84
- Park JW, Kim KS, Jin HS, et al. Der p 2 isoallergens have different allergenicity and quantification with 2-site ELISA using monoclonal antibodies is influenced by the isoallergens. *Clin Exp Allergy* 2002;32: 1042-7
- Hales BJ, Thomas WR. T cell sensitization to epitopes from the house dust mites *Dermatophagoides pteronyssinus* and *Euroglyphus maynei*. *Clin Exp Allergy* 1997;27:266-875
- Hales BJ, Shen HD, Thomas WR. Cross reactivity of T cell responses to *Dermatophagoides pteronyssinus* and *D. farinae*: studies with group 1 and 7 allergens. *Clin Exp Allergy* 2000;30: 927-33
- Wambre E, Bonvalet M, Bodo VB, et al. Distinct characteristics of seasonal (Bet v 1) vs. perennial (Der p 1/Der p 2) allergen-specific CD4+ T cell responses. *Clin Exp Allergy* 2010;41:192-203
- Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol* 2011;32:402-11
- Comprehensive analysis of mite-induced allergy at a molecular level.**
- Jacquet A. Innate immune responses in house dust mite allergy. *IRSN Allergy* 2013;2013:1-18.735031 Hindoui publishing Ed
- Hammad H, Lambrecht B. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol* 2008;8:193-204

24. Hammad H, Chieppa M, Perros F, et al. House dust mite allergen induces asthma via TLR4 triggering of airway structural cells. *Nat Med* 2009;15:410-16
- **One of the first studies documenting an interaction of allergens with innate immunity.**
25. Valerio RC, Murray P, Arlian LG, Slater JE. Bacterial 16S ribosomal DNA in house dust mite cultures. *J Allergy Clin Immunol* 2006;118:1296-300
26. Da Silva CA, Pochard P, Lee CG, Elias JA. Chitin particles are multifaceted immune adjuvants. *Am J Respir Crit Care Med* 2010;182:1482-91
27. Thomas WR, Smith W, Hales BJ. The allergenic specificities of the house dust mite. *Chang Gung Med J* 2004;27:563-9
28. Thomas WR, Hales BJ, Smith WA. House dust mite allergens in asthma and allergy. *Trends Mol Med* 2010;16:321-8
29. Bessot JC, Pouli G. Mite allergens: an overview. *Eur Annu Allergy Clin Immunol* 2011;43:141-56
30. WHO/IUIS allergen nomenclature database. Available from: www.allergen.org
31. Batard T, Nony E, Chabre H, et al. Molecular and functional characterization of natural allergen extracts. *Proceedings of the 13th PEI meeting (14-17 September 2011)*. 2013. pp 54-6
32. Zhang YY, Sun X, Liu ZG. Morphology and Three-dimensional reconstruction of the digestive system of dermatophagoides farinae. *Int Arch Allergy Immunol* 2008;146:219-26
33. Batard T, Hrabina A, Bi XZ, et al. Production and proteomic characterization of pharmaceutical grade Dermatophagoides pteronyssinus and Dermatophagoides farinae extracts for allergy vaccines. *Int Arch Allergy Immunol* 2006;140:295-305
34. Weghofer M, Grote M, Resch Y, et al. Identification of Der p 23, a peritrophin-like protein, as a new major Dermatophagoides pteronyssinus allergen associated with the peritrophic matrix of mite fecal pellets. *J Immunol* 2013;190:3059-67
35. Hales BJ, Elliot CE, Chai LY, et al. Quantification of IgE binding to the Chitinase and Chitinase-like house dust mite allergens Der p 15 and Der p 18 compared to the major and mid range allergens. *Int Arch Allergy Immunol* 2013;160:233-40
36. Kidon M, Chin CW, Kong LW, et al. Mite component-specific IgE repertoire and phenotypes of allergic disease in childhood: the tropical perspective. *Pediatr Allergy Immunol* 2011;22:202-10
37. Chapman MD, Wunschmann S, Pomes A. Proteases as Th2 adjuvants. *Curr Allergy Asthma Rep* 2007;7:363-7
38. Roelandt T, Heughebaert C, Hachem JP. Proteolytically active allergens cause barrier breakdown. *J Invest Dermatol* 2008;128:1878-80
39. Trompette A, Divanovic S, Visintin A, et al. Allergenecity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009;457:585-8
40. Mueller GA, Edwards LL, Aloor JJ, et al. The structure of the dust mite allergen Der p 7 reveals similarities to innate immune proteins. *J Allergy Clin Immunol* 2010;125:909-17
41. Epton MJ, Dilworth RJ, Smith W, Thomas WR. Sensitization to the lipid-binding apolipoprotein allergen Der p 14 and the peptide Mag-1. *Int Arch Allergy Immunol* 2001;124:57-60
