
Identification of allergens in the venom of the common striped scorpion

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Background: The common striped scorpion, *Centruroides vittatus*, is endemic to the southwestern United States and causes thousands of human stings annually. Immediate hypersensitivity reactions to *C vittatus* venom have been reported.

Objectives: To identify specific IgE in 11 patients with immediate hypersensitivity to *C vittatus* and to characterize the allergens present in the venom.

Methods: Skin testing to dialyzed, filtered venom was performed in 5 patients. Immunoglobulin E immunoblots to whole milked venom was accomplished with serum samples from 8 patients. Enzymatic properties of whole venom were also determined.

Results: *C vittatus* venom was found to contain 150 µg/µL of protein. Four of 5 patients tested had positive skin test reactions to the purified venom extract, with no late reactions. In all 8 patients, sodium dodecyl sulfate–polyacrylamide gel electrophoresis demonstrated multiple proteins, 9 of which were identified as allergens on IgE immunoblots, ranging in size from 30 to 170 kD. Enzymatic activity was found to include phospholipase A, alkaline phosphatase, esterase, esterase lipase, and acid phosphatase.

Conclusions: *C vittatus* envenomation may result in immediate hypersensitivity reactions in susceptible individuals. Venom specific IgE can be identified by using skin tests and IgE immunoblots. The allergens identified in these patients had molecular weights distinct from those of known scorpion neurotoxins. A safe and effective skin testing extract can be prepared from dialyzed pure venom and may lead to the widespread ability to diagnose *C vittatus* venom allergy.

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INTRODUCTION

Much is known about the characteristics of the Hymenoptera venoms,¹ and it is well accepted that these venoms can cause hypersensitivity reactions in humans.² However, many arthropods produce venoms with toxic and allergenic properties.^{3–5} Scorpions, which belong to the class Arachnida (Fig 1),⁶ produce venoms of varying toxicities that can be fatal to humans.^{3–5,7,8}

The common striped scorpion, *Centruroides vittatus*, is the most frequently encountered scorpion in the United States and is responsible for thousands of human stings each year (Fig 2).⁹ *C vittatus* is commonly encountered indoors, and many people are stung after stepping on the scorpion while barefooted.⁹ *C vittatus* is a highly adaptable species, and although its geographic distribution is currently limited to the south-central area of the United States and northeastern Mexico (Fig 3), migration to other areas is possible.⁹ It produces

low-toxicity venom that causes a moderate amount of pain but is otherwise harmless. However, the venom contains low-molecular-weight (LMW) neurotoxins that have been known to result in paresthesia and muscle spasms.^{9,10}

Hypersensitivity reactions to the venom of *C vittatus* have also been reported.^{5,10} In the largest series of patients, Demain and Goetz¹⁰ demonstrated immediate, late, and delayed skin test responses to various concentrations of *C vittatus* venom. They demonstrated immediate skin reactivity in only 1 patient, however, and showed an irritant effect at 1:1,000 dilution of an undialyzed venom extract. Furthermore, many of their patients and controls showed late-phase cutaneous reactions to skin testing.¹⁰

In recent years, we have had numerous individuals arrive at the Allergy and Immunology Clinic at Wilford Hall USAF Medical Center with symptoms consistent with hypersensitivity reactions after being stung by *C vittatus*. We sought to identify in vitro specific IgE to *C vittatus* venom in these individuals and to develop a skin testing extract from the venom. In addition, we sought to characterize the venom by determining allergen sizes and enzymatic properties.

