

Allergy to bumblebee venom

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Allergy to bumblebee venom is a rare form of Hymenoptera venom allergy. Because bumblebees are increasingly used for the pollination of greenhouse plants, the prevalence of this Hymenoptera allergy has increased during the past decade. The clinical presentation, diagnosis and therapy of bumblebee venom allergy are similar to other Hymenoptera venom allergies. There is a significant immunological cross-reactivity between bumblebee and honeybee venom. It has been claimed that immunotherapy with honeybee venom can protect patients with bumblebee venom allergy. This concept, however, has been called into question after the finding of bumblebee venom-specific IgE lacking cross-reactivity to honeybee venom, and three cases of bumblebee venom-allergic patients in whom immunotherapy with honeybee venom was unsuccessful. Immunotherapy with pure bumblebee venom has been shown to be effective and safe, and is currently the treatment of choice in individuals who cannot avoid contact with bumblebees. Immunotherapy with honeybee venom, however, should be considered in patients with severe reactions to bumblebee stings and concurrent sensitization to honeybee venom. *Curr Opin Allergy Clin Immunol* 1:361–365. © 2001 Lippincott Williams & Wilkins.

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Current Opinion in Allergy and Clinical Immunology 2001, 1:361–365

Abbreviations

CAST cellular antigen stimulation test
RAST radioallergosorbent test

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1528-4050

Introduction

Hymenoptera venom allergy is a common problem in many parts of the world. It is most commonly caused by members of the families Apidae, Vespidae and Myrmicidae [1]. Bumblebees belong to the family of Apidae. Allergy to bumblebee venom has attracted some interest recently: an increase in prevalence has been observed, as a result of a newly developed method of pollinating greenhouse plants by domesticated bumblebees. This article reviews current knowledge on bumblebee allergy with special reference to recent progress and unsolved problems in this particular field of Hymenoptera venom allergy.

Biology of bumblebees

Bumblebees are a morphologically homogeneous group of social Aculeata. In comparison with honeybees, bumblebees are usually bigger, more hairy and often have a more varied color. Like honeybees, they live in colonies. Bumblebee colonies, with few exceptions, are annual, with only the young, inseminated queens surviving the winter. In spring, the queens construct a nest, commonly in an abandoned rodent burrow, and begin to lay eggs, from which adult workers emerge in approximately 5 weeks. The colony size increases continuously during the summer as successive broods of workers are reared, and can reach up to several hundred workers, depending on the species. Later in summer, males and queens are reared. After emerging from the nests, these reproductive forms soon mate. Inseminated queens prepare for hibernation, whereas the other members of the bumblebee colony die [2–4]. Taxonomically, bumblebees are classified in one single genus *Bombus*, which belongs to the family of Apidae (Fig. 1). Bumblebees prefer cool and temperate climates and are most abundant in Europe, Asia and north America. Michener [3] estimated the total number of different bumblebee species at 250. They can extend northwards as far as there is land. In the tropics, bumblebees are only found in mountainous regions. In Africa bumblebees are almost absent except for a narrow band in the north. Australia and New Zealand are devoid of indigenous bumblebees, but for economic reasons some species were imported during the 19th century. Bumblebees feed on pollen and nectar collected from plants, and by foraging they pollinate plants. The pollination by bumblebees is of great economic importance [4,5].

Stinging apparatus and venom of bumblebees

Female bumblebees (workers and queens) have a stinging apparatus, constructed similarly as in other hymenopterans. It consists of an acid (venom-producing) gland, a venom reservoir (sac), an alkaline gland (Dufor's gland), and the sting itself. In contrast to honeybees, the stinging apparatus in bumblebees has a stronger connection to the abdomen, and less pronounced barbs. Therefore, bumblebees can retract their sting from human skin and, in contrast to honeybees, do not usually die after having stung. Bumblebees are not aggressive and do not sting unless disturbed in the proximity of their nests or if squeezed [4,6]. The venom of bumblebees has not been analysed as thoroughly as that of honeybees. Hymenopteran venom consists of low molecular weight substances (biogenic amines, sugars, amino acids, etc.), peptides, and proteins. Table 1 summarizes the information on bumblebee venom published to date [1,7–14]. In contrast to honeybee venom, bumblebee venom contains no mellitin [13], but instead polypeptides with a similar function called bombolitins. These amphiphilic substances have a unique structure and can, similar to mellitin or mastoparan and crabrolin from vespid venoms, degranulate

mast cells and increase the enzymatic activity of phospholipases [15,16].

