

Expert Opinion

1. Introduction
2. Mechanism of immunomodulation
3. Indications
4. Protocols
5. Adverse effects
6. Effectiveness
7. Perspectives
8. Expert opinion and conclusion

For reprint orders, please
contact:
reprints@ashley-pub.com

Ashley Publications
www.ashley-pub.com



Vaccines & Antibodies

Immunotherapy of hypersensitivity to hymenoptera venom

Axel Roers & Nicolas Hunzelmann[†]

[†]University of Cologne, Department of Dermatology, Josef Stelzmann Str. 9, 50931 Cologne, Germany

Insect sting allergy is a common condition with a risk of life-threatening anaphylaxis. After a severe reaction, the fear of being restung can significantly reduce quality of life. Venom immunotherapy (VIT) is a highly effective treatment of the underlying type I-sensitisation. This review addresses the mechanisms of immune modulation by VIT and outlines current clinical application. Although highly effective in the majority of patients, VIT fails in a few individuals. It can also cause systemic allergic side effects, restricting its application to physicians trained in the treatment of anaphylaxis. This review discusses several new strategies to overcome these problems, which are presently a promising focus of research. These include the use of new adjuvants, of recombinant and genetically engineered venom allergens, as well as vaccination with peptides.

Keywords: anaphylaxis, hymenoptera, insect sting, venom immunotherapy

Expert Opin. Biol. Ther. (2005) 5(10):1349-1358

1. Introduction

IgE-mediated reactions to hymenoptera venom are among the most common causes of anaphylaxis. Although lethal outcome is rare, there is an average of 0.14 – 0.45 deaths per million per year following hymenoptera stings reported for various European countries and the US [1,2]. Approximately 0.15 – 3.3% of the general population have a history of a systemic anaphylactic reaction to honeybee or vespid stings [1]. The species that most frequently cause allergic reactions are the *Vespidae*, which comprise wasps, yellow jackets and hornets, the *Apinae* (honeybees) and the *Formicidae* (fire ants) [3]. The severity of a systemic anaphylactic sting reaction can vary from merely cutaneous symptoms, such as pruritus, urticaria or angioedema, to severe cardiopulmonary problems, including bronchospasm, hypotension and arrhythmia [3]. Initially mild symptoms may unpredictably progress to life-threatening conditions within seconds [3]. The three major allergenic proteins of wasp venom are phospholipase A1 [4], hyaluronidase [4] and antigen 5 [5], designated Ves v 1, Ves v 2 and Ves v 5, respectively, for *Vespula vulgaris* according to the allergen nomenclature [6]. The most important allergens of honeybee venom are phospholipase A2 and hyaluronidase, designated Api m 1 and Api m 2, respectively, for *Apis mellifera* [7].

The idea of controlling the symptoms of what is now known as allergic disease by gradually adapting the organism to the precipitating agent was first propagated by L Noon and J Freeman in 1911, who successfully treated hay fever with pollen extracts [8,9]. The concept was soon transferred to insect venom allergy; however, as long as whole body extracts from ground vespid or honeybees were employed, success was very limited. After immunotherapy of vespid and bee venom allergy with whole body extracts had been the standard of treatment for decades, a controlled study demonstrated that it was no more effective than placebo in 1978 [10]. In the

same study, the use of pure vespid and honeybee venom was evaluated systematically for the first time and was shown to be highly effective in protection from systemic anaphylactic reactions [10,11]. A similar result was obtained in another study on immunotherapy with pure honeybee venom [12]. In the following years, subcutaneous injections of increasing amounts of these venoms became the standard of therapy for hypersensitivity to vespid and bee stings [13]. Today, venom immunotherapy (VIT) for allergy to hymenoptera stings represents the most successful immunotherapy for allergic disease and has become a model for allergen-specific therapy [14,15]. In contrast to extracts from flying hymenoptera, whole body extracts from fire ants may be effective in therapy of fire ant venom hypersensitivity [16,17]. A recent double-blind, placebo-controlled study clearly demonstrated the effectiveness of immunotherapy with ant venom [18]. The production of pure hymenoptera venom is a highly labour- and cost-intensive process, involving the insect stinging through a membrane into a venom reservoir in the case of the honeybee, and the dissection of the venom organ from individual frozen insects in the case of vespids.

2. Mechanism of immunomodulation

The allergic sensitisation is based on the production of allergen-specific IgE, which binds to Fcε receptors on mast cells and eosinophils. Antigen crosslinks the surface-bound IgE and, via complex signal transduction pathways, induces the release of preformed mediators stored in the mast cell granules, which are responsible for immediate symptoms. In addition, mast cell activation by IgE crosslinking results in the *de novo* production of prostaglandins, leukotrienes and cytokines such as tumour necrosis factor-α, IL-4, IL-5 and IL-6, which are involved in the induction of prolonged reactions. The production of specific antibodies of the IgE class depends on genetic recombination of the immunoglobulin heavy chain locus in B cells. This so-called 'class switch' recombination to IgE is thus a prerequisite for allergic sensitisation and is controlled by T helper (Th) type 2 cells secreting IL-4 and IL-13.

Although VIT and allergen immunotherapy for other atopic conditions have been shown to be highly effective and have been the standard of treatment for > 20 years, our understanding of the mechanisms of immune modulation underlying the protective effect of immunotherapy is still incomplete. Among the effects of VIT is the induction of a specific IgG4 response, which has long been thought to be the prime reason for protection. Specific IgG4 binds allergen and, thus, competes with IgE. It also binds to mast cells and eosinophils via surface FcγRIIb. Coaggregation of these receptors and the high-affinity Fcε receptors by antigen results in an inhibitory signal blocking mast cell degranulation [19]. In addition, specific IgG also contributes to the immunomodulatory effect by inhibiting IgE-facilitated antigen presentation to T cells [20]. Although induction of specific

IgG4 is a prominent finding in the course of VIT, correlation of IgG4 titres to VIT-induced protection has not always been found, and it was suggested that VIT-induced changes in antibody specificity rather than mere IgG4 concentrations play a role for the protective effect. Increased capacity of IgG to block IgE–allergen binding has been observed after VIT and may show a better correlation with protection [21].

