

Successful immunotherapy for *Triatoma protracta*-induced anaphylaxis

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A successful program of immunotherapy for Triatoma protracta-induced anaphylaxis was developed. This program included a new passive extract-antigen preparation standardized by RAST inhibition. This antigen facilitated the development of a reliable skin test protocol for in vivo diagnosis of Triatoma protracta allergy. Five patients with T. protracta-induced anaphylaxis underwent a rapidly increasing dosage schedule of immunotherapy. The IgE- and IgG-antibody responses during immunotherapy were followed with solid-phase RIA. Protection against anaphylaxis was confirmed in all patients with a "bite challenge" by T. protracta. This is the first report of completely successful T. protracta immunotherapy. (J ALLERGY CLIN IMMUNOL 73:369-375, 1984.)

Allergic reactions to the bite of insects of the genus *Triatoma* have been known since 1894.¹ There have been numerous individual case reports of generalized urticaria and/or anaphylaxis from the bite of various species of *Triatoma*.²⁻⁵ The true incidence of immediate hypersensitivity to *Triatoma* is unknown. A questionnaire survey by the California State Health Department found a 5% incidence of allergic reactions to *Triatoma* in Mariposa County. Some patients had as many as 15 systemic allergic reactions in one season.⁶ Most physicians are not aware of the problem because of the surreptitious habits of the bug; furthermore, the patients are generally thought to have had idiopathic reactions.²

There are 10 *Triatoma* species found in the United States. However, there are only six species that are likely to be encountered in the continental United States (Fig. 1). Allergic reactions to *T. protracta*, *T. gerstaeckeri*, *T. sanguisuga*, *T. rubida*, and *T. rubro-*

fasciata have been reported.⁷ Little is known about the cross-reactivity of allergic reactions to the various species of *Triatoma*. *Triatoma* bugs have many common names, such as the kissing bug or the cone-nosed bug. These names are very imprecise for identification and are best avoided as they generally lead to confusion with other harmless insects. *Triatoma* species found in the United States range in size from 12 mm to 30 mm as adults. *T. protracta* and *T. rubida* are typical examples (Fig. 2). All *Triatoma* bugs are exclusive blood feeders in both sexes and in all developmental stages. *T. protracta* live in the nest of its primary host, the wood rat (genus *Neotoma*). Each summer the adult bugs discur. It is during this time that most human bites occur. *T. protracta* generally flies when the air temperature is above 20° C (65° F) and usually does not fly more than 1 mile.⁸ The flights are primarily nocturnal, and the insects are attracted by light to houses. Most bites occur when the host is asleep.

In a nonallergic person, a *Triatoma* bite is painless. Even though it may take 10 to 25 min for a feeding, the host is usually not awakened. The sensitive person typically awakens from sleep with symptoms of impending anaphylaxis. However, the *Triatoma* may go unnoticed since the bite itself is painless, and the bite site may resemble other urticarial lesions. If patients are aware of the problem of *Triatoma* allergy, the insect may be found on a careful search of the bedding. Because of the stealth involved in the feeding habits of *Triatoma*, the bug may be an important cause of idiopathic anaphylaxis or allergic reactions, especially if there is a nocturnal pattern.

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Abbreviations used

I125:	Iodine 125
PBS:	Phosphate-buffered saline
RAST:	Radioallergosorbent test
RIA:	Radioimmunoassay

Triatoma feed by capillary puncture and locally anticoagulate the blood by injecting saliva into the bite site. The salivary gland secretions contain the antigen(s) that are responsible for allergic reactions.⁹ Up to the present time, there has been no generally accepted or readily available antigen preparation for standardized *Triatoma* skin testing or immunotherapy. By use of a passively derived extract from *T. protracta* salivary glands, we have established in vivo and in vitro diagnostic tests. Furthermore, a formal regimen for immunotherapy was developed that provided complete protection upon provocative challenge.

CASE REPORTS

Only patients with histories of life-threatening allergic reactions were selected for immunotherapy; generalized urticaria alone was not sufficient. Two of the five case histories are presented as typical examples.

