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Studies of kissing bug-sensitive patients: Evidence for the lack of cross-reactivity between *Triatoma protracta* and *Triatoma rubida* salivary gland extracts

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In the southern and western sections of the United States, bites from the reduviid bug, commonly known as the kissing bug, genus Triatoma, may induce serious life-threatening allergic reactions. This study was undertaken to identify the allergens responsible for patient sensitization and to determine the extent of cross-reactivity of these allergens. The Triatoma spp. most commonly encountered in California and Arizona, T. protracta and T. rubida, were obtained, maintained in the laboratory, and dissected to prepare extracts for testing. Extracts were prepared from T. protracta and T. rubida for study by RAST, lymphocyte transformation, leukocyte histamine release, and RAST inhibition. Sera and cells were collected from patients who had generalized reactions to Triatoma bites. Our results indicate that T. protracta and T. rubida antigens to which patients are sensitized are present in extracts that contain saliva and that human responses are specific for T. protracta or T. rubida, i.e., allergic cross-reactivity could not be demonstrated. (J ALLERGY CLIN IMMUNOL 77:364-70, 1986.)

Anaphylactic reactions in man may result from stings from Hymenoptera, particularly bees, wasps,

yellow jackets, hornets, and ants. Life-threatening and fatal reactions may also follow repeated bites from insects in the order Hemiptera, particularly from bites of the reduviid bug, *Triatoma*, commonly called kissing bug.¹⁻³ The range of *Triatoma* includes a major portion of the southern United States and Latin America⁴ as illustrated in Fig. 1. There are at least 15 species of *Triatoma* in the United States. The two most commonly encountered species in California, Texas, and Arizona are *T. protracta* and *T. rubida*. Adult *Triatoma* are 15 to 30 mm in length and brown to black in color.

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Abbreviations used

WBEs:	Whole body extracts
HEs:	Head extracts
TSGEs:	Thorax with salivary glands
TOGs:	Thorax without salivary glands
AEs:	Abdomen extracts
LHR:	Leukocyte histamine release
LT:	Lymphocyte transformation
PHA:	Phytohemagglutinin
SGEs:	Salivary gland extracts

The exact incidence of systemic allergic manifestations after the bite of several species of the blood-feeding *Triatoma* insects is unknown, even though serious reactions such as anaphylaxis and delayed hypersensitivity reactions have been recognized for more than 80 years.⁵ Bites from *Triatoma* usually take place at night with the insect standing beside the host, inflicting multiple bites, up to 15 at one feeding, with engorgement occurring over a 3- to 30-minute period. Characteristic features of these bites include their usually painless nature and clustering on an exposed extremity, trunk, or face, thereby accounting for the common name, kissing bug. Walsh and Jones¹ and Mortenson and Walsh⁶ analyzed 110 case histories and noted two distinct types of hypersensitivity reactions: (1) a localized reaction at the site of the bite accompanied by an intense itching and the formation of a large wheal that may persist for a week or more and (2) a systemic reaction manifested by nausea, diarrhea, urticaria, angioedema, dyspnea, or syncope.

Preliminary work of Pinnas et al.,⁷ Lindberg et al.,^{8,9} and Marshall and Street¹⁰ presented evidence that *Triatoma* salivary glands contain the antigens responsible for sensitization. Saliva is produced in salivary glands that are located in the thorax and travels through the salivary ducts to the proboscis through which it is injected into the bite victim. Lindberg et al.⁹ also presented evidence for species specificity of the responses to *T. protracta* and *T. rubida*. The data presented here confirm and extend these observations. Sera from patients with local or systemic reactions to *T. protracta* and *T. rubida* were evaluated by RAST and RAST-inhibition assays. *T. rubida* and *T. protracta* specimens collected in the field and raised in the laboratory were used to prepare extracts. Results confirmed that the antigens responsible for allergic reactions are found in saliva and that sensitization is species specific since no antigenic cross-reactivity between saliva preparations from different species was observed.

