

Clinical and immunological studies of venom immunotherapy

ROBERT E. REISMAN, CARL E. ARBESMAN and
MARY LAZELL

*Allergy Research Laboratory, Buffalo General Hospital, Division of Allergy,
Department of Medicine, State University of New York at Buffalo, N. Y., U.S.A.*

*(Received 28 August 1978; revision received 20 November 1978; accepted for publication
20 November 1978)*

Summary

Clinical and immunological studies were conducted in fifty insect-sensitive patients receiving bee and yellow jacket venom immunotherapy. All patients had had anaphylactic reactions and had elevated serum levels of venom-specific IgE (RAST) prior to therapy. Venom injections were given weekly starting at a dose of about 0.001 μ g and aiming for a maximum of 50 μ g or greater. Maintenance injections were administered monthly.

In the majority of patients, venom-specific IgG was produced by immunizing doses above 1 μ g. Highest titres were related to higher doses. Almost all patients receiving doses of 50 μ g of venom had an IgG antibody response.

Sequential measurements of venom-specific IgE were done in thirty-seven patients. Twenty-two had a declining titre, ten had a persistently elevated titre and five patients had a rise in antibody titre. There was no relationship between IgE and IgG antibody titres except for four patients who had a marked rise of both antibodies following venom therapy. Ten of the fifty patients received subsequent stings with no reactions. At the time of the subsequent uneventful sting, all but one had detectable serum IgG antibodies.

These studies suggest: (1) venom immunotherapy appears effective for immunization of insect-sensitive individuals; and (2) considerable variability in individual immunological response to therapy and thus the probable need to individualize the degree and extent of venom therapy.

Introduction

In recent years, both clinical and immunologic studies have strongly suggested that venom is the appropriate therapeutic agent for administration to patients who are

* This study was presented in part at the 34th annual meeting of the American Academy of Allergy, 25 February-1 March 1978, Phoenix, Arizona.

Correspondence: Robert E. Reisman, M.D. Allergy Research Laboratory, Buffalo General Hospital, 100 High Street, Buffalo, New York 14203, U.S.A.

anaphylactically sensitive to stinging insects (Loveless & Fackler, 1956; Busse *et al.*, 1975; Lichtenstein, Valentine & Sobotka, 1974; Hunt *et al.*, 1978; Amodio *et al.*, 1978; Markley *et al.*, 1978). The purpose of this report is to describe our experience with fifty patients who received venom immunotherapy over a period of 1–2 years.

Materials and methods

Venom collection

Venom was collected locally from honey-bees and yellow jackets by the method of electrical stimulation (Benton, Morse & Stuart, 1963). Bee hives of local bee keepers were used as the source for the honey-bee collection. Yellow jacket nests were found in the surrounding areas and in all cases specimens were taken for positive entomological identification of the insect.

After collection, the venom was dissolved in a small volume of borate buffered saline, filtered and the protein concentration determined on the filtrate. A 0.1% venom solution was then prepared (1000 µg/ml in TRIS buffer with 0.3% human serum albumin).

Patients

All patients had a clinical history of anaphylaxis immediately following an insect sting. These patients were usually seen shortly after the sting reaction. For inclusion into the study, patients also had elevated titres of venom-specific IgE as determined by the radioallergosorbent test (RAST—see below).

Therapy

Therapy was administered using the specific venom to which the patient was sensitive, as determined by the RAST. Initial doses of venom were in the range of 0.001 µg. Injections were given weekly, with an approximate doubling of the successive doses. Desired maintenance doses were in the range of 50–100 µg, the approximate equivalent of that deposited by one natural sting. Maintenance injections were given once monthly.

Evaluation of therapy

Clinical. With one exception no attempt was made to intentionally re-sting individuals receiving treatment. Observations were made on those patients who received inadvertent stings.