Patient 1 is a 48-year-old man with a history of mild allergic rhinitis but otherwise no allergic problems. In November of 1980, he awoke from sleep with generalized pruritus and shortness of breath. On attempting to arise from the bed, he experienced postural hypotension and collapsed. His blood pressure was 60 mm Hg when paramedics arrived and administered epinephrine. The patient responded promptly. No etiology for the anaphylactic episode was determined. Similar events occurred in Aug. and Sept. of 1981. However, on one of these occasions, the patient was able to identify an insect bite, and subsequently the insect was found in the bedding. The insect was brought to UCLA (University of California Los Angeles) and identified as *T. protracta*.

Patient 2 is a 32-yr-old man with no previous history of allergic problems. On Aug. 5, 1981, he experienced generalized urticaria that progressed to hypotension that he thought started after observing an insect bite. The patient was taken to a local emergency room and brought the insect with him. The patient responded well to epinephrine. The insect was disregarded as a cockroach. The patient was told it had nothing to do with his symptoms. Approximately 8 hr later that evening, the patient had a recurrence of symptoms while sleeping and again required epinephrine administration. Three days later, the patient again experienced generalized urticaria and hypotension while sleeping and was referred to UCLA. After the earlier history was obtained, the patient was asked to bring in the insect that he originally suspected. It was identified at UCLA as *T. protracta*.

MATERIAL AND METHODS

Triatoma colonies were maintained, and the salivary glands were dissected as described by Marshall and Street.⁹ However, the glands were not freeze-thawed as originally described but were passively extracted in PBS (0.015M Na₂HPO₄, 0.015M NaH₂PO₄, 0.65M NaCl, pH = 7.5) for 24 hr. Salivary glands were mixed with PBS at a ratio of one set of glands per 1 ml of PBS. Antigen lots were made from the glands of at least 50 insects. The passive extract was then passed through a 0.2 μ m cellulose-acetate filter. The antigen content of the passively extracted material was compared by RAST-inhibition assay to the freeze-thaw preparation. The antigen concentration is expressed in RAST-inhibition units.

RIA

Solid phase RIAs were performed according to the method of Klinman and Taylor.¹⁰ Polyvinyl chloride 96 well plates (Dynatech, Alexandria, Va.) were coated with 100 μ l of the freeze-thaw derived antigen at 100 U/ml (previous experiments have demonstrated this to be the optimum concentration). After an overnight incubation, the plates were washed with PBS with azide (0.015M Na₂HPO₄, 0.015M NaH₂PO₄, 0.65M NaCl, 0.003M NaN₃, pH = 7.0) with 0.05% Tween 20 (PBS-Tween) and blocked with 1% ovalbumin. After ½ hr the plates were washed again with PBS-Tween. Serum samples were added in a 1:10 dilution for IgE and 1:100 for IgG. Dilutions were made with 1% ovalbumin. After a 4-hour incubation the plates were washed with PBS-Tween and bound immunoglobulin was detected by the addition of radiolabeled I125 either anti-IgE (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) or anti-IgG (Tago Diagnostics, Burlingame, Calif.). In order to quantify the amounts of IgG and IgE represented by the counts per minute bound, standard curves were run simultaneously on the plates with the serum samples. The standard curve for IgE was done by coating the wells with anti-human IgE (Fc epsilon specific, CIA/E-4.15 and CIA/E-7.12 mouse monoclonal antibodies prepared in our laboratory). Known amounts of purified human IgE were added and incubated, and bound immunoglobulin was detected with I125 anti-human IgE (Kirkegaard & Perry). A similar procedure was used for IgG (Cappel Laboratories, Cochranville, Pa.). By use of these standard curves, the counts bound in the *Triatoma*-specific assays were converted to nanograms and micrograms of antibody for IgE and IgG, respectively. The standard curves for purified IgE and IgG were each parallel to their respective *Triatoma*-specific assays over the range of concentrations used. The IgE- and IgG-antibody responses to *Triatoma* antigen(s) were measured on each patient before and throughout immunotherapy. All sera from individual patients were tested in the same assay.