Switching of the humoral response to IgG4 is a consequence of the complex alterations of T cell responses occurring as a result of VIT. Protection from anaphylaxis was shown to be associated with immune deviation of the allergen-specific Th2 response towards a more Th1-biased response in VIT [22,23] as well as in immunotherapy for inhalant allergy [24–27]. Recently, research on the mechanism of allergen immunotherapy focused intensely on a possible role of regulatory T (T_{reg}) cells, which, over the past few years, have been identified as pivotal regulators of immune responses. The so-called 'natural T_{reg} cells' differentiate under the control of the transcription factor foxp3 [28–30]. They are CD4-positive cells, which, unlike conventional T cells, already express the IL-2 receptor alpha chain (CD25) prior to activation. Natural T_{reg} cells have been shown to control T cell responses as well as innate immunity by cell contact-dependent mechanisms and by secretion of IL-10 and transforming growth factor-β. Allergologists became increasingly interested in these cells, as their absence in patients with a null mutation of the *foxp3* gene results in a complex syndrome characterised by autoimmunity, but also by elevated IgE levels, severe atopic eczema and food allergy [31]. Another subset of T_{reg} cells designated 'IL-10-producing T_{reg} cells' can be differentiated *in vitro* or *in vivo* by various regimens of antigen exposure, do not express foxp3 and were shown to suppress T cell responses by the release of IL-10 [32]. Increased IL-10 production was demonstrated in skin biopsy specimens after intradermal allergen administration in patients receiving VIT [33], and VIT is associated with an increase in T cells that secrete IL-10 [22,23,34,35] consistent with the induction of T_{reg} cells [36]. These results are supported by similar findings in immunotherapy for inhalant allergens [37–40]. Among the cytokines mediating the immunomodulatory effect of VIT, IL-10 is considered to be of particular importance [34]. IL-10 stimulated IgG4 production and inhibited IgE production *in vitro* [34], and was shown to have a role in controlling mast cell function and homeostasis in animal models [41,42]. In addition to the long-term effects on T cell and humoral responses, VIT already has pronounced protective effects detectable after completion of the first week of rush immunotherapy, which may be explained in part by the inhibitory effects of IL-10 and interferon-γ on mast cells and basophilic granulocytes [43]. Thus, VIT may suppress type I allergy by both direct effects on the effector cells and by alterations of the T cell and humoral responses. However, the elucidation of the mechanism of immunotherapy still requires intense research, as to date our understanding of the processes involved remains vague.

3. Indications

The decision to commence VIT is based on the patient's history, on the results of skin tests and/or on the result of laboratory tests. In general, only patients who report sting reactions, which are indicative of a systemic IgE-mediated anaphylactic reaction, are eligible for specific immunotherapy [44]. Even large local reactions do not justify therapy, as these reactions are associated with a low risk of systemic anaphylaxis upon subsequent stings [44]. Only patients who suffered at least systemic urticaria (with wheals occurring distant to the site of the sting) should be subjected to further testing [45]. The presence of venom-specific IgE is assayed for by direct determination in serum [45] or by skin testing with venom applied first by pricking of the skin with a lancet through a droplet of venom solution and, if negative, also by intradermal injections of venom dilutions. The predictive value of the detection of specific IgE for systemic anaphylaxis should not be overestimated, as venom-specific IgE is found in up to 26% of the healthy adult population and only 17% of these will show a systemic reaction if stung [15]. If serum IgE and skin testing remain negative despite a history, which is suggestive of anaphylaxis, additional *in vitro* tests may be useful, such as histamine or leukotriene release, basophil activation assay, western blot or RAST/FEIA-inhibition assays. Immunotherapy should be offered to patients with a history of a systemic anaphylactic reaction to an insect sting if specific IgE is detected by skin testing or by *in vitro* assays. However, negative *in vitro* or skin tests do not exclude venom allergy [46]. Even the somewhat more sensitive skin test may be negative in patients who report a systemic sting reaction. In this situation, an *in vitro* assay for specific IgE should be performed. If both assays are negative, a repeat test may be done some weeks later. Serum tryptase levels should also be determined to detect mastocytosis as a cause of non-IgE-mediated anaphylaxis upon an insect sting. In contrast to older guidelines, absence of detectable IgE is no longer considered a guarantee of safety [46]. There are no clear recommendations for the management of history-positive but IgE-negative patients, but VIT can be recommended to some of these patients depending on the severity of the initial sting reaction. In children, earlier work suggested that the often merely cutaneous symptoms of an anaphylactic reaction do not justify immunotherapy, as these children should have only a low risk of developing severe, that is, more than cutaneous, anaphylaxis following subsequent stings and would presumably lose the allergy over time [47]. However, the same group now reported that an important fraction of children do not 'outgrow' the hypersensitivity and that VIT should be offered to children who developed a severe sting reaction with more than cutaneous symptoms [48]. For patients with elevated serum tryptase and/or mastocytosis, VIT may be of particular importance, as these conditions are associated with a high risk of severe systemic anaphylaxis [49,50]. However, some authors do not offer VIT to this group of patients due to concerns regarding efficacy and safety [51].

4. Protocols

Several different protocols have been established for VIT that all follow the general scheme of an initial phase with incrementally increasing doses and maintenance treatment. Whereas the maintenance dose, which is in general applied once a month, is usually 100 µg, the various protocols differ in the duration of the initial build-up stage. The classical regimen entails the weekly administration of increasing doses, and the maintenance dose is reached after 4 months [44]. In various different shorter regimens, so-called 'rush VIT', the initial build-up is faster and takes ~ 1 week [52,53]. 'Ultra rush' protocols for VIT were described that allow one to reach the maintenance dose after 2 days or even after 210 min [54-56]. Whereas the traditional schedule is usually performed in an out-patient setting with monitoring of the patients for 20 – 30 min or 30 – 60 min after each injection (guidelines in the US and Europe, respectively), hospitalisation is recommended in general during the build-up phase for rush and ultra rush protocols in some European countries. In contrast, these faster protocols do not necessarily entail hospitalisation according to current guidelines in the US. So far, there is no reliable parameter for monitoring the success of VIT in the individual patient since systemic reactions can occur despite a negative skin test result or negative specific IgE tests. Some centres offer sting challenge tests to their patients after VIT has been stopped. Although this test cannot definitely prove protection of the individual patient as a result of VIT, the sting challenge can be used as a reliable indicator of therapeutic efficacy in larger groups of patients [57]. Discontinuation of VIT is usually recommended after 5 years; however, in patients with elevated serum tryptase levels and/or mastocytosis lifelong therapy is recommended by some authors [49]. Even in patients with severe initial reaction without elevated serum tryptase levels and/or mastocytosis, some allergists recommend continued immunotherapy [44]. Some authors suggest that the decision on the duration of VIT should be based on the severity of the initial sting reaction. They consider 2 – 3 years of VIT sufficient in the case of mild-to-moderate initial reactions and recommend longer therapy for patients who had experienced severe anaphylaxis and who persistently show positive skin test results [58].

