

Recent developments and future strategies for immunotherapy of insect venom allergy

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Purpose of review

Hymenoptera venom allergy may cause life-threatening, sometimes even fatal, allergic reactions and thus may be associated with a serious reduction in the quality of life. Venom immunotherapy is effective in the majority of patients with this allergy. Treatment failures do however occur and immunotherapy may cause frequent systemic allergic side effects, especially in honeybee venom-allergic patients. New strategies to improve safety and efficacy of this treatment are therefore of general interest.

Recent findings

Among these new strategies are premedication with antihistamines: this definitely reduces side effects and based on recent in-vitro experiments and one clinical controlled study may even improve efficacy by modulating the T-cell response through interference with histamine receptors on these cells. Furthermore, during recent years the cDNA of most major allergens of bee and vespine venoms has been cloned and these allergens have been expressed in recombinant form. This allows for the preparation of patient-tailored extracts, with or without reduction of their allergenicity, for example by using unrefolded or point-mutated recombinant allergens. Yet another approach is the use of non IgE binding peptide fragments of the allergen with preserved T-cell epitopes for immunotherapy. Such preparations of bee venom phospholipase A2 have been used successfully in pilot studies. Finally, DNA vaccination with phospholipase A2 sequence plasmids has proved effective in one experimental study in sensitized mice.

Summary

A number of new strategies for venom immunotherapy, mostly based on genetic engineering, have been described and we await their use in clinical medicine.

Keywords

antihistamines, recombinant venom allergens, peptide immunotherapy, DNA vaccination

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Abbreviation

PLA phospholipase A2

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Introduction

Allergy to stinging insects is often considered as a model for immunotherapy of IgE-mediated allergy [1]. According to a sting challenge during Hymenoptera venom immunotherapy, about 95% of patients allergic to *Vespula* stings are protected, in contrast to only 80–90% of those allergic to honeybee venom [2,3]. Systemic allergic side effects to immunotherapy injections may occur in 20–40% of patients on immunotherapy with honeybee venom and up to 5–10% on *Vespula* venom [3]. Thus there remains considerable potential for further improvement in safety and efficacy of venom immunotherapy. Among the recent developments in this field the following will be discussed: (1) premedication with antihistamines; (2) recombinant venom allergens; (3) immunotherapy with T-cell epitope peptides; and (4) DNA vaccination. Other recent developments in venom immunotherapy, such as the quality of life aspects of patients with venom allergy and their modification by this treatment [4], as well as the association of an elevated basal serum tryptase [5] or mastocytosis [6] with severe anaphylaxis requiring long-term immunotherapy are discussed elsewhere.

Premedication with antihistamines

Due to the frequent side effects, especially in the dose increase phase of immunotherapy with honeybee venom, premedication with antihistamines during the initial phase of this treatment has been proposed. The efficacy of such a treatment in reducing local and generalized cutaneous allergic side effects like itching, urticaria and angioedema is documented in a number of double blind placebo-controlled trials as shown in Table 1 [7–10]. The effect of the premedication on more severe allergic side effects, including respiratory or cardiovascular symptoms, because of their infrequent occurrence, could not be judged from these trials.

Premedication with antihistamines has been questioned for two reasons: (1) it was claimed that, by masking mild side effects, it could prevent a dose reduction and thus further the occurrence of severe allergic reactions. This phenomenon has not been observed in any of the controlled and many uncontrolled studies on antihistamine premedication. (2) The effect of immunotherapy is generally thought to be mainly due to an allergen-specific change of the T-cell response [11] and it is well known that T cells bear histamine receptors [12]. Could it be, therefore, that

Table 1. Premedication with antihistamines during early immunotherapy: double blind, placebo-controlled trials

Author (premedication)	No. patients	Venom	Protocol	Reduction in side effects	
				Local	Systemic
Berchtold (1992) [7] (terfenadine)	52	Bee	Rush	Yes	Yes (U, AE)
Herman (1996) [9] (cetirizine)	140	Bee	Rush	Yes	No
Brockow (1997) [8] (terfenadine)	121	Bee/Vespula	Rush	Yes	Yes
Reimers (2000) [10] (fexofenadine)	54	Bee	Ultra-rush	Yes	Yes (U, AE)

U, urticaria; AE, angioedema.

antihistamine premedication influences the long-term efficacy of venom immunotherapy – for the better or the worse? In order to answer this question we analysed the long-term results of our first double blind placebo-controlled premedication study [7]. The 52 bee venom-allergic patients of this trial had been receiving premedication with terfenadine or placebo for 21 days during the dose increase phase of a rush protocol. Forty-seven of them were reevaluated after 3 years of venom immunotherapy. Seventeen had been reexposed to a field sting by a honeybee and 24 agreed to a sting provocation test during reassessment [13]. None of the 41 patients was on antihistamine treatment before the reexposure.