RAST-inhibition assay

Microtiter plates were coated as described above. A sample of a known positive serum was mixed 1:5 with 1%

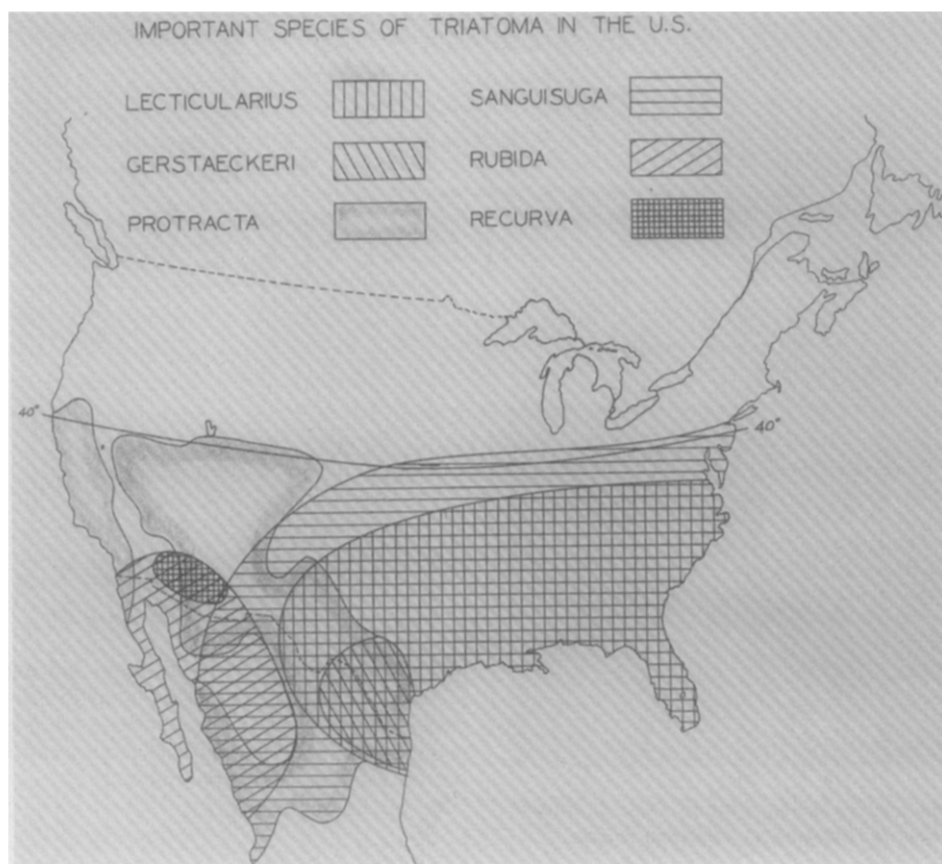


FIG. 1. Distribution of important species of genus *Triatoma* in United States.

ovalbumin. Additional samples of this positive serum were mixed 1:5 with tenfold dilutions of each of the antigen preparations. All dilutions were made with 1% ovalbumin. These samples were then applied to the antigen-coated plates at 50 μ l/well. After a 4-hour incubation the plates were washed with PBS-Tween and labeled with I125 anti-human IgE. Control sera from nonallergic persons were run simultaneously to determine the background counts. These background counts were subtracted from the raw counts and then were plotted on Fig. 3. All samples were done in triplicate, and the experiment was repeated twice.

Antigen standardization

Antigen units were defined by RAST-inhibition assay in reference to a known pretreatment serum. The amount of antigen that inhibited 50% of the maximum binding of 10 μ l of the reference serum is defined as 0.4 U. All antigen batches were standardized to the reference serum.

