

Imported fire ant hypersensitivity

Studies of human reactions to fire ant venom

Frank K. James, Jr., M.D., Colonel, MC, USA, Hobert L. Pence, M.D., Major, MC, USA, Donald P. Driggers, M.S., Captain, MSC, USA, Robert L. Jacobs, M.D., Major, USAF (MC), USA, and Donald E. Horton, M.S., First Lieutenant, MSC, USA

Fort Sam Houston and Lackland Air Force Base, Texas

It is now apparent that venom and venom components of the Hymenoptera superfamilies of Apida (honeybee) and Vespida (wasps, yellow jackets, and hornets) are becoming increasingly important in the diagnosis and treatment of hypersensitivity reactions. Stings from fire ants (superfamily Formicidae, family Myrmicinae) have also been recognized as causes of systemic reactions in man. Fire ant venom is unique in its composition, consisting mainly of alkaloids in aqueous suspension with only trace amounts of protein. This study compares the skin reactivity of fire ant venom and synthesized alkaloid components with the whole body extract (WBE) of fire ants and other Hymenoptera. The venom as well as the WBE of fire ants was found useful for skin test diagnosis of sensitive individuals. There appear to be cross-reactive or shared antigens between fire ant venom, WBE, and WBE of other Hymenoptera. Successful passive transfer of skin reactivity to nonsensitive individuals was accomplished with sera from sensitive individuals. Loss of this passive transfer by heating sera at 56° C for 4 hr is evidence in favor of IgE mediating the positive skin test to the venom.

Severe and sometimes fatal human reactions from stings of winged Hymenoptera have been described since antiquity. The importance of Hymenoptera sensitivity in this country is emphasized by the fact that during the past 25 years approximately 40 recorded deaths each year are caused by allergic reactions from winged stinging insects.¹ Most of these deaths and other severe generalized reactions to stinging insects have been attributed to the members of the superfamily Apida (honeybees) and Vespida (wasps, hornets, and yellow jackets). Recently, there has been an increased interest in systemic reactions from fire ants (superfamily Formicidae, family Myrmicinae) which are of considerable medical importance, particularly in the United States Gulf Coast area.^{2, 3} Most

From the Department of Clinics and the Department of Medicine, Allergy and Clinical Immunology Service of Brooke Army Medical Center, 485th Medical Detachment (Preventive Medicine), and the Allergy and Clinical Immunology Service of Wilford Hall USAF Medical Center.

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Reprint requests to: Frank K. James, Jr., M.D., Colonel, MC, USA, Department of Medicine, Beach Pavilion, Brooke Army Medical Center, Fort Sam Houston, Texas 78234.

systemic reactions to ants are felt to be due to the stings from the two species of imported fire ants, *Solenopsis richteri* and *Solenopsis invicta*.² Fire ants are also of great economic importance, as it has been estimated that they are found on more than 150 million acres of land and do 40 million dollars damage to crops each year with a land value loss of more than half a billion dollars.⁴

In the medical management of systemic allergic reactions to stinging insects, it has become increasingly apparent that the commercial whole body extract (WBE) is inconsistent in diagnosing hypersensitivity and not always effective in treatment.⁵ This has led investigators to evaluate the pure venom of the different species as possible agents for more definitive diagnosis and treatment. In a number of recent studies, investigators have shown that pure venom or venom components are much more reliable for in vitro and in vivo diagnosis of IgE-mediated hypersensitivity than the WBE.⁶⁻¹⁵ In addition, there are recent reports of two treatment failures with WBE in patients who responded to treatment both immunologically and clinically with immunotherapy from the pure venom of the specific species involved.^{16, 17}

Although formic acid was isolated over 300 years ago, specific studies of the products of importance from the superfamily Formicidae (ants) have only been undertaken during the past two decades. These substances, known as pheromones, are considered key elements for regulating social interactions which occur in colonies. They can be classified as attractants, repellants (such as formic acid), and venoms.¹⁸

Ant venoms have been studied in a comparatively small group of the Myrmecines. Fire ants are representative of this group and produce a unique venom unknown in any other animal species.