5. Adverse effects

Local reactions with erythema, swelling and pruritus occur in most patients and can usually be controlled by premedication with antihistamines and by topical application of corticosteroids. However, VIT can also trigger the systemic allergic reaction that it is meant to prevent [59]. In a multi-centre study compiling data for different VIT regimens, anaphylactic side effects of variable intensity, including urticaria, angioedema, hypo- or hypertension or bronchospasm, occurred in up to 20% of the patients, a third of whom required medical treatment [60]. However, for patients on VIT

following the well-established rush protocols, the rate of systemic side effects is considerably lower [52,61]. Systemic side effects occur more frequently during phases of dose increase and are more common in immunotherapy of honeybee venom allergy as compared with wasp venom allergy [60,62].

Concomitant medication with beta-blockers does not seem to increase the incidence of systemic side effects of VIT, but should be avoided in general as it may enhance allergic reactions and can interfere with the effect of potentially life-saving adrenaline injections in cases of severe anaphylaxis. A recent study did not find an increased rate of severe side effects or sting reactions in patients on beta-blockers during VIT [63]. The authors concluded that the combination of VIT and beta-blockers can be an option for individual patients with severe cardiovascular disease. Based on individual case reports, angiotensin-converting enzyme inhibitors have been implicated as a risk factor for severe anaphylaxis in patients receiving VIT; however, convincing evidence supporting this notion is missing [21]. Premedication with antihistamines is effective in reducing local and systemic side effects of VIT [61,64]. Concerns that antihistamines may constitute a safety risk because of masking of systemic reactions, and that they might reduce the effectiveness of VIT, have recently been settled. A double-blind, placebo-controlled, long-term study demonstrated a trend towards an enhancement of the effectiveness of VIT by premedication with terfenadine [65].

6. Effectiveness

The protecting effect of VIT against anaphylactic reactions to insect stings has been demonstrated by a large number of independent studies. As venom allergy is a potentially fatal condition, it is not possible to perform a prospective, double-blind, placebo-controlled study. A recent meta-analysis clearly showed that VIT is effective [13]. The effect of immunotherapy must be judged in the context of the natural course of venom hypersensitivity. An individual who has experienced anaphylaxis following a sting has a risk of 50 – 70% for a second systemic reaction after a subsequent sting in the first few years after the initial sting without VIT [66]. However, this risk gradually decreases, reaching ~ 20% after 10 – 20 years. In wasp venom-allergic patients, immunotherapy reduces the risk of a second anaphylactic event to 2 – 8% while the patient is on maintenance therapy [57,66], whereas this risk remains 15 – 25% in patients on bee VIT [57]. Many of the patients who still react to stings while receiving VIT can effectively be protected by an increase of the venom dose [67]. After therapy is discontinued, there seems to be a slow increase of the cumulative risk for systemic reactions. In wasp venom allergy, the risk for a systemic sting reaction was < 10% within the first few years after discontinuation of therapy [68], but approached 20% 10 years after immunotherapy was stopped, as determined by an evaluation of field stings [66]. Thus, the value of VIT is that it quickly reduces the likelihood of a severe reaction to low levels during

Box 1. New strategies for venom immunotherapy.

Modern adjuvants (TLR ligands):

- 3-deacylated monophosphoryl lipid A
- CpG oligodeoxynucleotides

Peptides derived from venom allergens:

- Short peptides containing T cell epitopes
- Long overlapping peptides covering complete proteins

Recombinant venom allergens:

- Mutated allergens with reduced antigenicity
- Patient-tailored allergen cocktails

TLR: Toll-like receptor.

those first years with a high risk for untreated patients. When therapy is discontinued, this benefit may be gradually lost, and after some 10 – 20 years the risk may finally become similar for treated and untreated patients [66]. In general, however, post-treatment anaphylaxis is milder than pretreatment reactions, although rare life-threatening reactions after immunotherapy have been reported. In children, VIT has proven particularly effective in that the protective effect is more long-lasting as compared with adult patients [48]. VIT reduced the risk of a systemic sting reaction from 17% in untreated children to 3% even 10 – 20 years after discontinuation of therapy. VIT is less effective in patients with elevated serum tryptase levels and mastocytosis [51].

7. Perspectives

Although immunotherapy with hymenoptera venoms is highly effective, there is great potential for improvement. Most importantly, VIT unpredictably fails in individual patients. Therefore, VIT cannot definitely relieve the fear of insect stings and does not abrogate the necessity to carry adrenaline injectors. Furthermore, VIT still carries a low risk of severe systemic reactions, which limits its application to physicians trained in the management of anaphylaxis. In addition, the large number of injections required for successful therapy makes VIT an expensive and time-consuming procedure. Finally, the production of pure venom preparations is highly cost-intensive. Various novel concepts are followed to overcome these problems (Box 1). Several new approaches under investigation for immunotherapy of inhalant allergy have not yet been tested for the treatment of hymenoptera venom allergy, but may hold great potential for the improvement of VIT [69], as discussed in the following sections.