Table 2 shows the results of these reexposures. Of the 41 reexposed patients – 20 on original premedication with terfenadine and 21 on placebo – six developed a systemic allergic reaction. All were in the placebo group. These results indicate that antihistamine premedication during early immunotherapy does not adversely affect the efficacy of this treatment, but may rather increase it. Prospective studies are needed to confirm this effect and to study its exact mechanism, thought to be due to interference with histamine receptors on T cells and antigen presenting cells [12,14•]. Stimulation of H1 receptors on T cells furthers while stimulation of H2 receptors downregulates proliferation and cytokine expression by these cells. Because H1 receptors are abundant on Th1 cells while H2 receptors prevail on Th2 cells, blockage of H1 receptors induces down-regulation of Th2 and upregulation of Th1 cells [12]. This observation also raises the possibility of influencing efficacy and safety of immunotherapy by premedication with other immune modulating drugs.

Recombinant allergens

The cDNA of a number of major venom allergens from the honeybee (*Apis mellifera*), various vespids (*Vespula vulgaris*, *Dolichovespula maculata*, *Polistes annularis*) and from the fire ant (*Solenopsis invicta*) has been cloned and recombinant allergens were expressed in various prokar-

Table 2. Terfenadine premedication during rush-immunotherapy with bee venom: long-term results

	Premedication with	
	Fexofenadine	Placebo
No. patients originally treated	26	26
No. controlled after 3 years	24	23
No. reexposed	20	21
Field sting/challenge	9/11	8/13
Systemic reaction	0	6

Terfenadine versus placebo: $\chi^2 = 7.41$; $P < 0.01$. Data from Müller *et al.* [13].

yotic and eukaryotic systems. The data concerning the respective allergens are summarized in Table 3 [15•,16].

The biologic properties of bee venom phospholipase A2 (PLA), a 16–20 kDa enzyme expressed in *Escherichia coli* [17], were found to be comparable to those of natural purified PLA, both with regard to enzymatic activity and IgE binding properties in intracutaneous skin test endpoint titration, RAST (Radio-Allergo-Sorbent-Test), and histamine release assay [18–20]. Similar results were obtained with recombinant bee venom hyaluronidase, a 43 kDa enzyme expressed in a eukaryotic system, baculovirus-infected insect cells [21].

The three major allergens of vespid venoms, PLA, hyaluronidase and antigen 5, have also been cloned and expressed in recombinant form [16].

To date, recombinant venom allergens have not been used for immunotherapy. Theoretically, however, a number of promising approaches are conceivable:

- (1) Once all relevant allergens of a venom are available in recombinant form, the sensitization pattern of an individual patient can be determined exactly by estimating specific IgE antibodies to them all. A patient-tailored cocktail containing all the allergens to which the patient has IgE antibodies could then be prepared for immunotherapy [22•].

Table 3. Recombinant Hymenoptera venom allergens

Species	Allergen		MW (kDa)	Expression system
<i>Apis mellifera</i>	Api m 1	phospholipase A2	16–20	Prokaryotic
	Api m 2	hyaluronidase	43	Eukaryotic
	Api m 3	acid phosphatase	49	
<i>Vespula vulgaris</i>	Ves v 1	phospholipase A1	35	Prokaryotic
	Ves v 2	hyaluronidase	45	Prokaryotic
	Ves v 5	antigen 5	25	Prokaryotic and eukaryotic
<i>Dolichovespula maculata</i>	Dol m 1	phospholipase A1	35	Prokaryotic
	Dol m 2	hyaluronidase	45	Prokaryotic
	Dol m 5	antigen 5	25	Prokaryotic and eukaryotic
<i>Polistes annularis</i>	Pol a 5	antigen 5	25	Prokaryotic
<i>Solenopsis invicta</i>	Sol i 2		30	Eukaryotic
	Sol i 3	antigen 5	25	Eukaryotic
<i>Myremcia pilosula</i>	Myr p 1		7.5	Eukaryotic

MW, molecular weight. Data from Müller *et al.* [15••].

- (2) The mostly conformational B-cell epitopes have been shown to be strongly reduced in unfolded recombinant allergens [17–19]. Such preparations will have a strongly reduced reactivity to IgE antibodies fixed on effector cells because they are devoid of conformational B-cell epitopes, and will therefore induce much less mediator release and be better tolerated. Alternatively, all relevant T-cell epitopes of the allergen are preserved, and so is their capacity to interact with T cells and thus to induce protective immunologic effects.
- (3) A similar concept consists of point mutations of conformational B-cell epitopes resulting in hypo-allergenic mutants. Such point mutations have been described for various inhalant allergens [22••] and also of bee venom PLA [15••] and hyaluronidase [23].

