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# Cross-reactivity between allergens in the venom of the common striped scorpion and the imported fire ant

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**Background:** The common striped scorpion, *Centruroides vittatus*, and the imported fire ant (IFA) are endemic to the south-central United States. There is evidence of venom-specific IgE in patients experiencing hypersensitivity reactions to scorpion stings. The infrequency of repeated scorpion stings and the presence of immediate reactions to an initial sting suggest prior sensitization.

**Objective:** In the present study we evaluated the cross-reactivity of *C vittatus* venom with IFA whole-body extract (WBE).

**Methods:** Sera were obtained from patients with symptoms of immediate hypersensitivity to *C vittatus* stings and from scorpion sting-naïve patients allergic to IFA venom. Inhibition IgE immunoblots were performed by using scorpion venom and IFA WBE. Skin testing with scorpion venom was performed on scorpion sting-naïve patients allergic to IFA venom.

**Results:** Sera from patients with scorpion venom allergy demonstrated IgE binding to multiple allergens of similar sizes against both scorpion venom and IFA WBE. This binding was completely inhibited by preincubation of the sera with scorpion venom and IFA WBE. Pooled sera from patients with IFA venom allergy demonstrated similar bands on IgE immunoblotting against both IFA WBE and scorpion venom, with the latter being completely inhibited by preincubation of the sera with IFA WBE. Skin testing with scorpion venom was positive in 6 of 9 patients with IFA venom allergy.

**Conclusion:** Significant cross-reactivity exists between the venom of *C vittatus* and IFA WBE. The high sensitization rate to IFA venom in endemic areas may therefore be a risk factor for subsequent immediate reactions to an initial scorpion sting. Patients with immediate hypersensitivity reactions to scorpion stings may potentially benefit from immunotherapy with IFA WBE. (J Allergy Clin Immunol 2004;114:383-6.)

**Key words:** *Centruroides vittatus*, scorpion, venom, cross-reactivity, imported fire ant

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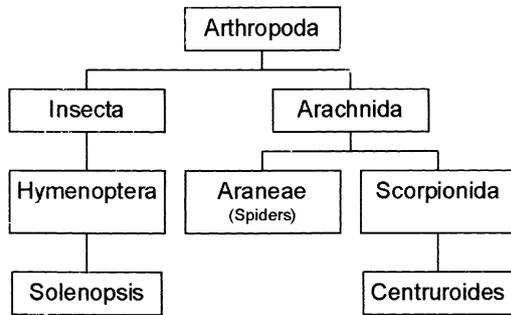
## Abbreviations used

IFA: Imported fire ant

WBE: Whole body extract

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.<sup>1</sup> Stings may elicit hypersensitivity reactions in some individuals,<sup>2,3</sup> and there is evidence of venom-specific IgE through skin testing.<sup>3</sup> Some of these patients experienced large local and even systemic reactions with their initial scorpion sting,<sup>3</sup> which suggests the presence of either cross-sensitization or cross-reactivity with a previously encountered similar antigen. Cross-reactivity has been well established for many Hymenoptera venoms, such as that in vespids, which show cross-reactivity among all major proteins.<sup>4</sup> Similarly, Sol i 1 from the imported fire ant (IFA) has been demonstrated to cross-react with both honeybee and vespid venoms,<sup>5</sup> and sera from patients sensitive to bee or vespid venoms who had no IFA exposure have demonstrated IgE binding to Sol i 1.<sup>5</sup>

Among the many potential sensitizing exposures that may be cross-reactive with scorpion venom, IFA stings have many features that make them an ideal candidate, even though the species are phylogenetically distinct (Fig 1). First, the endemic areas in which *C vittatus* and IFAs are found have a high degree of overlap (Fig 2).<sup>1,6,7</sup> Second, stings from IFAs are exceedingly common; up to 60% of the population in an endemic area is stung per year.<sup>7</sup> Third, IFA stings commonly elicit both sensitization and IgE-mediated clinical reactions, with 17% to 56% of patients stung experiencing large local reactions and anaphylaxis occurring in 0.6% to 6%.<sup>5</sup> The prevalence of IFA-specific IgE is as high as 16% to 24% in a population in which the ants are endemic.<sup>5</sup> Finally, among 11 patients with scorpion venom allergy evaluated at our institution, 4 (36%) patients had a history of anaphylaxis (with positive skin test responses) to IFA venom, and at least 2 others had a history of large local reactions, suggesting that this association may be clinically relevant. The goal of this study was thus to evaluate cross-reactivity between allergens in *C vittatus* and IFA venom through inhibition IgE immunoblots and skin testing.



**FIG 1.** Phylogenetic relationship between the IFA (*Solenopsis* species) and the common striped scorpion (*Centruroides vittatus*).