METHODS

Patients

The protocol for this study was approved by the Wilford Hall USAF Medical Center Investigational Review Board. Patients were identified from allergy clinic records or were actively recruited from other clinics (primary care practices and emergency departments). All patients had a history of a

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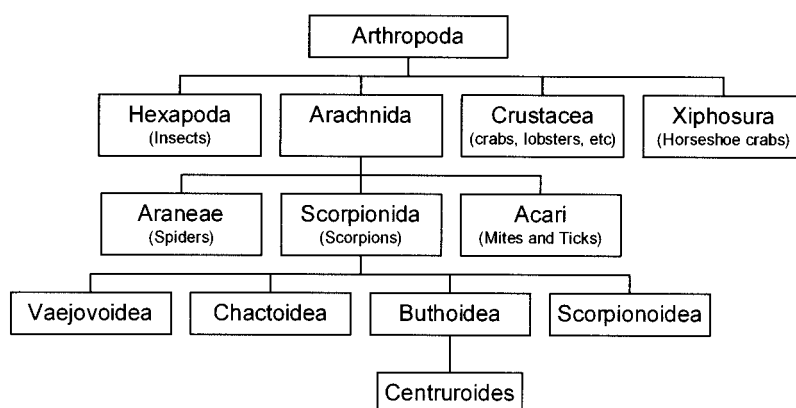


Figure 1. Phylogeny of the genus *Centruroides*.



Figure 2. The common striped scorpion, *Centruroides vittatus*.

scorpion sting experienced in the San Antonio, TX, area with symptoms suggestive of an IgE-mediated reaction. A systemic reaction was defined as diffuse urticaria, pruritus, flushing, angioedema, upper airway obstruction, asthmalike symptoms, rhinorrhea, sneezing, gastrointestinal tract symptoms, cardiovascular decompensation, or alteration of consciousness. A large local reaction was defined as localized swelling of at least 8 to 10 cm beginning within 4 to 6 hours and lasting for at least 24 hours. Patients were excluded if they were pregnant; had any serious concurrent disease processes, such as chronic cardiopulmonary disease; or were actively taking a β -blocker or other medication known to interfere with skin testing (ie, antihistamines). Written informed consent was obtained from each patient, and serum samples were drawn and stored at 4° C.

Venom Collection and Preparation

Centruroides vittatus was collected from the San Antonio area, and identification was confirmed by an entomologist. The venom was collected directly by milking the venom from the truncated stinger into capillary tubes. As previously described by Yahel-Niv and Zlotkin,¹¹ 1 μ L of venom was diluted 1:1,000 in sterile diluent, resulting in approximately 100 μ g/mL of venom. Neurotoxin fractions were removed via dialysis using 12,000-Da dialysis tubing in hopes of decreasing irritant and late-phase reactions. The resultant solution was rendered sterile by filtration through a 0.20- μ m syringe filter.

Skin Testing

Skin testing was performed to the purified *C vittatus* venom extract as previously described by Demain and Goetz.¹⁰ A