7.1 Modern adjuvants

Inflammatory responses mediated by innate mechanisms at the site of antigen entry have an important impact on the regulation of a T cell response. Adjuvants trigger local inflammatory responses and, thereby, enhance the adaptive immune

responses to co-injected antigen. The finding that protection in allergen immunotherapy is associated with altered regulation of T cell responses has raised hopes that coadministration of novel adjuvants along with allergen in immunotherapy might potentiate a shift from allergen-specific Th2 to Th1 responses. The adjuvant effect might allow for reduced numbers of injections and lower venom doses. Two new types of adjuvant have gained particular attention in this context over the past few years. A derivative of lipopolysaccharide, 3-deacylated monophosphoryl lipid A (MPL), stimulates IL-12 production by dendritic cells and is a potent promoter of Th1 responses [70-72]. Grass pollen immunotherapy with a mixture of allergen and MPL proved effective in the treatment of hay fever and may be superior to conventional pollen immunotherapy [73]. MPL has not yet been tested in VIT. A second class of adjuvant is based on the finding that particular sequence (CpG) motifs of double-stranded DNA are methylated in mammalian but not bacterial genomic DNA. Unmethylated CpG motifs are recognised by a specialised receptor, Toll-like receptor (TLR)-9 [74]. Ligation of TLR-9 by bacterial DNA or, alternatively, short synthetic oligodeoxynucleotides (ODNs) containing the relevant motifs triggers an inflammatory response [75-78]. Through the induction of IL-12 secretion by macrophages and dendritic cells, CpG ODNs stimulate Th1 differentiation. CpG ODNs have also been shown to reduce IgE-production *in vitro* as well as *in vivo* [79,80]. In mice, CpG ODNs inhibited allergic airway disease [81-83]. The immunomodulatory effect seems to be significantly enhanced by direct conjugation of CpG ODNs to the allergenic protein [84-86]. Although several studies of CpG ODNs in inhalant allergen immunotherapy are under way [87], a trial investigating a possible enhancing effect of CpG ODNs on VIT is still missing.

7.2 Recombinant allergens

The genes encoding the most important allergens contained in honeybee and vespid venom have been identified. Several allergens, such as bee and vespid hyaluronidases and phospholipases, were successfully expressed in recombinant form in different prokaryotic and eukaryotic cells [88]. Although recombinant allergens have not yet been tested as vaccines in immunotherapy, this development holds great promise. In contrast to complete venom, vaccines composed of pure recombinant allergens do not contain ill-defined mixtures of additional and in part toxic molecules. Moreover, availability of recombinant allergens should allow determination of the exact spectrum of allergens towards which a patient is sensitised. Immunotherapy may then be performed with these individual allergens only in a patient-tailored fashion [89]. The recombinant approach also allows genetic modification of the allergenic proteins [90,91]. In order to reduce the allergenicity of the vaccine while preserving immunogenicity, mutant allergens were generated that contain the linear T cell epitopes, but not the conformational B cell epitopes responsible for IgE binding. Birch pollen allergen Bet v-1 engineered to meet

these requirements displayed a much reduced allergenicity, but retained the capacity to stimulate Bet v-1-specific T cells and induced production of blocking IgG [92-94]. Likewise, the major honeybee venom allergen, phospholipase A2, was mutated to reduce IgE binding, but leaving T cell epitopes intact [95]. Furthermore, a fusion protein composed of the two major bee venom allergens, phospholipase A2 and hyaluronidase, was expressed in *Escherichia coli*. All T cell epitopes were present in the fusion protein; B cell epitopes, however, were disrupted due to incorrect folding. Following injection of the fusion protein into mice, IgE production in response to sensitisation with the native allergens was reduced [96]. So far, these new vaccines have not been tested for efficacy in immunotherapy of bee venom allergy. Problems regarding production and stability, as well as the enormous number of bee and vespid species with considerable diversity of venom allergens, might limit broader application of recombinant allergens.

7.3 Peptides

A different approach, which follows the same concept of bypassing IgE-mediated effects, is the treatment with short synthetic peptides that contain the relevant T cell epitopes, but do not bind allergen-specific IgE [97]. In animal models of inhalant allergy, vaccination with peptides induced peripheral T cell tolerance [98,99]. A recent study showed that injection of short peptides containing T cell epitopes of Fel d 1, the major allergen in hypersensitivity to cat dander, could reduce the cutaneous response. Of the patients receiving Fel d 1 peptides, 25% developed late asthmatic reactions to initial low doses of the vaccine, despite tolerating higher doses of peptides [100]. For the treatment of bee venom allergy, the peptide vaccination relies on the thorough mapping of T cell epitopes of the major honeybee venom allergen, phospholipase A2, which had been performed earlier by different groups [101-103]. A mixture of three peptides encompassing major phospholipase A2 T cell epitopes was used as a vaccine in five bee venom-allergic patients with a sensitisation to phospholipase A2. Weekly injections of increasing doses over 2 months did not cause allergic side effects, but resulted in protection from anaphylaxis upon subcutaneous provocation and bee stings [104]. Another study employed three long overlapping synthetic peptides, altogether covering the entire length of the phospholipase A2 protein, in a double-blind, placebo-controlled clinical trial in patients hypersensitive to honeybee venom. A maintenance dose of 100 µg per peptide was given 6 times over the 2-month duration of the study. This treatment was well-tolerated and induced peptide-specific *ex vivo* T cell hyporesponsiveness in patients with bee venom allergy [105]. Protection from systemic reactions was not reported in this Phase I study. In a mouse model of phospholipase A sensitisation, successful immunotherapy by intranasal or intraperitoneal administration of long overlapping peptides was described [106,107]. The treatment resulted in full protection from anaphylaxis, a drop in phospholipase A-specific IgE levels, an increase in IgG2 levels (the murine equivalent of

human IgG4) and antigen-specific T cell anergy. Larger studies addressing clinical efficacy of these new approaches to VIT are needed.