Immunological. Whenever possible, sequential sera were obtained during the course of treatment and the levels of venom-specific IgE and venom-specific IgG determined. The methods for these determinations have been described previously (Reisman, Wypych & Arbesman, 1975; Shimizu *et al.*, 1978).

Venom-specific IgE values were expressed in comparison to a positive serum standard which was empirically designated as 100%. RAST levels above 10% were considered significant. Values for venom-specific IgG were expressed in arbitrary units based on a positive standard assigned a value of 100 units. Values greater than 3 units were considered significant.

All serum samples from the same patient were analysed at the same time.

Results

Characteristics of treatment group (Table 1)

Venom immunotherapy was administered to fifty patients for a period of 1–2 years. Thirty-five patients received bee venom and sixteen received yellow jacket venom. One patient was treated with both bee and yellow jacket venoms.

The patients ranged in age from 5 to 68 years. There were thirty-six males and fourteen females. Twenty-one patients were over 20 years of age and twenty-nine patients less than 20 years. Fifteen had concomitant atopic disease.

Table 1. Venom immunotherapy—treatment group (fifty patients)*

Therapy	No. of patients treated	Age (years)	Sex	Atopic
Bee venom	35	5–68	36 males	15
			14 females	
Yellow Jacket venom	16			

* One patient received both bee and yellow jacket venom immunotherapy.

Reactions to venom immunotherapy

During the course of bee venom therapy, six patients had local reactions and three had systemic reactions, all of a very mild nature. The systemic reactions occurred following bee venom doses of 20, 500, and 600 μ g, respectively. Following yellow jacket therapy, there were no reactions.

Immunological response

Pre-treatment levels of venom-specific IgE are shown in Fig. 1. Bee venom RAST levels ranged from 12 to 183%. Yellow jacket specific IgE ranged from 17 to 185%.

Prior to therapy most patients had very low or undetectable levels of venom-specific IgG. There were six patients who did have fairly high levels of venom-specific IgG which we believe occurred in response to the same sting which caused the anaphylactic reaction (Light *et al.*, 1977).

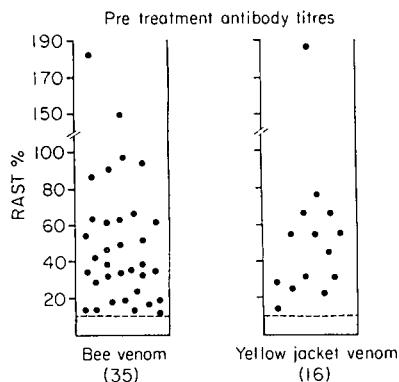


Fig. 1. Pre-treatment bee venom and yellow jacket venom specific IgE. Titres expressed in RAST %. Levels above 10% are considered significant.

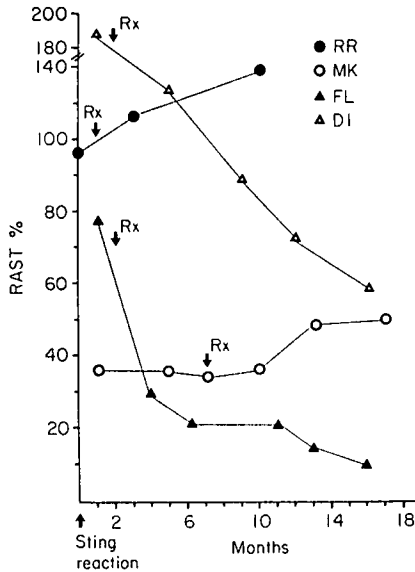


Fig. 2. Effect of venom immunotherapy upon venom specific IgE in four patients. Time in months following the sting reaction is plotted along the horizontal axis and venom RAST % along the vertical axis. Initiation of venom therapy for each patient is noted by the 'Rx' arrow.

Effect of therapy on venom-specific IgE. Over a period of at least one year following bee venom therapy, fourteen patients had a fall in IgE antibodies, seven remained essentially the same, and three had a rise in titre; following yellow jacket venom therapy, eight patients had a fall in IgE antibodies, three remained the same and two had a rise in titre. The change in titre was not directly related to the amount of therapy.