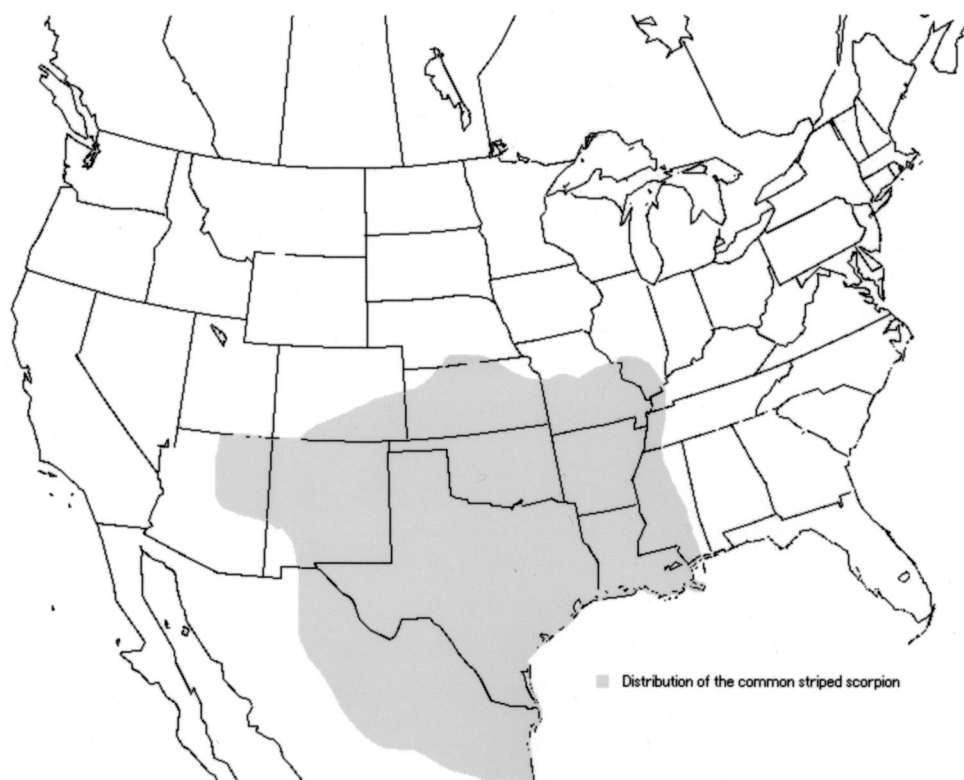


Figure 3. Approximate geographic distribution of *Centruroides vittatus*.

modified prick-and-wipe method was used to perform epicutaneous testing with 1:1,000 wt/vol strength extract. If the result was negative, testing continued with intradermal skin testing, first using 0.02 mL of 1:1,000,000 wt/vol concentration extract, and using log-fold increases in weight per volume strength until either (1) a positive result was obtained or (2) a final concentration of 1:10,000 wt/vol strength was reached with a negative result. The positive and negative skin test controls used were histamine (1 mg/mL for epicutaneous testing and 0.1 mg/mL for intradermal testing) and sterile diluent, respectively. All skin tests were read 15 minutes after placement. A positive result was defined as a wheal size 3 mm or greater in diameter with surrounding erythema for epicutaneous testing and a wheal size 5 mm or greater in diameter with surrounding erythema for intradermal skin testing.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and IgE Immunoblots

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of the venom extract was accomplished. Undialyzed *C vittatus* venom extract (5 μ g) was electrophoresed on 10% to 20% gradient gels (BMA-PAGE; Cambrex, Rockland, ME) along with LMW controls (Cambrex). Gels were stained with 0.025% Coomassie brilliant blue R-250. The molecular weight of each stained protein

was calculated by comparing the protein's gel position with a standard curve constructed from the molecular weight standards.

Immunoblots were accomplished in the following manner. Undialyzed *C vittatus* venom extract was transferred from unstained SDS-PAGE gels to 0.45- μ m nitrocellulose membranes. After transfer, the molecular weight standards were cut from the nitrocellulose membrane and stained with Coomassie brilliant blue. The nitrocellulose membranes were washed, blocked for 40 minutes with 20% fetal bovine serum (FBS), and incubated overnight at room temperature with either (1) 1:20 vol/vol dilution of each of the scorpion-allergic patients' serum samples in 10% FBS or (2) 1:20 vol/vol pooled human cord serum samples used as a negative control. The membranes were then washed and incubated for 6 hours at room temperature using an IgG mouse anti-human IgE monoclonal antibody (Sigma-Aldrich Corp, St Louis, MO) diluted 1:5,000 vol/vol in 10% FBS, followed by washing and incubation overnight in alkaline phosphatase–conjugated goat anti-mouse IgG (Chemicon International, Temecula, CA) diluted 1:2,000 vol/vol in 10% FBS. After a final washing, the alkaline phosphatase was developed using reagents (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's protocol. The molecular weight of visualized proteins was

determined using a standard curve constructed from the controls with known molecular weights.