Allergens of bumblebee venom

Protein allergens in hymenopteran venom are of prime importance to the allergologist. The total protein content in the bumblebee venom of the species *Bombus impatiens* and *Bombus pennsylvanicus* has been estimated to be 52 and 145 µg, and the amount delivered per sting 10 and 31 µg, respectively [17]. In 1996, Hoffman and Jacobson [14] summarized their studies on bumblebee venom. Pure venom of the American bumblebee *B. pennsylvanicus* was fractionated by gel filtration on a Sephadex G75 column, and fractions were further analysed using sodium dodecylsulphate–polyacrylamide gel electrophoresis and by determining enzyme activities. In sequence of descending molecular weight they identified a 95 000 M_r protein, an acid phosphatase of 39 000 M_r, a hyaluronidase of 38 000 M_r, a protease of 28 000 M_r, and a phospholipase A of 14 200 M_r molecular mass. They also identified four proteins (36 000, 33 000, 29 000 and 22 000 M_r) of unknown enzymatic activity (probably fragments of the 95 000 M_r protein), as well as some smaller peptides. The protease of 28 000 M_r was not found in honeybee venom. Sera from patients

Figure 1.

Simplified taxonomy of Aculeatae (order: Hymenoptera) according to Müller [1].

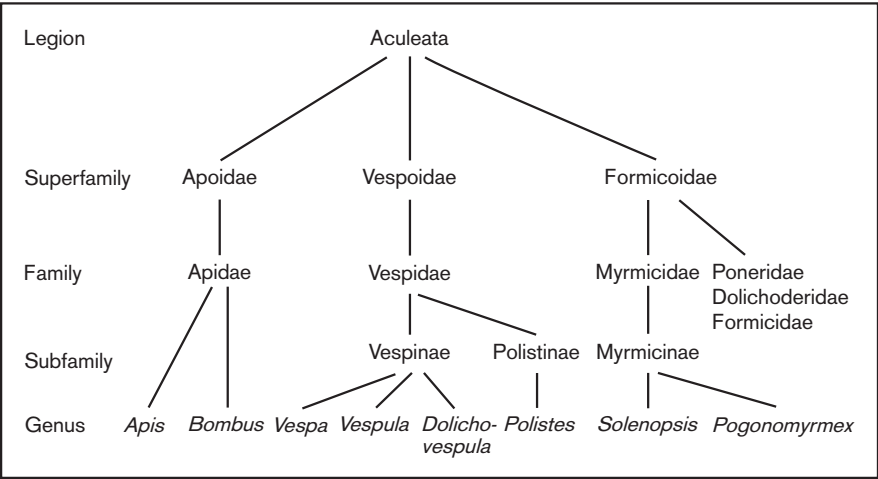


Table 1. Bumblebee venom components

Component	Quantity	Reference	Species
Serotonine	Traces	[7]	<i>Bombus terrestris</i>
Acetylcholine	30 µg/venom reservoir	[8]	<i>Bombus terrestris</i>
Slow muscle-contracting factor		[8]	<i>Bombus lapidarius</i>
Putrescine		[9]	<i>Bombus ignitus</i>
Mast cell degranulating peptide	<1% of dry weight	[10]	<i>Bombus pennsylvanicus</i>
Bombolitins	25% of dry weight	[11]	<i>Bombus pennsylvanicus</i>
Citrate	6.5–8.2% of dry weight	[12]	<i>Bombus fervidus</i> , <i>Bombus pennsylvanicus</i>
Proteins	15–30% of dry weight	[1,13,14]	<i>Bombus impatiens</i> , <i>Bombus pennsylvanicus</i>

allergic to bumblebee venom reacted most strongly to the hyaluronidase in four cases, to the acid phosphatase in one case, and to the phospholipase A in another case. The complementary DNA sequence of phospholipase A had 54% identity with phospholipase A from honeybee venom, and there were 33% additional conservative substitutions [14]. In 1998, Stapel and coworkers [18] published sodium dodecylsulphate–polyacrylamide gel electrophoresis and immunoblot studies of bumblebee venoms. They found a 95 000 M_r protein in the European, but not in the American bumblebee and honeybee venom preparation. Furthermore, all venom types had a strong band at 17 000 and a weak band at 45 000 M_r , which was attributed to the phospholipase A and the hyaluronidase, respectively. Both types of bumblebee venom, but not honeybee venom, contained a 31 000–33 000 M_r protein. Finally, a band of 28 000 M_r (probably representing the protease described and sequenced by Hoffman and Jacobson) was detected in the American bumblebee venom preparation alone. Immunoblotting studies [18] showed a heterogeneous pattern: one patient's serum reacted strongly only to phospholipase A, another exclusively to hyaluronidase, whereas the sera of two other patients reacted with several bumblebee polypeptides.