Examples of changes in venom-specific IgE in four patients are shown in more detail in Fig. 2. Following initiation of venom therapy, two of these patients had a fairly rapid fall in IgE antibody titre, one patient had an increase and the fourth patient had a modest rise, followed by a levelling off of the antibody titre. All patients in this illustration received identical doses of venom.

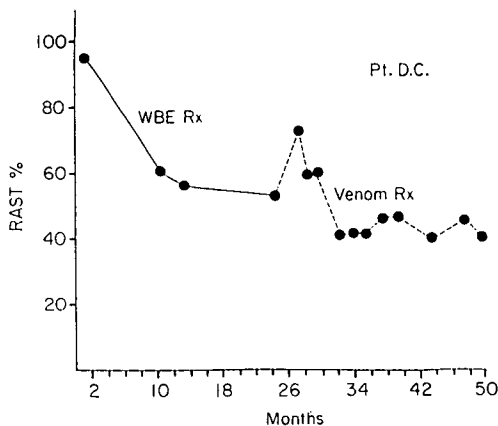


Fig. 3. Levels of bee venom specific IgE, expressed as RAST %, in a bee sensitive patient over a period of 4 years. Solid lines indicate period of whole body extract therapy and broken lines indicate period of bee venom therapy.

Table 2. Venom specific IgE and IgG responses in patients receiving doses of 50 μ g or greater of venom. Time between serum specimens was approximately 1 year

Patient	Venom specific IgE % STD		Venom specific IgG Units	
	Pre Rx	Post Rx	Pre Rx	Post Rx
Bee venom Rx				
D.B.	55	16	0	3
D.C.	53	46	10	16
T.E.	35	66	9	15
W.F.	37	20	0	10
P.I.	50	20	8	14
D.I.	187	73	5	32
L.M.	14	1	0	58
T.M.	19	35	0	5
D.P.	37	4	0	3
S.P.	32	36	0	7
M.P.	65	27	10	11
W.R.	47	23	4	9
R.R.	96	137	5	22
R.K.	40	11	8	16
R.S.	84	23	40	17
Yellow Jacket venom Rx				
M.D.	56	26	6	6
J.H.	67	90	0	5
J.S.	28	12	0	22
A.S.	185	127	0	3
S.Z.	25	11	5	5
A.M.	30	6	0	3

Bee venom-specific IgE data of another patient followed for over 4 years, are shown in Fig. 3. This patient received whole body extract therapy for the first 2 years. During this period IgE antibodies fell initially, and then levelled off for over 1 year (RAST about 60%). Shortly after venom therapy was initiated, there was a rise in antibodies followed by a decline and then a stable titre (about 40%) for almost 2 years.

Effect of therapy upon venom-specific IgG. Preliminary observations revealed that stimulation of venom-specific IgG usually did not occur until venom doses of at least 1 μ g were administered. Venom-specific IgG titres were analysed in the available sequential serum of those patients who were receiving a dose of at least 1 μ g. Following bee venom therapy, eleven patients had a rise in the IgG titre. Six failed to show any IgG antibody response. Of these, two had had a pre-existing titre which fell. There were two other patients who, prior to treatment, had elevated IgG antibodies which persisted but did not increase.

Of the patients receiving yellow jacket immunotherapy in doses greater than 1 μ g of venom, four had a rise in IgG antibodies and three had no response. There was one other patient who had a persistent titre with no change.