Venom Characterization

Venom from *C vittatus* was isolated in the manner described previously herein, and enzymatic activity was determined in the following manner. Phospholipase activity was determined according to the method of Habermann and Hardt¹² using egg yolk agarose suspension plates with radial diffusion of the venom into the substrate. Hyaluronidase activity was determined using a radial diffusion assay with bovine vitreous humor hyaluronic acid (Worthington Biochemical Corp, Lakewood, NJ) as substrate.¹³ Other enzymatic activities were determined using Api-Zym strips (bioMerieux, Hazelwood, MO), which detect up to 19 different enzymatic activities, including a variety of proteases and peptidases.¹⁴

RESULTS

The protein concentration of the whole venom, determined by using the method of Lowry et al,¹⁵ was found to be between 100 and 150 $\mu\text{g}/\mu\text{L}$. A total of 11 patients with histories consistent with IgE-mediated symptoms to a scorpion sting were enrolled. Five of these patients were skin tested with the venom extract, with 4 of the 5 showing positive skin test results (Table 1). Ten control subjects who had never been stung by *C vittatus* were skin tested with the venom extract. All the control subjects had negative skin test results using 1:10,000 wt/vol. An irritant effect was observed at 1:1,000 wt/vol in all control subjects. No late-phase cutaneous reactions were observed in patients or control subjects. None of the patients experienced systemic symptoms suggestive of hypersensitivity or neurotoxin effects as a result of skin testing. Serum samples were obtained for IgE immunoblots from 2 of the 5 patients undergoing skin testing and from an additional 6 patients who did not undergo skin testing. These 6 additional patients had their serum samples collected before initiation of this study, at the time of initial presentation for

evaluation of "scorpion allergy," and were not able to return to the clinic for skin testing.

Venom SDS-PAGE identified proteins of various sizes, 9 of which were shown to bind IgE on the immunoblots (Fig 4). All 8 patients whose serum samples were tested showed IgE to venom components on the immunoblots. The allergens identified were found to be of the apparent molecular weights 30, 36, 52, 58, 102, 127, 146, 153, and 170 kD.

Enzyme analysis of whole venom revealed alkaline phosphatase, esterase, esterase lipase, and acid phosphatase activities detected using the Api-Zym assays. In addition, phospholipase A activity was found via radial diffusion assay. No hyaluronidase activity was detected in the venom.

DISCUSSION

Systemic reactions to the stings of *C vittatus*, like those to Hymenoptera stings,² are probably underreported. This may in part be because patients and physicians are unaware of the potential for anaphylaxis as a result of scorpion envenomation. In addition, anaphylaxis may be confused with neurotoxic symptoms, a well-known manifestation of scorpion venom.^{4,16}

All patients participating in this study had demonstrable venom specific IgE—in vivo, in vitro, or both. Only 1 patient had a negative skin test reaction using the venom extract. However, in this particular patient, skin testing was performed 14 years after the last sting. Her serum had been obtained and stored 9 years before initiation of this study (5 years after her last sting), at the time of her initial visit to our clinic, likely explaining why her immunoblot reaction was positive.

None of the patients who underwent skin testing experienced late-phase reactions after skin testing. This was also true at 1:1,000 wt/vol, which was found to be the concentration resulting in an irritant effect. Late-phase reactions were a limitation in the past to developing a successful skin testing extract with the venom of *C vittatus*.¹⁰ We attribute the lack

Table 1. Patient Demographics and Skin Test Results

Patient No.	Age, y/sex	Reaction	Years since last sting	Skin test result (concentration, wt/vol)	Immunoblot result
1	44/M	A	5	ND	Positive
2	35/F	LH, S, U	1.5	ND	Positive
3	38/F	C, LH NV, W	1	Positive (1:100,000)	Positive
4	53/F	C, W	7	ND	Positive
5	66/F	ABD, I, LH, SOB	14	Negative	Positive*
6	51/F	LLR	3	ND	Positive
7	57/M	LLR	2	ND	Positive
8	46/F	LLR	2	ND	Positive
9	49/M	F, I, LH	1	Positive (1:100,000)	ND
10	41/F	U	2.5	Positive (1:10,000)	ND
11	48/M	LLR	1	Positive (1:10,000)	ND

Abbreviations: A, angioedema; ABD, abdominal cramping; C, chest tightness; F, flushing; I, impending doom; LH, lightheadedness; LLR, large local reaction; ND, not done; NV, nausea/vomiting; S, syncope; SOB, shortness of breath; U, urticaria; W, wheezing.