Epidemiology and clinical presentation of bumblebee venom allergy

Allergy to bumblebee venom is rare. Müller [19] found, in a total of 403 patients with Hymenoptera allergy who could identify the offending insect, only one case of bumblebee allergy. In an earlier study carried out by a single physician in the United States [20], however, 6% of Hymenoptera venom allergic patients attributed their reaction to bumblebee stings. In the mid-1980s, de Jonghe in Belgium established a method of pollination of tomato plants in greenhouses using bumblebee colonies [21]. This method of pollination expanded soon after to other countries in Europe, particularly the Netherlands, and is now used all over the world [22]. Gretenkord [23] wrote that in 1996 approximately 300 000–350 000 bumblebee colonies were kept worldwide for breeding, and that approximately 60 000 colonies had been imported into Japan in 1995. During the past decade, several cases of bumblebee allergy in greenhouse workers and bumblebee farm employees have been reported in Belgium and the Netherlands [24–26]. Another risk group for bumblebee allergy is scientists studying the behaviour of bumblebees [27]. In a search of the medical literature, we identified approximately 46 cases of bumblebee allergy [14,18,24–26,27•,28–31], with detailed information available on 31 patients. Of these, 18 were men (mean age 39 years, range 19–68 years) and 13 women (mean age 31 years, range 19–49 years). The clinical presentation of bumblebee venom allergy is similar to honeybee and

yellow jacket venom allergy. The reported severity of reaction (according to Mueller [32]) was grade I in three patients, grade II in five patients, grade III in 14 patients, and grade IV in nine patients. There was one report of a near fatal reaction [29], and one case with a fatal outcome after a bumblebee sting [28].

Diagnosis of bumblebee venom allergy

Diagnosis is based on history, skin tests and the demonstration of specific IgE in the patient's serum. All patients on whom skin test results with bumblebee venom have been published had positive skin tests [26,27•,31]. Unfortunately, bumblebee venom extracts are only available from a few commercial suppliers. Similarly, the measurement of IgE specific to bumblebee venom is not possible with all serological test systems. Furthermore, differences between bumblebee species (e.g. American and European species) could theoretically lead to false-negative test results. Therefore, even now it can be difficult to confirm a suspected bumblebee allergy because of the lack of suitable test material. In 1993, Kochuyt *et al.* [25] reported finding positive radioallergosorbent test (RAST) or positive skin tests to honeybee venom in all seven tested patients with systemic reactions to bumblebee stings. They proposed using honeybee venom for diagnostic and therapeutic purposes (venom immunotherapy) in bumblebee-allergic patients [25]. However, Hoffman and Jacobson [14] found two patients with negative RAST to honeybee among 13 bumblebee-allergic patients, and de Groot and coworkers [26] identified another bumblebee-allergic patient lacking detectable levels of IgE specific to honeybee venom. Altogether, out of 32 bumblebee venom-allergic patients in whom tests for IgE specific to honeybee venom have been published, four had negative results [14,25,26]. Skin tests for honeybee venom were positive in all patients tested. However, in most patients, the results with honeybee venom were a factor 100 or higher less sensitive than the tests with bumblebee venom [26,27•]. Therefore, although only rare individuals with bumblebee venom allergy have no cross-reacting antibodies to honeybee venom, the substitution of honeybee venom for skin testing and serological testing in cases of bumblebee venom allergy does not seem advisable. We recently successfully used another method to diagnose bumblebee venom allergy: in a 27-year-old female biologist with an occupational allergy to bumblebee venom, positive skin test and specific IgE to bumblebee venom, we performed a cellular antigen stimulation test (CAST) [33]. Skin tests and IgE specific to honeybee venom were also positive, but negative for yellow jacket venom. CAST was strongly positive for bumblebee venom, positive for honeybee venom and negative for yellow jacket venom. Three controls with a negative history and negative serology for Hymenoptera venom allergy all

tested negative with CAST for all three types of venom (unpublished results).