## METHODS

### IgE immunoblots and inhibition immunoblots

The protocol for this study was approved by the Wilford Hall US Air Force Medical Center Investigational Review Board. Sera from 2 groups of patients were obtained and pooled for the present analysis: the first group included 4 patients identified as having both a history of systemic reactions to scorpion stings and evidence of serum-specific IgE to scorpion venom on immunoblotting, and the second group included 10 known scorpion venom-naïve patients with IFA venom allergy. Native *C vittatus* were captured in San Antonio, Texas, and identification was confirmed by an entomologist. Venom was obtained by milking the truncated stinger directly into capillary tubes, and protein concentration of the venom was determined by using the method of Lowry.<sup>8</sup> Immunoblots were accomplished as follows: SDS-PAGE with 10% to 20% gradient BMA-PAGEr gels (BioWhittaker, Rockland, Me) was performed in multiple lanes for 5 µg of undialyzed *C vittatus* milked venom, 50 µg of commercial IFA whole-body extract (IFA-WBE; Hollister-Stier, Spokane, Wash), and known molecular weight controls (BioWhittaker). Proteins were then transferred to 0.45-µm nitrocellulose membranes by using the standard sandwich technique at 36 V for 2 hours at 4°C. After transfer, the nitrocellulose was cut to isolate individual lanes, and the lane containing the molecular weight standards was stained with 0.025% Coomassie Blue R-250.

The remaining nitrocellulose lanes were washed with PBS containing 0.5% Tween 20, blocked for 40 minutes with 20% FBS, and incubated overnight at room temperature with one of the following pooled patient sera (diluted 1:20 vol/vol in 10% FBS): (1) sera from patients with scorpion venom allergy; (2) sera from patients with scorpion venom allergy preincubated for 12 hours at 4°C with 1 mg/mL milked scorpion venom; (3) sera from patients with scorpion venom allergy preincubated for 12 hours at 4°C with 1 mg/mL IFA WBE; (4) sera from patients with IFA venom allergy; (5) sera from patients with IFA venom allergy preincubated for 12 hours at 4°C with 1 mg/mL IFA WBE; or (6) pooled human cord sera (negative control). The membranes were then washed and incubated for 6 hours at room temperature with an IgG mouse anti-human IgE mAb (Sigma, St Louis, Mo) diluted 1:5000 vol/vol in 10% FBS, followed by washing and incubation overnight in alkaline phosphatase-conjugated goat anti-mouse IgG (Chemicon International, Temecula, Calif) diluted 1:2000 vol/vol in 10% FBS. After a final washing, the alkaline phosphatase was developed by using Bio Rad (Hercules, Calif) reagents according to the manufacturer's protocol. The molecular weight of visualized proteins was determined by using a standard curve constructed from the known molecular weight controls.

## Skin testing

Skin testing was performed by using dialyzed and filtered *C vittatus* venom in patients with a history of systemic or large local reactions to IFA stings who also had positive skin test responses to IFA WBE but who had never been stung by a scorpion. Venom was dialyzed into distilled water by using 12,000-d dialysis tubing to remove low-molecular-weight neurotoxins in an effort to decrease irritant and late-phase reactions to skin testing<sup>3</sup> and filtered through a 0.22-µm syringe filter for sterilization before use. Patients were excluded if they were pregnant, had any serious concurrent disease process (eg, chronic cardiopulmonary disease), or were actively taking a β-blocker or medications known to interfere with skin testing (ie, antihistamines). A modified prick-and-wipe method was used to perform epicutaneous testing with 1:1000 wt/vol strength venom. If the result was negative, this was followed by intradermal testing, starting with 0.02 mL of a 1:1,000,000 wt/vol concentration and advancing with log-fold increases in concentration until either (1) a positive result was obtained or (2) a final concentration of 1:10,000 wt/vol was reached with a negative result. Positive and negative skin test controls were used with 1 mg/mL histamine for epicutaneous testing and 0.01 mg/mL for intradermal testing and sterile diluent, respectively. All skin test results were read at 15 minutes after placement. A positive result was defined for epicutaneous testing as a wheal size 3 mm in diameter or greater with surrounding erythema, and for intradermal testing, a positive result was defined as a wheal size of 5 mm or greater in diameter with surrounding erythema. Ten control patients were skin tested with the dialyzed and filtered venom, with negative results at 1:10,000 wt/vol administered intradermally.