8. Expert opinion and conclusion

VIT is a safe and effective treatment of hypersensitivity to hymenoptera stings and should be offered to patients who developed systemic anaphylaxis following a sting. Major disadvantages are the high frequency of injections and duration of treatment required for sustained protection. In addition, due to rare severe systemic allergic side effects, injections have to be administered by physicians experienced in the treatment of anaphylaxis. These factors make VIT an expensive and

time-consuming procedure. Therefore, modifications of the therapy regimens, which would increase the effectiveness, reduce the number of the injections required and largely eliminate treatment failures, would represent an important improvement. We feel that new protocols for VIT involving the coadministration of modern adjuvants, such as immunostimulatory DNA or monophosphoryl lipid A, with conventional whole venom preparations may result in a significant enhancement of therapeutic effectiveness in the near future. However, direct head to head comparison with existing VIT regimens in controlled studies will have to demonstrate equivalence or superiority of these new strategies with respect to safety, effectiveness and economic viability. Novel vaccines based on recombinant venom proteins still require considerable conceptual development.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

- SASVARY T, MUELLER U: Todesfälle an Insektenstichen in der Schweiz 1978 bis 1987. *Schweiz. Med. Wochenschr.* (1994) 124:1887-1894.
- CHARPIN D, BIRNBAUM J, VERVLOET D: Epidemiology of hymenoptera allergy. *Clin. Exp. Allergy* (1994) 24:1010-1015.
- FREEMAN TM: Hypersensitivity to hymenoptera stings. *N. Engl. J. Med.* (2004) 351:1978-1984.
- KING TP, LU G, GONZALEZ M, QIAN N, SOLDATOVA L: Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with their hornet and wasp homologs and possible implications for clinical allergy. *J. Allergy Clin. Immunol.* (1996) 98:588-600.
- LU G, VILLALBA M, COSCIA MR, HOFFMAN DR, KING TP: Sequence analysis and antigenic cross-reactivity of a venom allergen, antigen 5, from hornets, wasps, and yellow jackets. *J. Immunol.* (1993) 150:2823-2830.
- KING TP, HOFFMAN D, LOWENSTEIN H, MARSH DG, PLATTS-MILLS TA, THOMAS W: Allergen nomenclature. *Bull. World Health Organ.* (1994) 72:797-806.
- MULLER U: New developments in the diagnosis and treatment of hymenoptera venom allergy. *Int. Arch. Allergy Immunol.* (2001) 124:447-453.
- NOON L: Prophylactic inoculation against hay fever. *Lancet* (1911) 1:1572.
- FREEMAN J: Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. *Lancet* (1911) 2:814-817.
- HUNT KJ, VALENTINE MD, SOBOTKA AK, BENTON AW, AMODIO FJ, LICHTENSTEIN LM: A controlled trial of immunotherapy in insect hypersensitivity. *N. Engl. J. Med.* (1978) 299:157-161.
- This is a landmark study showing for the first time that, in contrast to the use of whole insect extracts, immunotherapy with pure venom is highly effective.
- GOLDEN DB, VALENTINE MD, KAGEY-SOBOTKA A, LICHTENSTEIN LM: Regimens of hymenoptera venom immunotherapy. *Ann. Intern. Med.* (1980) 92:620-624.
- MULLER U, THURNHEER U, PATRIZZI R, SPIESS J, HOIGNE R: Immunotherapy in bee sting hypersensitivity. Bee venom versus wholebody extract. *Allergy* (1979) 34:369-378.
- ROSS RN, NELSON HS, FINEGOLD I: Effectiveness of specific immunotherapy in the treatment of hymenoptera venom hypersensitivity: a meta-analysis. *Clin. Ther.* (2000) 22:351-358.
- MULLER U, MOSBECH H: Immunotherapy with hymenoptera venoms: EAACI position paper. *Allergy* (1993) 48:36-46.
- Guidelines of the European Academy of Allergology and Clinical Immunology.
- GOLDEN DB: Insect sting allergy and venom immunotherapy: a model and a mystery. *J. Allergy Clin. Immunol.* (2005) 115:439-447.
- FREEMAN TM, HYLANDER R, ORTIZ A, MARTIN ME: Imported fire ant immunotherapy: effectiveness of whole body extracts. *J. Allergy Clin. Immunol.* (1992) 90:210-215.
- TRIPLETT RF: Sensitivity to the imported fire ant: successful treatment with immunotherapy. *South. Med. J.* (1973) 66:477-480.
- BROWN SG, WIESE MD, BLACKMAN KE, HEDDLE RJ: Ant venom immunotherapy: a double-blind, placebo-controlled, crossover trial. *Lancet* (2003) 361:1001-1006.
- DAERON M, MALBEC O, LATOUR S, AROCK M, FRIDMAN WH: Regulation of high-affinity IgE receptor-mediated mast cell activation by murine low-affinity IgG receptors. *J. Clin. Invest.* (1995) 95:577-585.
- WACHHOLZ PA, DURHAM SR: Mechanism of immunotherapy: IgG revisited. *Curr. Opin. Allergy Clin. Immunol.* (2004) 4:313-318.
- An excellent review of the role of IgG in VIT-induced immunomodulation.
- RUEFF F, PRZYBILLA B: Venom immunotherapy: adverse reactions and treatment failure. *Curr. Opin. Allergy Clin. Immunol.* (2004) 4:307-311.
- A thorough review of data on systemic allergic side effects of VIT.
- BELLINGHAUSEN I, METZ G, ENK AH, CHRISTMANN S, KNOP J, SALOGA J: 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.* (1997) 27:1131-1139.