*Serum sample was obtained 9 years before skin testing.

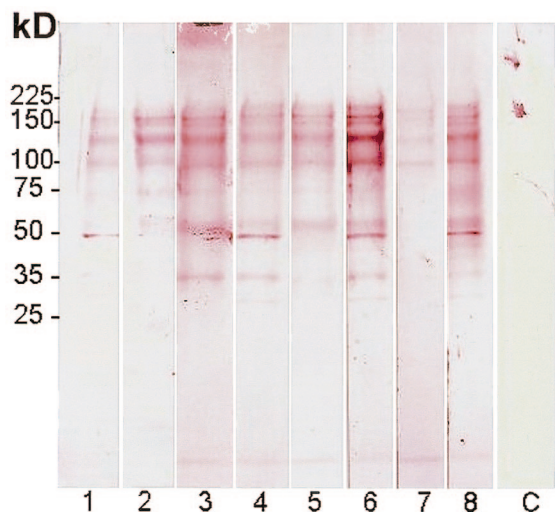


Figure 4. Immunoglobulin E immunoblot against *Centruroides vittatus* venom for patients 1 through 8 showing binding to multiple allergens. The cord blood lane (C) shows no IgE binding.

of late-phase reactions in our study to the removal of LMW proteins before skin testing via dialysis. Scorpion neurotoxins have previously been characterized as small proteins, typically less than 10,000 Da.⁸ Despite being small, these neurotoxins have been found to stimulate IgE production in rabbits and mice.¹⁷

The serum samples from each patient on which immunoblots were performed demonstrated specific IgE to multiple venom proteins. The percentages of patients with specific IgE to the various proteins were as follows: 38% showed IgE to the 30-kD protein, 62% to the 36-kD protein, 62% to the 52-kD protein, 50% to the 58-kD protein, and 100% to the 102-, 127-, 146-, 153-, and 170-kD proteins. Not all these proteins are likely to be distinct allergens; many of the larger proteins may be multimers of the smaller allergens. Because the immunoblots did not reveal specific IgE against proteins measuring less than 12,000 Da, it does not seem that LMW neurotoxins are significant *C vittatus* allergens in humans. Similarly, removal of LMW neurotoxins did not seem to affect the diagnostic value of the skin testing extract.

One patient (patient 9) has experienced numerous additional stings since the conclusion of this study. Many episodes have resulted in systemic reactions, some requiring self-administration of epinephrine. Symptoms with subsequent reactions have been similar to past reactions and include flushing, lightheadedness, and a sense of impending doom. In his job as a rancher in southern Texas he is at high risk of being stung by *C vittatus*, especially because he chooses not to wear work gloves during his daily duties of clearing brush. At the time of this study, however, he refused an attempt at experimental specific immunotherapy using *C vittatus* venom.

We did not study patients stung by *C vittatus* who did not experience immediate hypersensitivity reactions. However, given the high allergenicity of scorpion venom in animals,¹⁷ we would expect a high percentage of atopic patients to make specific IgE to *C vittatus* venom. In addition, we did not find any obvious relationship between severity of reactions and intensity of immunoblot results or concentration of skin test reactivity. Studies in Hymenoptera-allergic patients show an imperfect correlation between venom IgE levels and the severity of clinical reactions.¹⁸

CONCLUSION

We demonstrated the presence of venom specific IgE in individuals who experienced hypersensitivity reactions after envenomation by *C vittatus*. We developed a successful skin testing extract using pure venom, with the removal of LMW neurotoxins via dialysis. Our findings may lead to the widespread ability to diagnose patients with hypersensitivity to scorpion venom and eventually to treat them using specific venom immunotherapy.

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