Skin testing

Graded prick and intradermal skin testing was performed that started with a prick test at 10 U/ml concentration. If this was equivocal (wheal less than 4 mm), then intradermal testing was started at 0.01 U/ml. If the skin test was negative, intradermal testing was started at 0.1 U/ml. The intradermal testing was continued with tenfold increases in con-

centration up to a maximum of 100 U/ml or until a positive test was obtained. A positive test was defined as a wheal size greater than or equal to 4 mm. Appropriate histamine and diluent controls were performed. The injected volume of the intradermal tests was 0.01 to 0.02 ml. Dilutions of the passive extract for skin testing and immunotherapy were made in a 0.9% saline, 0.4% phenol, and 0.03% human serum albumin buffer.

Bite challenge

The protection against anaphylaxis was assessed by a bite challenge with an adult *T. protracta*. The bite challenges were done in the UCLA Hospital with appropriate monitoring. The bug was placed in a 50 ml beaker that was covered with a single layer of gauze. The beaker was inverted on the palmar aspect of the patient's forearm, and the insect was allowed to feed until completion of a blood meal (15 to 25 min). Patients were observed in the hospital after the bite.

RESULTS

Antigen preparation

The passive extract was demonstrated to have equivalent amounts of antigenic activity compared to the original freeze-thaw material (Fig. 3). The complete inhibition achieved by the passive extract at

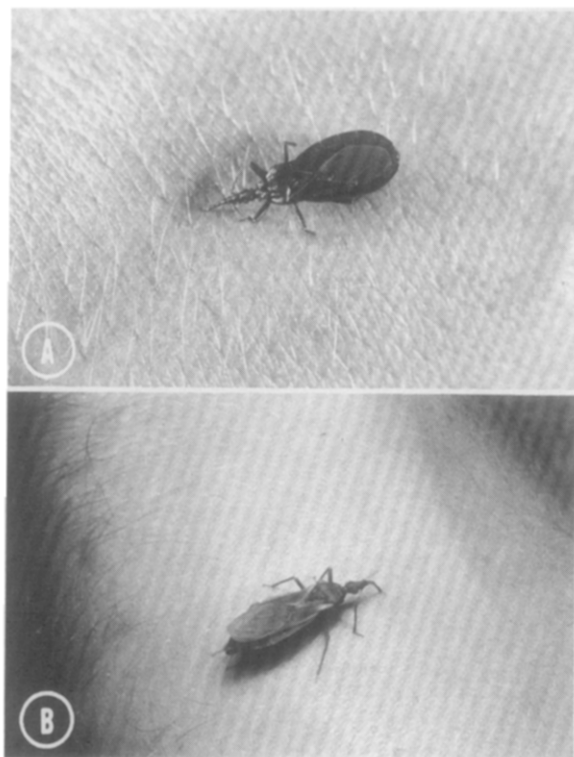


FIG. 2. *Triatoma* species taking a blood meal on human forearm. A, *Triatoma protracta*. B, *Triatoma rubida*.

1000 U/ml indicates that the passive extract contains all the antigens of the freeze-thaw preparation represented on the microtiter plates. In addition, the passive extract demonstrated equivalent inhibition to the freeze-thaw preparation over the 1 U/ml to 100 U/ml concentration range. However, the passive extract had fourfold less total protein that may account for our findings of marked reduction in local reactions during immunotherapy compared with the findings of Marshall and Street.⁹

Diagnosis

All five patients had positive skin tests at an intradermal concentration of 0.01 U/ml. Sixty-nine of 71 history-negative patients have been negative with a 100 U/ml intradermal skin test. The two positives at 100 U/ml were negative at 10 U/ml intradermal concentration. The IgE RIA with each patient's serum before immunotherapy gave binding of 200% to 400% greater than control sera that included sera from Eskimos who had never left Alaska (no possible *Triatoma* exposure).