42. Smith WA, Hales BJ, Jarnicki AG, Thomas WR. Allergens of wild house dust mites: environmental Der p 1 and Der p 2 sequence polymorphisms. *J Allergy Clin Immunol* 2001;107:985-92
43. Ayuso R, Reese G, Leong-Kee S, et al. Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins. *Int Arch Allergy Immunol* 2002;129:38-48
44. Van Ree R, Antonicelli L, Akkerdaas JH, et al. Possible induction of food allergy during mite immunotherapy. *Allergy* 1996;51:108-13
45. Pajno GB, La Grutta S, Barberio G, et al. Harmful effect of immunotherapy in children with combined snail and mite allergy. *J Allergy Clin Immunol* 2002;109:627-9
46. Tourdot S, Airouche S, Berjont N, et al. Evaluation of therapeutic sublingual vaccines in a murine model of chronic house dust mite allergic airway inflammation. *Clin Exp Allergy* 2011;41:1784-92
47. Passalacqua G, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol* 2007;119:881-91
48. Canonica GW, Bousquet J, Casale T, et al. Sub-lingual immunotherapy: World Allergy Organization position paper. *Allergy* 2009;64:1-59
49. Cox L, Jacobsen L. Comparison of allergen immunotherapy practice patterns in the United States and Europe. *Ann Allergy Asthma Immunol* 2009;103:451-60
50. Zuberbier T, Bachert C, Bousquet PJ, et al. GA2LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. *Allergy* 2010;65:1525-30
51. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol* 2011;127(Suppl):S1-S5
52. Akdis M, Akdis C. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol* 2014;133:621-31
- **Recent review on the mechanisms of allergen immunotherapy performed via the subcutaneous or sublingual route.**
53. Moingeon P. Update on immune mechanisms associated with sublingual immunotherapy: practical implications for the clinician. *J Allergy Clin Immunol Pract* 2013;1:228-41
- **Recent review focusing on the specifics of the oral immune system and sublingual immunotherapy mechanisms.**
54. Frati F, Incorvaia C, David M, et al. Requirements for acquiring a high-quality house dust mite extract for allergen immunotherapy. *Drug Design Dev Ther* 2012;6:117-23
55. Moingeon P, Batard T, Nony E, et al. Quality control of House Dust Mite extracts for allergen immunotherapy. *Int Arch Allergy Immunol* 2013;161:285-6
56. Calderon MA, Simons FE, Malling HJ, et al. Sublingual allergen immunotherapy: mode of action and its relationship with the safety profile. *Allergy* 2012;67:302-11
57. Bernstein DI, Epstein T, Murphy-Berends K, Liss GM. Surveillance of systemic reactions to subcutaneous immunotherapy injections: year 1 outcomes of the ACAAI and AAAAI collaborative study. *Ann Allergy Asthma Immunol* 2010;104:530-5
58. James LK, Shamji MH, Walker SM, et al. Long term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol* 2011;127:509-16
59. Vanbervliet B, Tourdot S, Rouzair P, et al. SLIT prevents the development of Eczema in percutaneous allergen-sensitized mice. *J Invest Dermatol* 2012;132:244-6

60. Fernandez-Caldas E. Towards a more complete standardization of mite allergen extracts. *Int Arch Allergy Immunol* 2013;160:1-3
61. Brunetto B, Tinghino R, Braschi MC, et al. Characterization and comparison of commercially available mite extracts for in vivo diagnosis. *Allergy* 2010;65:184-90
62. Casset A, Mari A, Purohit A, et al. Varying allergen composition and content affects the in vivo allergenic activity of commercial *Dermatophagoides pteronyssinus* extracts. *Int Arch Allergy Immunol* 2012;159:253-62
63. Moingeon P. Sublingual immunotherapy: from biological extracts to recombinant allergens. *Allergy* 2006;61(S81):15-19
64. Valenta R, Niespodziana K, Focke-Tejkl M, et al. Recombinant allergens: what does the future hold? *J Allergy Clin Immunol* 2011;127:860-4
65. Cromwell O, Hafner D, Nandy A. Recombinant allergens for specific immunotherapy. *J Allergy Clin Immunol* 2011;127:865-72
66. Campbell JD, Buckland KF, Mc Millan SJ, et al. peptide immunotherapy in allergic asthma generaltes IL-10-dependent immunological tolerance associated with linked epitope suppression. *J Exp Med* 2009;206:1535-47