## RESULTS

The results of the IgE immunoblots are shown in Fig 3, with nitrocellulose lanes 1 to 6 each containing electrophoresed scorpion venom, and lanes 7 to 9 each containing IFA WBE. Lane 1, which was incubated with sera from patients with scorpion venom allergy, demonstrated allergens of apparent molecular weights of 36, 52, 58, 102, 127, 153, and 170 kd. This binding was completely inhibited by preincubation of the sera of patients with scorpion venom allergy with either scorpion venom (lane 2) or IFA WBE (lane 3). Lane 4, which was incubated with sera from patients with IFA venom allergy with no known history of scorpion sting demonstrated allergens to scorpion venom of apparent molecular weights of 58, 75, 102, 127, 170, and 188 kd, which were completely inhibited by preincubation of the sera with IFA WBE (lane 5). Lane 7, in which sera from patients with IFA venom allergy were incubated with electrophoresed IFA WBE, demonstrated IgE binding to bands of apparent molecular weights of 25, 52, 58, 75, 89, 102, 127, 146, and 188 kd. Incubation of sera from patients with scorpion venom allergy against the same IFA WBE demonstrated many bands of similar size, including 25, 75, 89, 102, and 127 kd.

Table I shows the results of skin testing with scorpion venom in scorpion venom-naïve patients with IFA venom allergy. All patients had positive skin test responses to IFA WBE, and 6 (67%) of 9 had a positive skin test response to scorpion venom at concentrations at or less than 1:10,000 wt/vol administered intradermally.

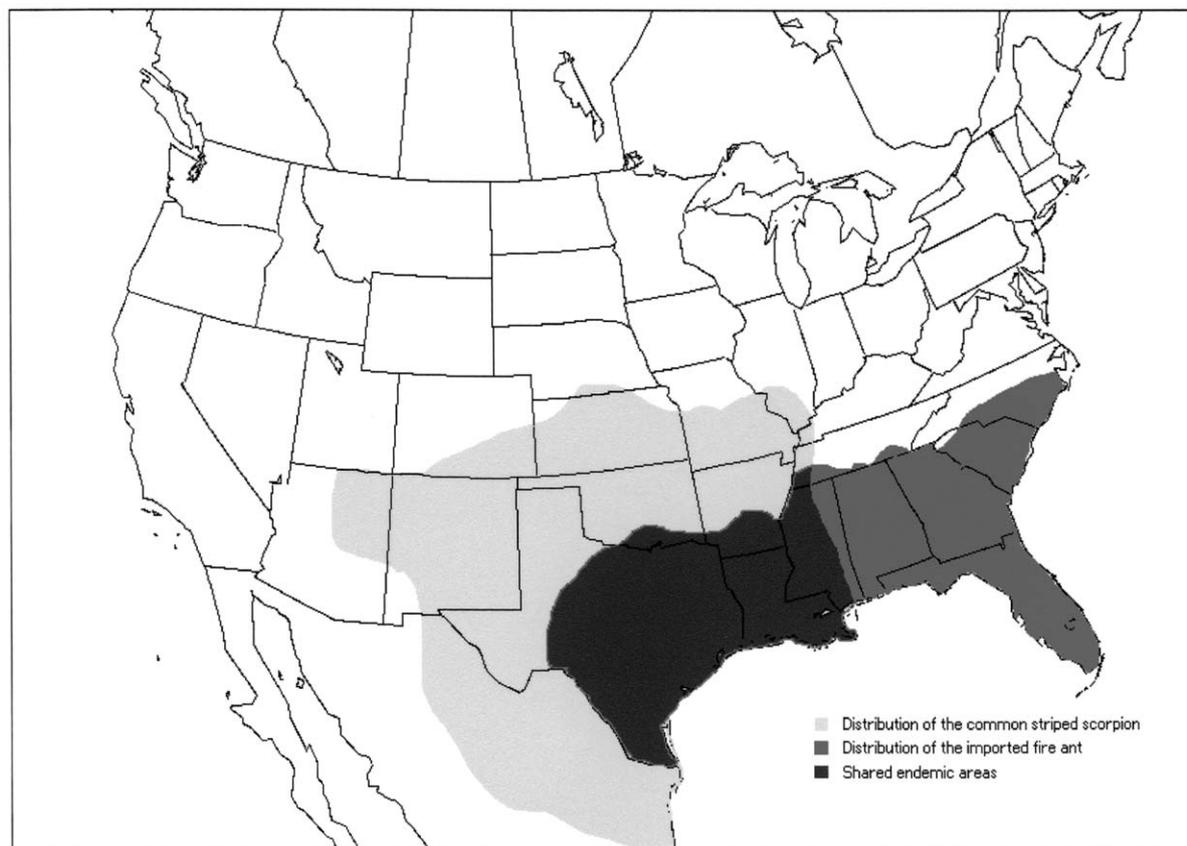


FIG 2. Approximate distributions of the common striped scorpion and the IFA in the United States.<sup>1,6,7</sup>