Immunotherapy

The immunotherapy regimen was designed with a rapidly increasing dosage schedule as shown in Table I. All patients experienced several moderate local

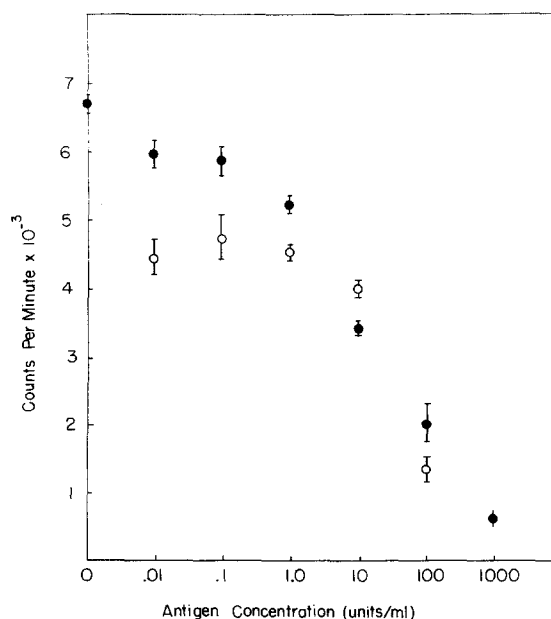


FIG. 3. Comparison of freeze-thaw and passive-extract antigen preparations by RAST-inhibition assay. ● = passive extract antigen; ○ = freeze-thaw antigen. Results are expressed as raw counts minus control sera counts (control sera = 1200 counts per minute).

reactions (wheal less than 3 cm) before reaching maintenance levels. Patient 1 had generalized urticaria beginning 4 hr after the injection at week 10. This did not recur after pretreatment with antihistamines on subsequent doses. The other four patients experienced no generalized reactions. The initial two patients have been on monthly maintenance for 15 mo without adverse reactions.

The IgG and IgE anti-*Triatoma* responses over the course of immunotherapy are shown in Fig. 4. All patients demonstrated a marked rise in IgG anti-*Triatoma* that started in the 4th wk and reached a plateau by about week 12. The IgG level remained fairly constant after 30 wk. The IgE response to immunotherapy for all patients demonstrated a rise at about 4 wk with plateaus at 12 to 14 wk that continued to week 30.

Provocative challenges

After observing an appropriate rise in the anti-*Triatoma* IgG level, the first bite challenge was done at week 14 of immunotherapy in patient 1. He developed a local reaction with a wheal of 7 cm and a peculiar wheal-and-flare response up the lymphatic channels on the inner aspect of the arm. Forty-five minutes after the bite, he then developed scattered urticaria lesions in his ipsilateral axilla. Similar lesions occurred in the contralateral axilla 15 min later. There were no other adverse reactions, specifi-

TABLE I. *Triatoma* treatment schedule

Week no.	Dose/day ½ hr apart	Concentration (U/ml)	Volume (ml) injected	Amount (units) injected
1	1	0.01	0.1	0.001
	2	0.1	0.1	0.01
	3	1.0	0.1	0.1
2	1	1.0	0.1	0.1
	2	1.0	0.5	0.5
	3	10	0.1	1.0
3	1	10	0.1	1
	2	10	0.5	5
	3	10	1.0	10
4	1	100	0.1	10
	2	100	0.2	20
5	1	100	0.2	20
	2	100	0.5	50
6	1	100	0.5	50
	2	100	1.0	100
7	1	1000	0.1	100
	2	1000	0.2	200
8	1	1000	0.2	200
	2	1000	0.5	500
9	1	1000	0.5	500
	2	1000	0.5	500
10	1	1000	1.0	1000
12	1	1000	1.0	1000
15	1	1000	1.0	1000
Monthly	1	1000	1.0	1000

cally no nausea, vomiting, wheezing, or hypotension.

The next bite challenge was done on patient 2 at week 32 of immunotherapy. Within 2 min the patient noticed pruritus and stinging at the bite site. After the *Triatoma* had been feeding for 10 min, there was a 12 mm wheal and 50 mm of erythema. The bug was allowed to continue feeding until engorged at 15 min. The patient's reaction proceeded no further. There was no generalized urticaria or hypotension as had happened during his bite exposures before immunotherapy. Several weeks after this bite challenge, the patient awoke from sleep with a pruritic wheal and flare below his knee. Although the patient could not find a *Triatoma* after searching through the bedding, this probably represented a natural exposure.