Sequential sera were available from twenty-one patients receiving doses of 50 μ g or greater of venom after 1 year of therapy. Following venom injections, all had detectable levels of venom specific IgG (Table 2). Eleven patients had IgG antibodies prior

Table 3. Serum levels of venom specific IgE and IgG prior to an uneventful re-sting in bee venom treated patients

Patient	Re-sting insect	Time interval between prior serum specimens and re-sting	Venom IgE (% STD)	Antibody* IgG (Units)
W.B.	Bee	2 weeks	3	0
R.K. **	Bee	5 min	18	16
D.M.	Bee	10 months	1	58
W.R.	Bee	1 month	30	7
T.E.	Unidentified	4 months	56	14
S.P.	Unidentified	3 months	33	7
M.P.	Unidentified	2 months	30	11
R.S.	Unidentified	1½ months	39	21
M.K.	Unidentified	2 months	36	7
D.B.	Unidentified	3 weeks	16	3

* Venom specific IgE expressed as % RAST standard.

Venom specific IgG expressed in units, also in relationship to a positive standard.

** Patient R.K. was intentionally re-stung.

to venom therapy, probably as the result of the sting exposure. In these patients, following treatment, antibody titres increased in seven, remained the same in three, and fell in one. The other patients who had undetectable venom-specific IgG prior to therapy all made antibodies in response to venom injections.

Relationships of venom-specific IgE and IgG. The changes in venom-specific IgE and IgG were compared in those patients receiving venom doses of 50 µg or greater after 1 year of therapy (Table 2). Venom-specific IgG changes are described above. Venom-specific IgE dropped in fifteen patients, remained about the same in two, and rose in four. As can be seen in Table 2, there was no relationship between the changes in the venom-specific IgE and IgG titres.

Clinical response to re-sting during therapy. During the course of immunotherapy, nine of these patients were inadvertently stung and one patient was stung intentionally. All had been bee sensitive. The honey-bee could be clearly identified in four of the ten re-sting episodes. In the others, the insect could not be identified. The levels of venom-specific IgE and IgG in the last serum obtained prior to the re-sting are shown in Table 3. Eight of the ten patients had elevated venom-specific IgG, one patient had a borderline titre (3), and one patient had no detectable IgG antibodies. This latter patient had discontinued treatment and had low levels of venom specific IgE. Eight of the patients had substantial levels of venom-specific IgE, well into the range in which reactions might be anticipated.

Discussion

In this study, ten of the fifty patients were re-stung. At least four positively identified the insect as being the same one responsible for their initial reaction. None of these four patients had reactions. Similarly, there were no reactions in the other six patients, either as a result of the immunization or because they were stung by an insect other than the one to which they were sensitive.

Perhaps the most interesting part of this study was the analysis of the immunological response. As noted, all patients had elevated levels of IgE-specific antibodies at the onset of the study. Sequential determinations of these IgE-specific antibodies revealed a great variation in the degree of response. Following therapy, the majority of patients had a decrease in venom-specific IgE. Several of these patients had a rapid fall in antibodies to the point where therapy was actually discontinued in three. It should be noted that this decrease may occur spontaneously and may not be a reflection of venom treatment (Reisman *et al.*, 1976). There were only five patients who had a marked rise in IgE following initiation of therapy. This type of response has been noted previously with venom therapy (Hunt *et al.*, 1978), particularly following the rapid method of desensitization (Sobotka, Valentine & Lichtenstein, 1978), and is also quite common in those individuals receiving ragweed pollen extracts (Reisman, Wypych & Arbesman, 1975). Finally, there were other patients who had a slow decline in IgE suggesting that it might be necessary to continue treatment for indefinite periods of time. These patients are presently being monitored on a regular basis.

The IgG response to venom treatment was usually found once doses greater than 1 μ g were administered. Most patients did produce IgG antibodies and it appeared that these antibodies were responsible for protection from subsequent stings. This conclusion had been reached previously from studies of bee keepers (Light *et al.*, 1975). Almost all of the patients had an IgG antibody response when doses of 50 μ g were administered. As this is in the range injected in the course of a normal sting, it appears to be the recommended therapeutic dose as well.