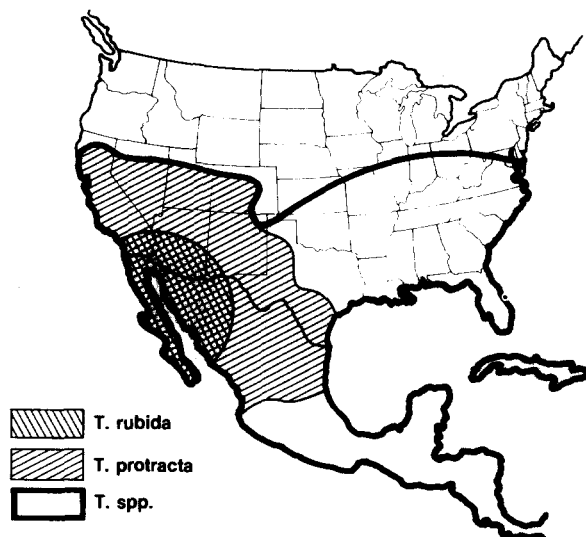


FIG. 1. Range of *Triatoma* spp. in the United States and Mexico (heavy line). Distribution of *T. protracta* and *T. rubida*.

MATERIAL AND METHODS

Collection, identification, and culturing of *Triatoma*

Triatoma were collected locally in Tucson, Arizona. *Triatoma* were removed from nests of *Neotoma* spp. (wood rats) and from homes of patients who had been bitten. *Triatoma* were identified according to the classification of Lent and Wygodzinsky¹¹ and were cultured with techniques similar to those outlined by Ryckman and Ryckman.¹² *Triatoma* were reared in plastic boxes covered with nylon mesh. The bugs were allowed to feed through screening material on mice placed in a holding device that allowed the bugs to be brought into contact with their abdomens. This prevented the escape of the bugs during the feeding operation and the feces of the bugs, which may be contaminated by *Trypanosoma cruzi*, from coming in contact with either the host animal or the screening material.

Antigen preparation

Live *T. rubida* (1:10 w/v) for WBEs were homogenized by means of a Polytron 10 ST (Brinkmann Instruments Inc., Westbury, N. Y.) for 30 seconds in an ice bath as described previously.¹³ Homogenates were passed through 0.22 μ m micropore filters before storage at 4° C in sterile vials.

In addition to WBEs, *T. rubida* were dissected to prepare HEs, TSGEs, TOGs, and AEs. Homogenization was performed as for WBEs.

SGEs were prepared from *T. protracta* (kindly supplied by Neil Marshall at University of California at Los Angeles, Los Angeles, Calif.) and *T. rubida*. Live adult *Triatoma*, immobilized in wax, were dissected, and their salivary glands were removed under a stereomicroscope and transferred into distilled water, 10 glands per milliliter, in a Petri dish. The salivary sacs were severed from the salivary ducts,

TABLE I. Summarized clinical data from *Triatoma*-sensitive patients

Patient	Age (yr)	Sex	Location	Reaction	RAST SGEs	
					<i>T. protracta</i>	<i>T. rubida</i>
P. S.	41	F	A	Generalized urticaria and pruritus, vomiting, nausea	----	4+
E. M.	16	F	A	Generalized urticaria, hypotension	—	3+
C. M.	40	F	A	Generalized urticaria, laryngeal edema, nausea, vomiting	—	2+
D. P.	42	M	A	Large local urticarial lesions	—	2+
P. L.	40	M	A	Large local urticarial lesions	—	±
M. G.	38	M	A	Dyspnea, nausea, urticaria, syncope, hypotension	—	2+
B. G.	46	F	A	Generalized urticaria, nausea, uterine bleeding	—	4+
S. H.	32	F	A	Diarrhea, syncope	ND	1+
M. F.	55	F	A	Generalized urticaria, pruritus	ND	1+
G. C.	50	M	A	Generalized urticaria, syncope	ND	1+
V. H.	63	F	A	Generalized urticaria, laryngeal edema, uterine bleeding, vomiting, diarrhea	—	1+
F. H.	30	M	A	Urticaria, periorbital edema	—	2+
O. M.	39	M	A	Generalized angioedema, syncope, nausea	—	4+
V. E.	36	F	A	Convulsions, urticaria, anaphylaxis	—	4+
H. E.	61	F	A	Laryngeal edema, urticaria, nausea	—	3+
J. R.	40	F	A	Generalized pruritus, angioedema, nausea, vomiting, headache	—	1+
B. S.	55	M	A	Syncope, pruritus, dyspnea, urticaria	—	3+
P. B.	35	F	A	Generalized pruritus, hypotension, abdominal cramps, dizziness	—	3+
H. T.	78	M	A	Generalized urticaria	—	2+

ND = not done; NA = not available; A = Arizona; C = California; T = Texas.

rinsed twice with distilled water, and gently pulled apart while they were in distilled water. The debris was removed by centrifugation, and the extract was transferred to pre-weighed micropolypropylene tubes and dried over desiccant at room temperature. The dried saliva was stored at -20°C and reconstituted in distilled water for use.