23. JUTEL M, PICHLER WJ, SKRBIC D, URWYLER A, DAHINDEN C, MULLER UR: 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.* (1995) 154:4187-4194.
 24. VARNEY VA, HAMID QA, GAGA M *et al.*: Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J. Clin. Invest.* (1993) 92:644-651.
 25. HAMID QA, SCHOTMAN E, JACOBSON MR, WALKER SM, DURHAM SR: Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J. Allergy Clin. Immunol.* (1997) 99:254-260.
 26. DURHAM SR, YING S, VARNEY VA *et al.*: Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J. Allergy Clin. Immunol.* (1996) 97:1356-1365.
 27. WILSON DR, NOURI-ARIA KT, WALKER SM *et al.*: Grass pollen immunotherapy: symptomatic improvement correlates with reductions in eosinophils and IL-5 mRNA expression in the nasal mucosa during the pollen season. *J. Allergy Clin. Immunol.* (2001) 107:971-976.
 28. KHATTTRI R, COX T, YASAYKO SA, RAMSDELL F: An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* (2003) 4:337-342.
 29. FONTENOT JD, GAVIN MA, RUDENSKY AY: Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* (2003) 4:330-336.
 30. HORI S, NOMURA T, SAKAGUCHI S: Control of regulatory T cell development by the transcription factor Foxp3. *Science* (2003) 299:1057-1061.
 31. CHATILA TA, BLAESER F, HO N *et al.*: JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic dysregulation syndrome. *J. Clin. Invest.* (2000) 106:R75-R81.
 32. O'GARRA A, VIEIRA P: Regulatory T cells and mechanisms of immune system control. *Nat. Med.* (2004) 10:801-805.
 - **A comprehensive review of T_{reg} cell populations and current knowledge of their function.**
 33. NASSER SM, YING S, MENG Q, KAY AB, EWAN PW: Interleukin-10 levels increase in cutaneous biopsies of patients undergoing wasp venom immunotherapy. *Eur. J. Immunol.* (2001) 31:3704-3713.
 34. AKDIS CA, BLESKEN T, AKDIS M *et al.*: Role of interleukin 10 in specific immunotherapy. *J. Clin. Invest.* (1998) 102:98.
 35. BELLINGHAUSEN I, KNOP J, SALOGA J: Role of interleukin 10-producing T cells in specific (allergen) immunotherapy. *J. Allergy Clin. Immunol.* (2000) 12:20-25.
 36. AKDIS CA, BLASER K, AKDIS M: Genes of tolerance. *Allergy* (2004) 59:897-913.
 37. GARDNER LM, THIEN FC, DOUGLASS JA, ROLLAND JM, O'HEHIR RE: Induction of T 'regulatory' cells by standardized house dust mite immunotherapy: an increase in CD4+ CD25+ interleukin-10+ T cells expressing peripheral tissue trafficking markers. *Clin. Exp. Allergy* (2004) 34:1209-1219.
 38. FRANCIS JN, TILL SJ, DURHAM SR: Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J. Allergy Clin. Immunol.* (2003) 111:1255-1261.
 39. ROBINSON DS, LARCHE M, DURHAM SR: Tregs and allergic disease. *J. Clin. Invest.* (2004) 114:1389-1397.
 40. JUTEL M, AKDIS M, BUDAK F *et al.*: IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur. J. Immunol.* (2003) 33:1205-1214.
 41. BOUTON LA, RAMIREZ CD, BAILEY DP *et al.*: Costimulation with interleukin-4 and interleukin-10 induces mast cell apoptosis and cell-cycle arrest: the role of p53 and the mitochondrion. *Exp. Hematol.* (2004) 32:1137-1145.
 42. PALANIYANDI SS, WATANABE K, MA M, TACHIKAWA H, KODAMA M, AIZAWA Y: Inhibition of mast cells by interleukin-10 gene transfer contributes to protection against acute myocarditis in rats. *Eur. J. Immunol.* (2004) 34:3508-3515.
 43. PIERKES M, BELLINGHAUSEN I, HULTSCH T, METZ G, KNOP J, SALOGA J: Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-g production of T cells. *J. Allergy Clin. Immunol.* (1999) 103:326-332.
 44. MOFFITT JE, GOLDEN DB, REISMAN RE, RUFUS L, NICKLAS R *et al.*: Stinging insect hypersensitivity: a practice parameter update. *J. Allergy Clin. Immunol.* (2004) 114:869-886.
 45. HAMILTON RG: Diagnostic methods for insect sting allergy. *Curr. Opin. Allergy Clin. Immunol.* (2004) 4:297-306.
 - **A useful overview of diagnostic procedures for insect venom hypersensitivity.**
 46. GOLDEN DB, TRACY JM, FREEMAN TM, HOFFMAN DR: Negative venom skin test results in patients with histories of systemic reaction to a sting. *J. Allergy Clin. Immunol.* (2003) 112:495-498.
 47. VALENTINE MD, SCHUBERTH KC, KAGEY-SOBOTKA A *et al.*: The value of immunotherapy with venom in children with allergy to insect stings. *N. Engl. J. Med.* (1990) 323:1601-1603.
 48. GOLDEN DB, KAGEY-SOBOTKA A, NORMAN PS, HAMILTON RG, LICHTENSTEIN LM: Outcomes of allergy to insect stings in children, with and without venom immunotherapy. *N. Engl. J. Med.* (2004) 351:668-674.
 - **The first long-term follow-up study of VIT in children.**
 49. HAEBERLI G, BRONNIMANN M, HUNZIKER T, MULLER U: Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin. Exp. Allergy* (2001) 33:1216-1220.
 - **An important study addressing the risk associated with VIT in patients with mastocytosis.**
 50. LUDOLPH-HAUSER D, RUEFF F, FRIES C, SCHOPF P, PRZYBILLA B: Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to hymenoptera stings. *Lancet* (2001) 357:361-362.
 - **The first comprehensive study on the correlation of severe anaphylactic reactions and increased serum tryptase levels that are in part due to previously undiagnosed mastocytosis.**