Since patient 2 showed a much less extensive allergic reaction at week 32 than patient 1 at week 14, patient 1 was rechallenged at week 33. The *T. protracta* fed for approximately 20 min. At the end of the feeding, the patient had a 15 mm wheal with 40 mm of erythema, and the reaction progressed no further. Subsequently three more patients underwent bite challenges after 28 to 30 wk on immunotherapy. None of these patients developed anything more than a local wheal and flare at the bite site.

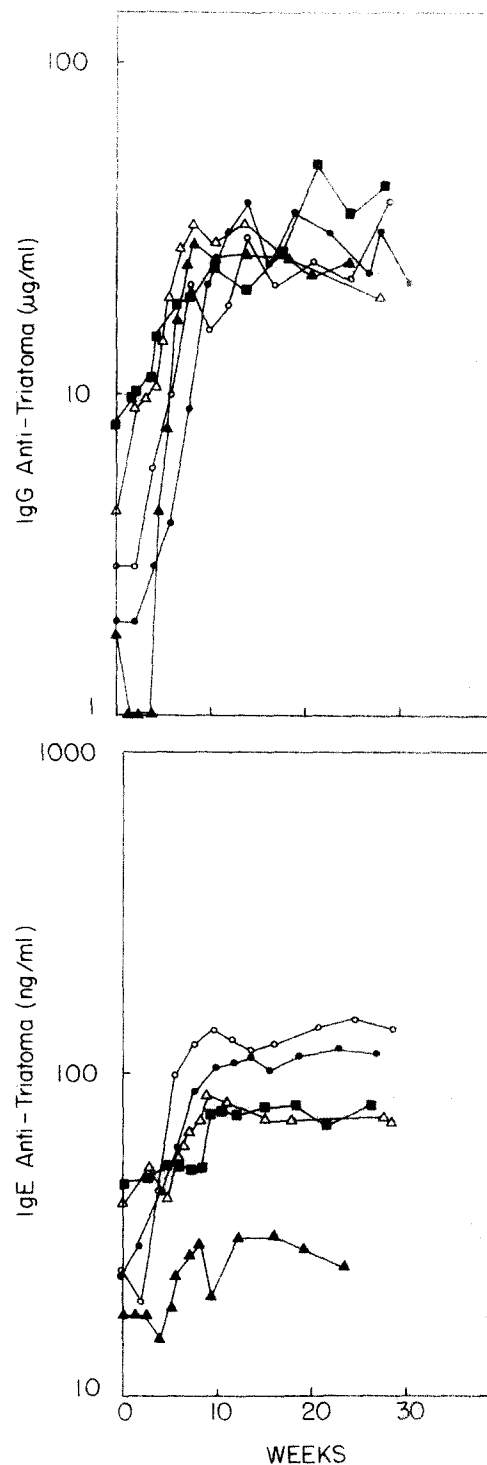


FIG. 4. IgG and IgE anti-*Triatoma* antibody responses during immunotherapy. ● = patient 1; ○ = patient 2; △ = patient 3; ■ = patient 4; ▲ = patient 5.

DISCUSSION

Five patients with repeated episodes of *T. protracta*-induced anaphylaxis were successfully treated with immunotherapy. The newly developed and standardized antigen preparation was demonstrated to be

safe and effective for diagnosis and treatment of *Triatoma* allergy. The availability of reliable diagnostic and treatment materials (Neil Marshall, 4038 Paula St., La Mesa, CA 92041) will be important as more physicians become aware of the problem of *Triatoma* allergy. With increasing reporting, *Triatoma*-induced anaphylaxis may well prove to have an incidence equal to that of Hymenoptera anaphylaxis in *Triatoma*-infested regions of the United States.

The patients with *Triatoma*-immediate hypersensitivity reacted on skin testing to a 1000- to 10,000-fold lower concentration of the passive extract than normal individuals. This correlated well with the patient's elevated pretreatment IgE anti-*Triatoma* antibody levels by RIA. Larger numbers of skin tests need to be done in order to accurately establish sensitivity and specificity. In addition, the skin testing results will be compared to the RIA for IgE anti-*Triatoma* antibodies as more patients are studied. This will define the relative sensitivities of the in vitro and in vivo methods for determining *Triatoma* allergy. Our preliminary data suggest that the skin test is more sensitive and specific than the RIA for IgE anti-*Triatoma* antibodies.