RAST assay

Cyanogen bromide-activated paper discs were incubated with various *Triatoma* extracts (WBEs, HEs, TSGEs, TOGs, AEs, and SGEs). Antigen-coupled discs were stored in incubation buffer at 4°C until they were used. For RAST determinations individual *Triatoma* extract discs were placed in test tubes and incubated with $50\ \mu\text{l}$ of patient sera at room temperature for 3 hours. Discs were washed three times at 10-minute intervals with 0.9% saline; then $50\ \mu\text{l}$ of ^{125}I -labeled anti-IgE (Pharmacia Diagnostics, Piscataway, N. J.) was placed on each disc. Tubes were capped and incubated overnight at room temperature. The liquid was

removed, and the discs were washed as before with saline. The bound radioactivity was measured in a Packard (Packard Instrument Co., Inc., Downers Grove, Ill.) gamma counter for 1 minute.

RAST-inhibition assay

With the RAST procedure described above, $200\ \mu\text{l}$ of sera from individual patients as well as serum pools from patients with 3 to 4+ *T. protracta* or *T. rubida* SGE RAST values were incubated overnight at 2°C with $100\ \mu\text{l}$ of RAST incubation buffer containing varying amounts of SGEs of suspected inhibitors, *T. protracta* or *T. rubida*. Aliquots of these mixtures ($50\ \mu\text{l}$) were added to RAST discs and assayed according to the Phadebas procedure. Serum diluted with buffer only was used as control, and determinations were carried out in duplicate. *T. rubida* pool was prepared by mixing equal volumes of serum from five highly allergic *T. rubida* subjects (patients 1, 2, 7, 13, and 14). Sera from patients 28, 33, and 34 made up the *T.*

TABLE I. Cont'd

Patient	Age (yr)	Sex	Location	Reaction	RAST SGEs	
					<i>T. protracta</i>	<i>T. rubida</i>
D. D.	32	M	A	Laryngeal edema	—	3+
C. V.	20	M	A	Generalized angioedema	—	4+
R. C.	40	M	A	Hypotension, laryngeal edema, syncope	—	2+
D. F.	26	F	C	Generalized pruritus, urticaria, nausea	2+	—
E. B.	71	M	C	Urticaria, syncope	1+	—
E. L.	36	F	C	Generalized erythema, pruritus	2+	—
M. M.	61	M	C	Generalized pruritus, urticaria, laryngeal edema, nausea	2+	—
D. V.	20	F	C	Angioedema, generalized erythema	—	—
D. H.	47	F	C	Generalized erythema, urticaria	3+	—
C. L.	49	M	C	Generalized pruritus, laryngeal edema, labored breathing	4+	1+
T. J.	12	M	C	Generalized urticaria, erythema, angioedema	3+	+
C. M.	42	M	C	Generalized urticaria, angioedema, pruritus	3+	—
F. S.	36	F	C	Generalized urticaria	3+	—
H. O.	65	F	C	Generalized urticaria	3+	—
P. P.	22	F	C	Generalized urticaria	3+	—
I. B.	40	M	C	Bronchospasm, hypotension, generalized urticaria	2+	—
L. W.	13	F	C	Generalized urticaria, syncope	3+	—
L. B.	NA	F	C	Generalized pruritus, edema, urticaria, nausea, vomiting, uterine bleeding, headache	2+	—
R. S.	46	M	T	Syncope, hypotension, laryngeal edema	2+	—

protracta pool. None of the patients had ever received specific immunotherapy. For inhibition studies SGEs from *T. rubida* and *T. protracta* were coupled to solid-phase discs at a concentration of 5 µg per disc. *T. protracta* and *T. rubida* SGEs were included as fluid-phase inhibitors at two or more high concentrations with all sera tested and with every solid-phase allergen.

Histamine-release studies

Protein content of the extracts was determined by the method of Lowry et al.¹⁴ Human peripheral blood leukocytes from six patients with a history of systemic reactions to *T. rubida* were isolated and incubated with *T. rubida* extract preparations. Leukocytes from four patients (patients 3, 7, 8, and 9) were studied for LHR with HEs, TSGEs, and AEs as antigens. Patients 1 and 5 were studied for LHR with the use of SGEs, TSGEs, and TOEs. Antigen-induced LHR assay was performed as described previously.¹³