51. DUBOIS AE: Mastocytosis and hymenoptera allergy. *Curr. Opin. Allergy Clin. Immunol.* (2004) 4:291-295.
 - **A comprehensive review of recent data concerning the management of venom allergy in mastocytosis.**
52. STURM G, KRANKE B, RUDOLPH C, ABERER W: Rush hymenoptera venom immunotherapy: a safe and practical protocol for high-risk patients. *J. Allergy Clin. Immunol.* (2002) 110:928-933.
 - **An interesting study demonstrating the low risk of systemic side effects of rush VIT.**
53. WENZEL J, MEISSNER-KRAEMER M, BAUER R, BIEBER T, GERDSEN R: Safety of rush insect venom immunotherapy. The results of a retrospective study in 178 patients. *Allergy* (2003) 58:1176-1179.
54. RUEFF F, PRZYBILLA B: Ultrarush immunotherapy in patients with Hymenoptera venom allergy. *J. Allergy Clin. Immunol.* (2001) 107:928-929.
55. BREHLER R, WOLF H, KUTTING B, SCHNITKER J, LUGER T: Safety of a two-day ultrarush insect venom immunotherapy protocol in comparison with protocols of longer duration and involving a larger number of injections. *J. Allergy Clin. Immunol.* (2000) 105:1231-1235.
 - **A large study of an improved and shortened protocol for VIT.**
56. BIRNBAUM J, RAMADOUR M, MAGNAN A, VERVLOET D: hymenoptera ultra-rush venom immunotherapy (210 min): a safety study and risk factors. *Clin. Exp. Allergy* (2003) 33:58-64.
57. RUEFF F, PRZYBILLA B, MULLER U, MOSBECH H: The sting challenge test in Hymenoptera venom allergy. Position paper of the Subcommittee on Insect Venom Allergy of the European Academy of Allergology and Clinical Immunology. *Allergy* (1996) 51:216-225.
58. REISMAN RE: Duration of venom immunotherapy: relationship to the severity of symptoms of initial insect sting anaphylaxis. *J. Allergy Clin. Immunol.* (1993) 92:831-836.
59. LOCKEY RF, TURKELTAUB PC, OLIVE ES, HUBBARD JM, BAIRD-WARREN IA, BUKANTZ SC: The hymenoptera venom study. III: Safety of venom immunotherapy. *J. Allergy Clin. Immunol.* (1990) 86:775-780.
60. MOSBECH H, MULLER U: Side-effects of insect venom immunotherapy: results from an EAACI multicenter study. European Academy of Allergology and Clinical Immunology. *Allergy* (2000) 55:1005-1010.
61. BROCKOW K, KIEHN M, RIETHMULLER C, VIELUF D, BERGER J, RING J: Efficacy of antihistamine pretreatment in the prevention of adverse reactions to hymenoptera immunotherapy: a prospective, randomized, placebo-controlled trial. *J. Allergy Clin. Immunol.* (1997) 100:458-463.
62. RZANY B, PRZYBILLA B, JARISCH R *et al.*: Clinical characteristics of patients with repeated systemic reactions during specific immunotherapy with hymenoptera venoms. A retrospective study. *Allergy* (1991) 46:251-254.
63. MULLER UR, HAEBERLI G: Use of beta-blockers during immunotherapy for hymenoptera venom allergy. *J. Allergy Clin. Immunol.* (2005) 115:606-610.
64. MULLER U: Recent developments and future strategies for immunotherapy of insect venom allergy. *Curr. Opin. Allergy Clin. Immunol.* (2003) 3:299-303.
65. MULLER U, HARI Y, BERCHTOLD E: Premedication with antihistamines may enhance efficacy of specific-allergen immunotherapy. *J. Allergy Clin. Immunol.* (2001) 107:81-86.
66. GOLDEN DB, KAGEY-SOBOTKA A, LICHTENSTEIN LM: Survey of patients after discontinuing venom immunotherapy. *J. Allergy Clin. Immunol.* (2000) 105:385-390.
 - **This is an excellent study discussing the effectiveness of VIT in the context of the natural course of venom hypersensitivity.**
67. RUEFF F, WENDEROTH A, PRZYBILLA B: Patients still reacting to a sting challenge while receiving conventional hymenoptera venom immunotherapy are protected by increased venom doses. *J. Allergy Clin. Immunol.* (2001) 108:1027-1032.
68. GOLDEN DB, KWITEROVICH KA, KAGEY-SOBOTKA A, VALENTINE MD, LICHTENSTEIN LM: Discontinuing venom immunotherapy: outcome after five years. *J. Allergy Clin. Immunol.* (1996) 97:579-587.
69. VALENTA R: The future of antigen-specific immunotherapy of allergy. *Nat. Rev. Immunol.* (2002) 2:446-453.
70. MIKLOSKA Z, RUECKHOLDT M, GHADIMINEJAD I, DUNCCKLY H, DENIS M, CUNNINGHAM AL: Monophosphoryl lipid A and QS21 increase CD8 T lymphocyte cytotoxicity to herpes simplex virus-2 infected cell proteins 4 and 27 through IFN-gamma and IL-12 production. *J. Immunol.* (2000) 164:5167-5176.
71. SALKOWSKI CA, GREGORY RD, VOGEL SN: Lipopolysaccharide and monophosphoryl lipid A differentially regulated interleukin-12, gamma interferon, and interleukin-10 mRNA production in murine macrophages. *Infect. Immun.* (1997) 65:3239-3247.
72. MARTIN M, MICHALEK SM, KATZ J: Role of innate immune factors in the adjuvant activity of monophosphoryl lipid A. *Infect. Immun.* (2003) 71:2498-2507.
73. DRACHENBERG KJ, WHEELER AW, STUEBNER P, HORAK F: A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* (2001) 56:498-505.
74. HEMMI H, TAKEUCHI O, KAWAI T *et al.*: A Toll-like receptor recognizes bacterial DNA. *Nature* (2000) 408:740-745.
75. KRIEG AM, WAGNER H: Causing a commotion in the blood: immunotherapy progresses from bacteria to bacterial DNA. *Immunol. Today* (2000) 21:521-526.
76. MEDZHITOV R, JANEWAY CA: Innate immunity: impact on the adaptive immune response. *Curr. Opin. Immunol.* (1997) 9:4-9.
77. KRIEG AM: CpG motifs in bacterial DNA and their immune effects. *Annu. Rev. Immunol.* (2002) 20:709-760.
78. BOHLE B, JAHN-SCHMIDT B, MAURER D, EBNER C: Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18 and IFN-gamma production in cells from allergic individuals and inhibit IgE synthesis *in vitro*. *Eur. J. Immunol.* (1999) 29:2344-2353.
79. FUJIEDA S, IHO S, KIMURA Y, YAMAMOTO H, IGAWA H, SAITO H: Synthetic oligodeoxynucleotides inhibit IgE