67. Yang L, Kajiura H, Suzuki K, et al. Generation of a transgenic rice seed-based edible vaccine against house dust mite allergy. *Biochem Biophys Res Commun* 2008;365:334-9
68. Walgraffe D, Matteotti C, el Bakkoury M, et al. A hypoallergenic variant of Der p 1 as a candidate for mite allergy vaccines. *J Allergy Clin Immunol* 2009;123:1150-6
69. Burtin D, Chabre H, Didierlaurent A, et al. Production of native and modified recombinant Der p 1 forms in tobacco plants. *Clin Exp Allergy* 2009;39:760-70
70. Bussi eres L, Bordas V, Bulder I, et al. Fusion proteins assembling Der p 1 and Der p 2 major allergens from *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol* 2010;153:141-51
71. Bordas V, Bussi eres L, Airouche S, et al. Expression and characterization of natural-like recombinant Der p 2 for sublingual immunotherapy. *Int Arch Allergy Immunol* 2012;158:157-67
72. Marth K, Focke-Tejkl M, Lupinek C, et al. Allergen peptides, recombinant allergens and hypoallergens for allergen-specific immunotherapy. *Curr Treat Options Allergy* 2014;1:91-106
73. Calamita Z, Saconato H, Pela AB, Atallah AN. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized clinical trials using the cochrane collaboration method. *Allergy* 2006;61:1162-72
74. Penagos M, Passalacqua G, Compalati E, et al. Metaanalysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest* 2008;133:599-609
75. Compalati E, Passalacqua G, Bonini M and Canonica GW. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. *Allergy* 2009;64:1570-9
76. Lin SY, Erekosima N, Kim JM, et al. Sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. *JAMA* 2013;309:1278-88
77. Abramson MJ, Puy RM, Weiner JM. Injection immunotherapy for asthma. *Cochrane Data base Syst Rev* 2010;8:CD001186
78. Calderon MA, Casale TB, Nelson HS, Demoly P. An evidence-based analysis of house dust mite allergen immunotherapy: a call for more rigorous clinical studies. *J Allergy Clin Immunol* 2013;132:1322-36
- **A comprehensive analysis of allergen immunotherapy clinical studies conducted in mite-associated allergies.**
79. Ameal A, Vega-Chicote JM, Fernandez S, et al. Double blind and placebo-controlled study to assess efficacy and safety of a modified extract of *Dermatophagoides pteronyssinus* in allergic asthma. *Allergy* 2005;60:1178-83
80. Garcia-Robaina JC, de la Torre F, Sanchez I, et al. Successful management of mite-allergic asthma with modified extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2006;118:1026-32
81. Bergmann KC, Demoly P, Worm M, et al. Efficacy and safety of sublingual tablets of house dust mite allergen extracts in adults with allergic rhinitis. *J Allergy Clin Immunol* 2014;133:1608-14
82. Mosbech H, Deckelmann R, de Blay F, et al. Standardized quality (SQ) house dust mite sublingual immunotherapy tablet (ALK) reduces inhaled corticosteroid use while maintaining asthma control: a randomized, double blind, placebo-controlled trial. *J Allergy Clin Immunol* 2014;In Press
83. Larch  M, Hickey P, Hebert J, Hafner R. Safety and tolerability of escalating doses of house dust mite-peptide antigen desensitization (HDM-PAD). abstract *J Allergy Clin Immunol* 2013;131:AB37
84. Kunding TM, Senti G, Schnetzler G, et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J Allergy Clin Immunol* 2006;117:1470-6
85. Chabre H, Gouyon B, Adam A, et al. Molecular variability of group 1 and 5 grass pollen allergens between Pooideae species: implications for specific immunotherapy. *Clin Exp Allergy* 2010;40:505-19
86. Moingeon P. The specificities of allergen recognition by CD4+ T lymphocytes at the epitopic level. *Clin Exp Allergy* 2014;44:898-900
87. Archila LD, DeLong JH, Wambre E, et al. Grass-specific CD4+ T cells exhibit varying degrees of cross-reactivity, implications for allergen-specific immunotherapy. *Clin Exp Allergy* 2014;44:986-98
88. Wambre E, Delong JH, James EA, et al. Specific immunotherapy modifies allergen-specific CD4+ T cell responses in an epitope-dependent manner. *J Allergy Clin Immunol* 2013;133:872-9