Immunological cross-reactivity between honeybee and bumblebee venom

The high prevalence of honeybee-specific IgE in bumblebee-allergic patients mentioned above is consistent with immunological cross-reactivity between honeybee and bumblebee venom. In 1982, Hoffman [13] reported the results of RAST inhibition experiments in which he used purified honeybee proteins to inhibit American bumblebee-specific IgE. Honeybee acid phosphatase, hyaluronidase, phospholipase A, and allergen C could all inhibit the binding of bumblebee-specific IgE to some extent. Additional studies by Hoffman and Jacobson [14] and Stapel *et al.* [18] demonstrated, however, that the allergens of both types of venom are not identical, and that bumblebee venom-specific epitopes do exist. Whether there is also some cross-reactivity between bumblebee and yellow jacket venom (hyaluronidase?) remains as yet a matter of conjecture.

Management of bumblebee venom allergy

The emergency treatment of acute anaphylactic reactions in patients allergic to bumblebee venom is similar to that for other hymenopteran allergies. Patients with bumblebee venom allergy should carry emergency medications (epinephrine, antihistamine, corticosteroid) with them. Patients with an allergy to honeybee venom should be informed about the possibility of a reaction to bumblebee stings, and vice versa. The best therapy and preventive measure is to avoid exposure to bumblebees, if necessary by a change of occupation. The risk of being stung by a bumblebee is very small in non-occupationally exposed individuals. Although the risk of bumblebee stings in greenhouse workers might be reduced in the future by the use of male bumblebees who possess no stinging apparatus [34], venom immunotherapy should be considered for allergic individuals with continued exposure to bumblebees. Two groups have reported successful venom immunotherapy using pure bumblebee venom preparations [26,27*,31]. Various induction schedules were used, including more conventional schedules with increasing doses over several weeks, and a 'rush' protocol of 4 days' duration in one patient. In total, 13 patients were desensitized with bumblebee venom. Immunotherapy with bumblebee venom was very safe, the only side-effects being two asthmatic reactions that occurred in a patient with unstable asthma. Further reactions in this patient could be prevented by treatment with inhaled steroids [31]. The protective effect of venom immunotherapy was tested either with field stings or with in-hospital sting challenges. Eleven out of 13 patients were completely protected, two patients had a total of three systemic

reactions. However, these reactions were milder than their previous reactions to bumblebee stings. Two other patients used epinephrine 20 min after a field sting, although they showed no signs of anaphylaxis. Similarly as in venom immunotherapy with honeybee and yellow jacket venom, skin sensitivity and specific IgE decreased during immunotherapy with bumblebee venom. These markers can be used to monitor the progress of the therapy [31]. Because bumblebee venom was unavailable and because they knew of the important immunological cross-reactivity between honeybee and bumblebee venom, Kochuyt and coworkers [25] treated seven patients with bumblebee allergy and sensitization to honeybee venom with honeybee venom immunotherapy. During immunotherapy, six out of seven patients experienced between one and more than 15 further stings by bumblebees. No systemic reactions and only two large local reactions occurred [25]. In contrast, van der Zwan and coworkers [24], and Stern *et al.* [27*] reported a total of three patients who, during immunotherapy with honeybee venom, experienced bumblebee stings and reacted with systemic symptoms of similar severity as before the initiation of immunotherapy. Stern and coworkers [27*] subsequently treated their two patients with bumblebee venom immunotherapy, and were able to demonstrate protection in an in-hospital sting challenge. It is currently unclear how large the risk of a reaction to a bumblebee sting is in a patient with primary sensitization to honeybee venom, and vice versa. We believe that immunotherapy with honeybee venom should be considered in selected patients with severe reactions to bumblebee venom and sensitization to honeybee venom. In rare patients with double sensitization, even a combined immunotherapy with both honeybee and bumblebee venom might be indicated. RAST inhibition tests could be helpful in choosing which venom extracts to use for immunotherapy [35*].

Conclusion

Venom immunotherapy with bumblebee venom is safe and efficacious. A total of 85% of patients treated hitherto could be completely protected by this treatment. As already stated by Stapel and coworkers [18], it does not currently seem advisable to use honeybee venom for diagnosis and immunotherapy in bumblebee-allergic patients. Venom immunotherapy with honeybee venom might be considered, however, in patients with severe reactions to bumblebee stings and concurrent sensitization to honeybee venom, in order to induce protection against honeybee stings. Because male bumblebees do not have a stinging apparatus, their usage for pollination instead of female bumblebees is a promising approach to prevent bumblebee venom allergy in the important risk group of greenhouse workers.

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