- induction in human lymphocytes. *Am. J. Respir. Crit. Care Med.* (2000) **162**:232-239.
80. LEE DJ, TIGHE H, CORR M *et al.*: Inhibition of IgE antibody formation by plasmid DNA immunization is mediated by both CD4+ and CD8+ T cells. *Int. Arch. Allergy Immunol.* (1997) **113**:227-230.
 81. BANERJEE B, KELLY KJ, FINK JN, HENDERSON JD JR, BANSAL NK, KURUP VP: Modulation of airway inflammation by immunostimulatory CpG oligodeoxynucleotides in a murine model of allergic aspergillosis. *Infect. Immun.* (2004) **72**:6087-6094.
 82. KOHAMA Y, AKIZUKI O, HAGIHARA K, YAMADA E, YAMAMOTO H: Immunostimulatory oligodeoxynucleotide induces TH1 immune response and inhibition of IgE antibody production to cedar pollen allergens in mice. *J. Allergy Clin. Immunol.* (1999) **104**:1231-1238.
 83. IKEDA RK, MILLER M, NAYAR J *et al.*: Accumulation of peribronchial mast cells in a mouse model of ovalbumin allergen induced chronic airway inflammation: modulation by immunostimulatory DNA sequences. *J. Immunol.* (2003) **171**:4860-4867.
 84. SHIROTA H, SANO K, KIKUCHI T, TAMURA G, SHIRATO K: Regulation of murine airway eosinophilia and Th2 cells by antigen-conjugated CpG oligodeoxynucleotides as a novel antigen-specific immunomodulator. *J. Immunol.* (2000) **164**:5575-5582.
 85. TIGHE H, TAKABAYASHI K, SCHWARTZ D *et al.*: Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. *J. Allergy Clin. Immunol.* (2000) **106**:124-134.
 86. TIGHE H, TAKABAYASHI K, SCHWARTZ D *et al.*: Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity. *Eur. J. Immunol.* (2000) **30**:1939-1947.
 87. CRETICOS PS, CHEN YH, SCHROEDER JT: New approaches in immunotherapy: allergen vaccination with immunostimulatory DNA. *Immunol. Allergy Clin. North Am.* (2004) **24**:569-581.
 88. MULLER U: Recombinant hymenoptera venom allergens. *Allergy* (2002) **57**:570-576.
 - **A comprehensive discussion of applications of recombinant venom allergens.**
 89. VALENTA R, LIDHOLM J, NIEDERBERGER V, HAYEK B, KRAFT D, GRONLUND H: The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin. Exp. Allergy* (1999) **29**:896-904.
 90. AKDIS CA, BLASER K: Bypassing IgE and targeting T cells for specific immunotherapy of allergy. *Trends Immunol.* (2001) **22**:175-178.
 91. WESTRITSCHNIG K, VALENTA R: Can we genetically engineer safer and more effective immunotherapy reagents? *Curr. Opin. Allergy Clin. Immunol.* (2003) **3**:495-500.
 92. VRTALA S, HIRTENLEHNER K, VANGELISTA L *et al.*: Conversion of the major birch pollen allergen, Bet v 1, into two nonanaphylactic T cell epitope-containing fragments: candidates for a novel form of specific immunotherapy. *J. Clin. Invest.* (1997) **99**:1673-1681.
 93. VRTALA S, AKDIS CA, BUDAK F *et al.*: T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v 1, induce blocking antibodies. *J. Immunol.* (2000) **165**:6653-6659.
 94. MAHLER V, VRTALA S, KUSS O *et al.*: Vaccines for birch pollen allergy based on genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1. *Clin. Exp. Allergy* (2004) **34**:155.
 95. BUHOT C, CHENAL A, SANSON A *et al.*: 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.* (2004) **13**:2970-2978.
 96. KUSSEBI F, KARAMLOO F, RHYNER C *et al.*: A major allergen gene-fusion protein for potential usage in allergen-specific immunotherapy. *J. Allergy Clin. Immunol.* (2005) **115**:323-329.
 - **An important study of the tolerising effect of genetically engineered bee venom allergens in mice.**
 97. LARCHE M, WRAITH DC: Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat. Med.* (2005) **11**:S69-S76.
 98. BRINER TJ, KUO MC, KEATING KM, ROGERS BL, GREENSTEIN JL: Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d 1. *Proc. Natl. Acad. Sci. USA* (1993) **90**:7608-7612.
 99. HOYNE GF, O'HEHIR RE, WRAITH DC, THOMAS WR, LAMB JR: Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J. Exp. Med.* (1993) **178**:1783-1788.
 100. OLDFIELD WL, LARCHE M, KAY AB: Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* (2002) **360**:47-53.
 - **An interesting study of the effectiveness of peptide vaccination for the treatment of cat dander allergy, including a discussion of side effects.**
 101. DHILLON M, ROBERTS C, NUNN T, KUO M: Mapping human T cell epitopes on phospholipase A2: the major bee-venom allergen. *J. Allergy Clin. Immunol.* (1992) **90**:42-51.
 102. KAEMMERER R, KETTNER A, CHVATCHKO Y *et al.*: Delineation of PLA2 epitopes using short or long overlapping synthetic peptides: interest for specific immunotherapy. *Clin. Exp. Allergy* (1997) **27**:1016-1026.
 103. CARBALLIDO JM, CARBALLIDO-PERRIG N, KAGI MK *et al.*: T cell epitope specificity in human allergic and nonallergic subjects to bee venom phospholipase A2. *J. Immunol.* (1993) **150**:3582-3591.
 104. MULLER U, AKDIS CA, FRICKER M *et al.*: 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.* (1998) **101**:747-754.
 - **A pioneering study of the effectiveness of peptide immunotherapy in patients with bee venom allergy.**
 105. FELLRATH JM, KETTNER A, DUFOUR N *et al.*: Allergen-specific T-cell tolerance induction with allergen-derived long synthetic peptides: results of a Phase I trial. *J. Allergy Clin. Immunol.* (2003) **111**:854-861.
 - **A Phase I study of immunotherapy with bee phospholipase A2 peptides. Ex vivo T cell tolerance induction is studied.**

106. VON GARNIER C, ASTORI M, KETTNER A *et al.*: Allergen-derived long peptide immunotherapy down-regulates specific IgE response and protects from anaphylaxis. *Eur. J. Immunol.* (2000) **30**:1638-1645.
107. ASTORI M, VON GARNIER C, KETTNER A, DUFOUR N, CORRADIN G, SPERTINI F: Inducing tolerance by intranasal administration of long peptides in naive and primed CBA/J mice. *J. Immunol.* (2000) **165**:3497-3505.

Affiliation

Axel Roers & Nicolas Hunzelmann^{†1}

[†]Author for correspondence

¹University of Cologne, Department of Dermatology, Josef Stelzmann Str. 9, 50931 Cologne, Germany

Tel: +49 221 478 5086; Fax: +49 221 478 6438;

E-mail: Nicolas.hunzelmann@uni-koeln.de