These studies suggest that venom is the appropriate therapeutic agent for the treatment of anaphylactically-sensitive individuals. The IgG antibody is apparently responsible for protection. We are continuing treatment, monitoring both IgE and IgG antibodies. When IgE antibodies are no longer elevated, therapy is discontinued. We have previously noted occasional anamnestic responses of IgE antibodies following subsequent stings and this possibility will have to be considered in the future. The marked difference in IgE responses suggests that the sensitivity to stinging insects may be lost within a short period of time or may continue on indefinitely; therefore, individuals will have to be monitored on a regular basis.

At the present time, there should be no difficulty with collection and processing of enough honey-bee venom for all patients requiring such treatment, although considerable problems do exist in the collection of other insect venoms.

Acknowledgment

This study has been supported in part by the United States Public Health Service Research Grant, 1-RO1-A1-14501, of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

References

- AMODIO, F., MARKLEY, L., VALENTINE, M.D., SOBOTKA, A.K. & LICHENSTEIN, L.M. (1978) Maintenance immunotherapy for hymenoptera sensitivity. *Journal of Allergy and Clinical Immunology*, **61**, 1934.
- BENTON, A.W., MORSE, R.A. & STUART, J.D. (1963) Venom collection from honey-bees. *Science*, **142**, 228.
- BUSSE, W.W., REED, C.E., LICHTENSTEIN, L.M. & REISMAN, R.E. (1975) Immunotherapy in bee-sting anaphylaxis. Use of honey bee venom. *Journal of the American Medical Association* **231**, 1154.

- HUNT, K.J., VALENTINE, M.D., SOBOTKA, A.K., BENTON, A.W., AMODIO, F.J. & LICHTENSTEIN, L.M. (1978) A controlled trial of immunotherapy in insect hypersensitivity. *New England Journal of Medicine*, **229**, 157.
- LICHTENSTEIN, L.M. VALENTINE, M.D. & SOBOTKA, A.K. (1974) A case for venom treatment in anaphylactic sensitivity to hymenoptera sting. *New England Journal of Medicine*, **290**, 1223.
- LIGHT, W.C., REISMAN, R.E., SHIMIZU, M. & ARBESMAN, C.E. (1977) Clinical application of measurements of serum levels of bee venom specific IgE and IgG. *Journal of Allergy and Clinical Immunology*, **59**, 247.
- LIGHT, W.C., REISMAN, R.E., WYPYCH, J.I. & ARBESMAN, C.E. (1975) Clinical and immunological studies of bee keepers. *Clinical Allergy*, **5**, 389.
- LOVELESS, M.H. & FACKLER, W.R. (1956) Wasp venom allergy and immunity. *Annals of Allergy*, **14**, 347.
- MARKLEY, L., AMODIO, F., SOBOTKA, A.K., VALENTINE, M.D. & LICHTENSTEIN, L.M. (1978) Immunotherapy with commercial venom preparations. *Journal of Allergy and Clinical Immunology*, **61**, 134.
- REISMAN, R.E. WYPYCH J.I. & ARBESMAN, C.E. (1975) Relationship of immunotherapy, seasonal pollen exposure and clinical response to serum concentrations of total IgE and ragweed specific IgE. *International Archives of Allergy and Applied Immunology*. **48**, 721.
- REISMAN, R.E., LIGHT, W.C., WYPCH, J.I. & ARBESMAN, C.E. (1976) Immunological studies on the effect of whole body insect extracts in the treatment of stinging allergy. *Journal of Allergy and Clinical Immunology*, **57**, 547.
- SHIMIZU M., WICHER, K., REISMAN, R.E. & ARBESMAN, C.E. (1978) A solid phase radioimmunoassay for detection of human antibodies. 1. Measurement of IgG antibody to bee venom antigens. *Journal of Immunological Methods*, **19**, 317.
- SOBOTKA, A.K., VALENTINE, M.D. & LICHTENSTEIN, L.M. (1978) The immune response to hymenoptera venoms. *Journal of Allergy and Clinical Immunology*, **61**, 136.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.