Lymphocyte transformation

T. rubida and *T. protracta* SGEs were used in LT studies performed on three occasions in triplicate with lymphocytes

from a *T. rubida*-sensitive patient as well as control lymphocytes. Heparinized (preservative-free heparin) peripheral blood was layered over Ficoll-Hypaque gradient (density 1.075) and spun at 200 × g for 20 minutes. Mononuclear cells, removed from the interface, were washed three times with medium RPMI 1640 (GIBCO Laboratories, Grand Island, N. Y.), adjusted to 1 × 10⁶ ml in RPMI 1640 with 20% fetal calf serum, and 0.1 ml of the cell suspension plus 20 µl of SGE were added to each well of a microtiter plate. Five days later, 0.05 ml of ³H-thymidine (specific activity 20 Ci/mmol) in RPMI 1640 was added, yielding a final concentration of 1 µCi/ml. Cells were harvested 8 hours later with a MASH II harvester (M. A. Bioproducts, Walkersville, Md.), and the glass fiber filter discs were suspended in 6 ml of Omnifluor (New England Nuclear, Boston, Mass.) in scintillation vials and counted.

Patients

Thirty-eight patients from Arizona, California, or Texas who manifested reactions after bites of bugs that were identified as *Triatoma* sp. were used for these studies. Clinical findings are summarized in Table I. The patients ranged in

TABLE II. RAST results as percent binding of ¹²⁵I-labeled IgE to various *Triatoma* extracts

Patient	Type reaction	<i>T. rubida</i>						<i>T. protracta</i>
		WBEs	HEs	TSGE	TOGs	AEs	SGE	SGE
		Percent binding*						Percent binding
P. S.	SR	3.6	1.6	18.6	2.2	5.2	18.3	1.9
E. M.	SR	1.6	2.5	12.2	1.7	1.7	12.4	1.5
C. M.	SR	1.5	1.6	6.9	1.6	1.7	7.2	1.5
M. G.	SR	1.5	1.6	8.0	1.5	1.6	7.5	1.4
R. S.	SR	1.4	1.9	1.5	1.4	1.6	1.6	7.1
D. P.	LR	1.6	2.4	7.3	1.5	1.8	8.8	1.5
P. L.	LR	3.1	1.7	3.2	2.7	3.2	2.4	1.9
J. Q.	NC	1.4	1.6	1.3	1.5	1.2	1.4	1.4
E. P.	NC	1.7	1.6	1.3	1.4	1.8	1.6	1.5

SR = systemic reaction; LR = local reaction; NC = normal control.

*Percent binding was determined by dividing counts bound by counts added times 100

TABLE III. Response of lymphocytes from *T. rubida*-sensitive and control subjects to SGEs from *T. rubida* and *T. protracta*

Antigen	Control (cpm)*	<i>T. r. sensitive</i> (cpm)*
None	257	376
Phytohemagglutinin	41,336	32,107
<i>T. r.</i> SGE (100 µg/ml)	276	27,107
<i>T. r.</i> SGE (50 µg/ml)	223	10,721
<i>T. r.</i> SGE (10 µg/ml)	196	1,792
<i>T. p.</i> SGE (100 µg/ml)	246	330

T. r. = *T. rubida*; *T. p.* = *T. protracta*.

*Counts per minute; mean values of triplicate cultures.

age from 12 years to 78 years and included 18 male and 20 female patients. Except for two patients with localized reactions, all patients had generalized reactions with a range of signs and symptoms listed from most to least prevalent as follows: urticaria, syncope, pruritus, laryngeal edema, angioedema, erythema, uterine bleeding, dyspnea, hypotension, convulsions, and diarrhea.

RESULTS

Most of several hundred *Triatoma* collected locally in the Tucson area were identified as *T. rubida*. Other species included six *T. recurva* and one *T. protracta*. Commercial whole body extracts that are no longer available were not of diagnostic value in RAST or LHR assays. These extracts produced positive skin tests in control subjects and inordinately high back-