The immunotherapy schedule that was developed uses rapid increases in antigen dosage. A rapid increase protocol was chosen because studies with Hymenoptera have shown fewer overall reactions during immunotherapy with greater and quicker rises in IgG antibodies.¹¹ The maintenance dosage for Hymenoptera immunotherapy is equivalent to two stings (100 µg venom). The maintenance dosage for *Triatoma* is difficult to establish since the amount of saliva injected during a bite is unknown. The 1000 U maintenance dosage is on the basis of calculations from the average wet weight of the salivary glands from an individual *Triatoma* and the limited protection by an equivalent of a 50 U maintenance dosage used by Marshall and Street.⁹ The 1000 U maintenance dosage gave excellent protection in all patients at 28 to 33 weeks. All patients retained a local reaction similar to a positive skin test reaction during bite challenges. This was expected as even with successful Hymenoptera immunotherapy, skin test reactivity is not lost.¹² Therefore we feel our patients had achieved complete protection. In addition, the fact that patient 2 experienced no systemic reaction to a subsequent natural exposure provides further evidence for protection caused by immunotherapy.

Control bite challenges similar to the Hymenoptera study¹³ were not performed for several reasons. First, many of the patients in the Hymenoptera control group had histories of generalized urticaria and nonlife-threatening reactions. *Triatoma*-allergic pa-

tients were eligible for study only with histories of life-threatening reactions. Second, the control phase of the Hymenoptera study was terminated after the second patient experienced a life-threatening systemic reaction in spite of adequate precautions before the sting challenge and vigorous resuscitation efforts.¹⁴

If we accept the Hymenoptera 40% false negative rate in the placebo therapy group as a historical control, five consecutively successful bite challenges in the treatment group indicate that the treatment is effective (95% confidence on the basis of binomial distribution). The 40% figure is probably too high in regard to *Triatoma protracta* anaphylaxis since our patients all have histories of repeated anaphylaxis, and many of the Hymenoptera patients did not. In spite of this, we now have statistical significance in the *T. protracta*-treatment group.

The bite challenge has been used to evaluate immunotherapy for *T. protracta* in only one other study.⁹ The bite challenge as we performed it represents a maximal exposure since the amount of antigen injected during a 20 to 25 min bite challenge is probably greater than a natural bite in a sensitized person. All patients had local pruritic reactions within 5 min of the bite challenge that suggested that they would have awakened and disrupted the natural *Triatoma* feeding before 20 min.

The fact that patient 1 had a more extensive reaction at week 14 than week 33 with similar IgG anti-*Triatoma* antibody levels has two possible explanations. First, there may be variability in a patient's response to an equivalent antigen challenge as with Hymenoptera-sting challenge.^{12, 15} Second, the IgG anti-*Triatoma* antibodies at 28 to 33 weeks, although quantitatively similar to week 14 levels, may be qualitatively much different in the ability to protect against anaphylaxis. In other words, the patient may be allergic to one or more antigens (or epitopes on the same antigen) in the *Triatoma* saliva and developed a more specific set of IgG antibodies directed against these antigens by 28 to 33 weeks.

Definition of the antigen(s) contained in the *T. protracta*-passive extract preparation awaits future study. The passive-extract antigen preparation probably contains as few extraneous compounds as possible as there is no practical way to induce *Triatoma* to bite an artificial membrane (e.g., honeybee venom collection).

Thus far we have worked with *T. protracta*. Studies of cross-reactivity and possible immunotherapy for other species need to be undertaken. Preliminary work of Lindberg et al.¹⁶ suggests species-specific responses to *T. protracta* and *T. rubida*. Species of the genus *Triatoma* have both a regional and a

worldwide distribution. Therefore *Triatoma*-induced immediate hypersensitivity may relate to a sizeable population of patients.

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