T-cell epitope peptides

T-cell epitopes consist of short linear sequences of 10–15 amino acids of an allergen which are recognized by specific T lymphocytes from allergic patients. By using overlapping peptides covering the whole 136 amino acid sequence of PLA, the most important bee venom allergen, major T-cell epitopes have been identified by several groups [24–26]. These linear peptides were unable to bind to PLA-specific IgE antibodies in sera from bee venom-allergic patients, but induced strong proliferation of the T lymphocytes of bee venom allergic patients and beekeepers *in vitro*. In a pilot study [27], five bee venom-allergic patients with predominant sensitization to PLA were treated by weekly injections of increasing doses of an equimolar mixture of three major T-cell epitope peptides of PLA thus identified, consisting of 11–18 amino acids each. Over 2 months, a total dose of 397 µg protein was given without causing any allergic side effects. This observation contrasts with the reported late side effects during peptide immunotherapy with T-cell peptides of the major cat allergen Fel d 1 [28] in a considerable proportion of patients.

This may be explained by the fact that much longer peptides were used in the cat study. The results of provocation tests 1–2 weeks after the last treatment injection are shown in Table 4. All five patients tolerated a first provocation test consisting of the subcutaneous injection of 10 µg of natural purified PLA, corresponding approximately to the PLA content of one to two bee stings, without any systemic allergic symptoms. Three patients also tolerated a second provocation test by a live bee sting, while two developed minor cutaneous symptoms consisting of singular urticarial wheals or mild angioedema, which were much less severe than before the treatment. In-vitro studies of lymphocyte cultures from the patients revealed a strong allergen-specific reduction of proliferation and secretion of both Th1 and Th2 cytokines following stimulation. This finding was less prominent in the two only partly protected patients, suggesting the induction of PLA-specific tolerance by this form of peptide immunotherapy [29]. During peptide immunotherapy, PLA-specific serum IgE antibodies decreased slowly while the quotient of specific IgG4 to specific IgE increased sharply. Further serological studies indicated that the two patients with incomplete protection were sensitized to bee venom allergens other than PLA. Immunotherapy with T-cell peptides from all allergens to which the patient is sensitized may thus be required in order to achieve complete protection.

Another group described successful immunotherapy of PLA-sensitized CBA/J mice with three long peptides of 44–60 amino acids of PLA, either by intraperitoneal or by intranasal administration [30,31]. As in the previous studies in humans [27,29], peptide immunotherapy in mice induced an abrogation of T-cell proliferation following PLA stimulation *in vitro*, a shift in the cytokine profile from Th2 to Th1 and a suppression of IgE (and IgG1 in mice) but enhancement of IgG2a in mice (the equivalent of IgG4 in man). Similar cellular and serological changes were also observed during

Table 4. Results of provocation tests after immunotherapy with phospholipase A2 (PLA) peptides

Patient	Reaction following	
	10 µg PLA s.c.	Live bee sting in forearm
1	None	Local immediate
2	None	Local immediate
3	Local immediate and delayed reaction	Local immediate, no systemic symptoms
4	None	Local immediate, after 15 min facial erythema, solitary urticarial lesions, mild angioedema
5	Local immediate	Local immediate, after 25 min single urticarial wheals, mild angioedema

s.c., subcutaneously. Data from Müller *et al.* [27].

immunotherapy with these long PLA peptides in human bee venom-allergic patients [32]. Efficacy of treatment with these long peptides, however, was not verified by a sting challenge.

DNA vaccination

Yet another fascinating experimental strategy for immunotherapy is DNA vaccination, consisting of the injection of DNA plasmids encoding the relevant allergens. In contrast to environmental allergen exposure and to classical immunotherapy, this kind of vaccination induces a Th1 response. Successful DNA vaccination of sensitized mice has been reported with plasmids from the house dust mite and pollen allergens [33•]. More recently, protection of PLA-sensitized mice from anaphylaxis induced by PLA through vaccination with PLA sequence DNA plasmids was reported [34]. Protection was complete if vaccination was done before sensitization, whereas only 65% of the animals with therapeutic vaccination after intraperitoneal sensitization to PLA survived.

As mentioned above, many Hymenoptera venom-allergic patients are sensitized to several different venom allergens from vespids or the honeybee. Treatment with one major allergen in recombinant unfolded or point mutated form, with peptides thereof, or with DNA plasmids encoding for it, may therefore be insufficient. One elegant solution for this problem has recently been presented [35]. These authors produced a chimeric protein consisting of two fragments each of the important bee venom allergens PLA, hyaluronidase and melittin by genetic engineering via directional fusion polymerase chain reaction technology. The fragments were designed in a way to preserve all relevant T-cell epitope peptides while conformational B-cell epitopes were destroyed. This chimeric protein indeed induced strong proliferation and cytokine secretion in lymphocyte cultures from bee venom-allergic

patients, but it did not react with specific IgE against either of the three allergens, nor did it induce mediator release from blood basophils or immediate wheal and flare reaction in intracutaneous skin testing of allergic patients.

Conclusion

A number of new and promising experimental approaches to improve efficacy and safety of venom immunotherapy have been described. The most fascinating are probably those based on modern technologies of genetic engineering. Much experimental and pre-clinical work remains to be done, however, before these new methods can become available for clinical treatment of our patients with venom allergy.

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