## DISCUSSION

Some individuals who have hypersensitivity reactions to scorpion stings appear to do so on their first sting,<sup>3</sup> suggesting existing IgE sensitization to allergens in scorpion venom. Data from this study suggest that venom from previous IFA stings may be the sensitizing exposure. Pooled sera from patients with IFA venom allergy demonstrated serum-specific IgE to multiple allergens in scorpion venom on immunoblots (Fig 3, lane 4), even though these individuals had undergone no known prior scorpion stings. Preincubation of their sera with IFA WBE before immunoblotting completely inhibited this binding, further supporting the notion that these epitopes were cross-reactive and related to IFA venom. We also found that 6 (67%) of 9 individual scorpion sting-naïve patients with IFA venom allergy had positive skin test responses for scorpion venom at intradermal concentrations at or less than 1:10,000 wt/vol. Thus even though these patients had never been stung by a scorpion, many patients with IFA venom allergy demonstrated serum-specific IgE to scorpion venom *in vitro* and *in vivo* and may be at risk for immediate hypersensitivity reactions to scorpion stings. This is also supported clinically by the fact that 4 of 11 patients with scorpion venom allergy had a history of prior systemic reactions to IFA stings, which is nearly 20-fold higher than would be expected for the general population

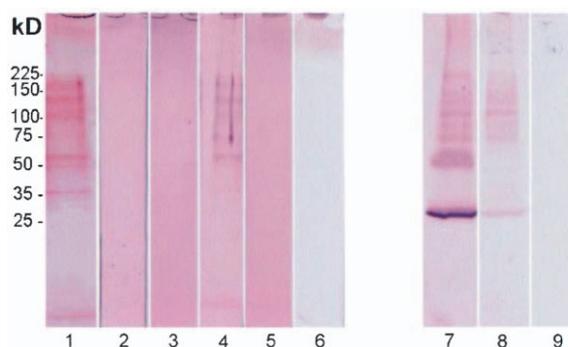


FIG 3. IgE immunoblots. Lanes 1 to 6 contain electrophoresed scorpion venom, 5 µg of protein per lane, incubated with the following: lane 1, sera from patients with scorpion venom allergy; lane 2, sera from patients with scorpion venom allergy preincubated with scorpion venom; lane 3, sera from patients with scorpion venom allergy preincubated with IFA WBE; lane 4, sera from patients with IFA venom allergy; lane 5, sera from patients with IFA venom allergy preincubated with IFA WBE; lane 6, human cord sera. Lanes 7 to 9 contain electrophoresed IFA WBE, 50 µg of protein per lane, incubated with the following: lane 7, sera from patients with IFA venom allergy; lane 8, sera from patients with scorpion venom allergy; lane 9, human cord sera.

in a fire ant-endemic area (36% vs 2%). The fact that IFA stings may cross-sensitize patients to scorpion venom significantly increases the number of patients at risk for immediate hypersensitivity reactions to scorpion stings

**TABLE I.** Results of scorpion venom skin testing in patients with IFA venom allergy who have never been stung by a scorpion

Patient age/sex	Reaction to IFA	Time since last IFA reaction	Time since last IFA sting	Scorpion ST, wt/vol dilution	IFA ST, wt/vol dilution
30/M	Systemic	6 mo	6 mo	+, 1:100,000 ID	+, 1:1,000,000 ID
48/M	Systemic	12 y	6 mo	-, 1:10,000 ID	+, 1:100,000 ID
34/M	LLR	6 mo	6 mo	+, 1:1,000,000 ID	+, 1:1000 prick
37/M	LLR	4 mo	4 mo	-, 1:10,000 ID	+, 1:1,000,000 ID
40/M	LLR	5 mo	5 mo	+, 1:1,000,000 ID	+, 1:1000 ID
44/M	Systemic	1 mo	1 mo	+, 1:100,000 ID	+, 1:1000 prick
21/F	Systemic	2 mo	2 mo	-, 1:10,000 ID	+, 1:10,000 ID
58/F	Systemic	2 y	<6 mo	+, 1:10,000 ID	+, 1:100,000 ID
59/M	Systemic	4 y	5 mo	+, 1:10,000 ID	+, 1:1000 prick

ST, Skin test; ID, intradermal; LLR, large local reaction.

because the prevalence of sensitization from IFA stings in an endemic area can be up to 24% of the general population,<sup>5</sup> and there is a broad degree of shared endemic distribution between *Centruroides* and *Solenopsis* species.

There is also evidence that most of the allergens identified in patients with a history of hypersensitivity reactions and specific IgE to *Centruroides* species venom are cross-reactive with IFA venom. All apparent IgE bands in the sera from patients with scorpion venom allergy against scorpion venom were completely inhibited by preincubation of the sera with IFA WBE.

Future studies may involve further investigation of the immunologic basis for the cross-reactivity between scorpion venom and IFA WBE and the potential treatment options for patients with immediate hypersensitivity reactions to scorpion stings. In these patients no definitive treatment is currently available, although on the basis of extensive cross-reactivity, immunotherapy with commercially available IFA WBE may be beneficial.

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