grounds in LHR. A variety of extracts prepared in our laboratory from *T. rubida* and *T. protracta* were used for all subsequent studies. Sera from patients with systemic allergic reactions after bites by *Triatoma* sp. were collected, and specific IgE antibodies to the *Triatoma* sp. were measured by RAST and LHR assays. Clinical data and RAST results for *T. rubida* and *T. protracta* reactivity are summarized in Table I. RAST values as reported in Table I correspond to standard Pharmacia classes 1 to 4 (presented as 1 to 4+) and were determined at different times throughout the study. Table II summarizes the RAST data, obtained in a study of reactivity to WBEs and partial body extracts of serum samples obtained from selected patients with suspected *Triatoma* sensitivity, and are presented as percent binding. Our results demonstrated that *Triatoma* extracts, TSGEs and SGEs, had the highest RAST activity. The HEs bound fewer counts, and AEs and WBEs had little or no activity. LHR results presented in Fig. 2 for patient B. G. are representative of the four patients studied with HEs, TSGEs, and AEs of *T. rubida* and demonstrate that the addition of TSGEs to peripheral blood leukocytes from sensitized individuals induced significant LHR, whereas HE induced less histamine release and AE induced little or no release. In other LHR studies, with SGEs and TOEs as antigens, only SGEs induced significant histamine release. Because the initial studies demonstrated that TSGEs were most active in both RAST and LHR, whereas TOGs were inactive, these results confirmed that the antigens responsible for the sensitivity reactions resided in the saliva.

We next sought to determine the extent of immunologic cross-reactivity between *T. protracta* and *T. rubida*. Patients who had allergic reactions to *T. ru-*

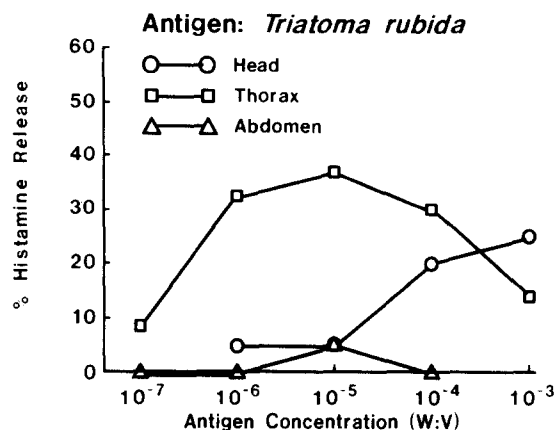


FIG. 2. *T. rubida* HEs (○), TSGEs (□), and AEs (△). Histamine-release dose-response curves induced by use of leukocytes from patient B. G. (Table I). Results are representative of all four *T. rubida*-sensitive patients studied.

bida bites had positive RAST results for *T. rubida* SGEs but negative results for *T. protracta* SGEs. Similarly, *T. protracta*-sensitive patients demonstrated positive RAST results when *T. protracta* SGEs were used but demonstrated negative results when *T. rubida* SGEs were used (Tables I and II). These results suggested that sensitization to *Triatoma* might be species specific, which was subsequently confirmed by RAST-inhibition assays as presented below.

SGE from *T. protracta* or *T. rubida* inhibited RAST of patients allergic to *T. protracta* or *T. rubida*, respectively, but not the other, thereby reaffirming species specificity (Fig. 3). Aqueous *T. protracta* SGE, 150 ng of protein, produced 50% inhibition with a *T. protracta* pool, whereas serum from *T. protracta*-sensitive patients 31, 32, and 34 required 35, 75, and 65 ng, respectively, for 50% inhibition. No inhibition was observed by the addition of up to 40 µg of aqueous *T. rubida* SGE. Aqueous *T. rubida* SGE produced 50% inhibition with 200 ng of allergen and *T. rubida* pool, whereas individual serum samples from *T. rubida*-sensitive patients 17 and 21 were inhibited by 90 ng and 130 ng of *T. rubida* SGE, respectively. No inhibition was observed with up to 50 µg of aqueous *T. protracta* SGE. In the absence of inhibitor, the serum pool *T. protracta* produced 27.4% total counts bound, and normal serum produced 0.6% total binding. Patients 31, 32, and 34 produced 13.4%, 10.9%, and 12% total binding, respectively, with solid-phase *T. protracta* SGE. In the absence of inhibitor, the serum pool *T. rubida* produced 44.9% total counts bound, and the normal serum pool bound 0.9% with solid-phase *T. rubida*. Patients 17 and 21 produced 40.8% and 45.7% binding, whereas total binding of the *T. protracta* serum pool was 1.5%. In ad-

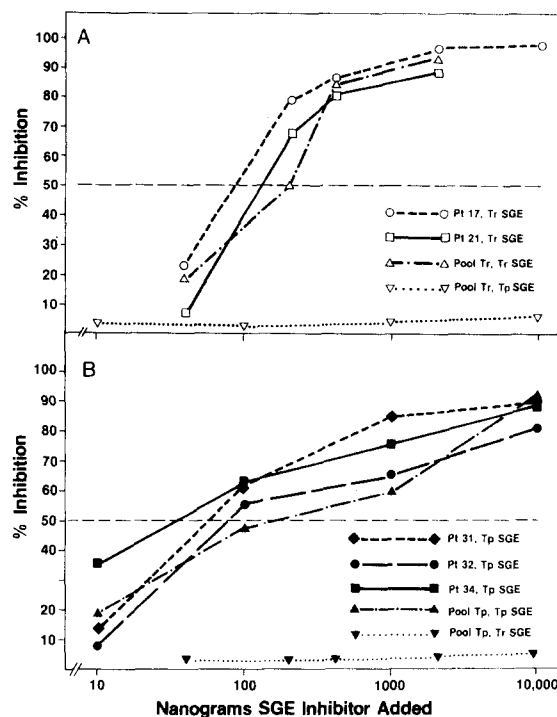


FIG. 3. A, RAST-inhibition curves in *T. rubida* SGE RAST. B, RAST-inhibition curves in *T. protracta* SGE RAST. Lack of cross-reactivity between *T. protracta* and *T. rubida* salivary gland extracts and the inhibition of individual *T. protracta* and *T. rubida* RASTs by corresponding SGEs is demonstrated. Percent RAST inhibition for each dilution of SGE was calculated as follows:

$$\frac{\text{Ave pos control} - \text{ave sample}}{\text{Ave pos control} - \text{ave neg sample}} \times 100\%$$

dition, SGEs were assayed for their ability to stimulate lymphocytes from allergic and control subjects as measured by ^3H -thymidine incorporation. Lymphocytes from a *T. rubida*-sensitive patient, as presented in Table III, incorporated ^3H -thymidine in the presence of *T. rubida* SGEs but not when *T. protracta* SGE were used. These results indicate that *T. rubida* and *T. protracta* SGE contain antigenically unique specificities, since significant inhibition occurred only with the homologous SGEs.

DISCUSSION

Kissing bugs may cause serious systemic reactions after bites that are often painless. These bugs are found across the entire southern United States, as well as in Central and South America. The species selected for examination were those most readily available in California and Arizona. Our results demonstrated that the allergens responsible for sensitization to *Triatoma* spp. are found in the saliva. Patients who had allergic reactions to *T. rubida* bites had positive RAST results for *T. rubida* SGEs but negative RAST results with

T. protracta SGEs. Similarly, results from LT studies demonstrated that lymphocytes from *T. rubida*-sensitive patients incorporated ^3H -thymidine in the presence of *T. rubida* SGEs but were not stimulated in the presence of *T. protracta* SGEs. RAST inhibition studies (Fig. 3) reaffirmed the species specificity of *T. protracta* and *T. rubida*. Previous RAST-inhibition studies with fractions of SGEs isolated by isoelectric focusing⁵ demonstrated the presence of several allergens in saliva and indicated that different antigens may be responsible for *Triatoma* sensitization in different individuals. Mathison et al.¹⁵ have studied patients with systemic reactions to *T. protracta* and confirmed our results that *Triatoma* SGEs induced positive RAST and LHR results. A recent study by Rohr et al.¹⁶ further confirmed that immunotherapy with *T. protracta* was protective against *T. protracta* bite challenges. Therefore, gland antigens may be responsible for sensitization to kissing bug bites in each susceptible individual, and immunotherapy with the involved species appears to be protective. The observed lack of cross-reactivity between species, as indicated by RAST, RAST inhibition, LHR, and LT studies in the present study, was unexpected, since studies in our laboratory of Hymenoptera-sensitive patients indicate substantial cross-reactivity, e.g., harvester ant venoms, *Pogonomyrmex*, demonstrated significant allergenic cross-reactivity among nine species tested.¹⁷ Furthermore, Mueller et al.¹⁸ compared the immunologic properties of venoms from four hornet species and found sufficient cross-reactivity among three of the four, suggesting that diagnosis and immunotherapy with commercially available *Vespa maculata* and *V. arenaria* should be adequate for most patients sensitive to *V. maculata*, *V. arenaria*, or *V. crabro* but probably not for those sensitive to *V. orientalis*.

Although all species of *Triatoma* have not yet been studied in this fashion, the apparent lack of cross-reactivity between *T. protracta* and *T. rubida* suggests that additional studies with each *Triatoma* species will be necessary to establish immunologic relationships. In view of the apparent lack of immunologic cross-reactivity of two species of *Triatoma* demonstrated in the present study, it appears that accurate entomological identification of the *Triatoma* species inducing the reaction in the patient, coupled with the preparation of SGEs from the offending species, may be essential for proper testing and treatment of *Triatoma*-sensitive individuals.

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