In view of the increasing importance of pure venom in the management of hypersensitivity from winged Hymenoptera, it was felt that evaluation of fire ant venom would be significant. This paper reports on the use of fire ant venom for skin testing in the diagnosis of systemic hypersensitivity to fire ants and compares the venom with WBE. In addition, skin testing was done with the standard mixed stinging insect (MSI) extract, a mixture of WBE of wasp, hornet, yellow jacket, and honeybee, to evaluate possible cross-reactivity between the winged species of Hymenoptera and fire ants. The possibility of cross-antigenicity has been suggested in three previous reports.^{2, 3, 19} In addition, testing was performed with two synthesized alkaloids, components of the pure venom. This material was synthesized and generously provided to us by Prof. Murray S. Blum, Athens, Ga. These compounds are *cis*-2-methyl-6-*N*-undecylpiperidine and *trans*-2-methyl-6-*N*-penadecyl piperidine. The method of synthesis of these components has been described.²⁰

MATERIALS AND METHODS

Eighteen patients were selected for this study from the Allergy Clinics of Brooke Army Medical Center and Wilford Hall USAF Medical Center on the basis of: (1) a clear-cut history of generalized or anaphylactic reactions to fire ant stings, and (2) the formation of a typical sterile pustule at the site of the sting a few hours after the sting. This sterile pustule is considered to be a very important diagnostic point, the key finding documenting a fire ant sting. The dermatologic changes noted in fire ant stings have been described in detail

TABLE I. Clinical features of fire ant-sensitive patients

Patients	Age (yr)	Sex	Inhalant allergy	Other Hymenoptera sensitivity
J. N. C.	17	M	Yes	No
J. T. C.	48	M	No	No
M. C.	47	F	Yes	No
W. G.	30	M	Yes	No
S. G.	45	F	Yes	No
R. J.	33	M	No	No
R. K.	25	M	No	No
T. K.	41	M	No	No
K. L.	24	M	No	Yes
M. M.	28	F	No	Yes
J. M.	32	F	Yes	No
E. M.	30	F	No	No
B. O.	40	F	No	No
R. P.	40	M	No	No
T. P.	?	M	No	No
W. S.	56	M	No	Yes
M. W.	30	F	No	No
G. W.	24	M	No	No
Mean	34.71	11 M 7 F	5 Yes 13 No	3 Yes 15 No

by Caro and colleagues.²¹ In addition, most of the patients could identify ants as coming from typical large mounds characteristic of the fire ant. The patients studied had been recently diagnosed as fire ant sensitive and were studied at the time of diagnosis and initiation of treatment. No medications were taken by the patients for 48 hr prior to testing. Evaluations were done between 30 days and 6 mo after the systemic reaction occurred. In addition, 13 normal control subjects were chosen who had no history of any type of systemic or severe generalized reaction to fire ant sting or who had no record of being stung at all. Informed consent was obtained from the patients and control subjects before testing with the venom.

Collection of the venom

Venom was collected from the workers of both *S. invicta* and *S. richteri* by the method of "milking" described by Blum and associates.²² Under a dissecting microscope, the abdomen was dissected free, then held by a remnant of the thorax. The abdomen was gently stroked with a 0.5 μ l pipette affixed to a cork. Venom would promptly appear at the tip of the extended stinger. Approximately 0.015 μ l was contained in each drop and it was possible to gain 4 or 5 drops of venom from each worker.

Skin testing with venom

The venom was added to a solution of 50% glycerin and normal saline to make a 1:400 volume to volume (V/V) dilution. This was then diluted with standard aqueous allergy diluent obtained from Greer Laboratories, Lenoir, N. C., to the appropriate skin testing concentrations. Initial skin tests were performed with a 1:4 million V/V dilution by the scratch technique. The tests were then carried out by intradermal (ID) injections of 0.05 cc of the same solution increasing by 10-fold stronger concentrations until a positive response was obtained up to a maximal concentration of 1:4,000 V/V.

A positive skin test was defined as a definite wheal-and-flare reaction occurring within 20 min following the skin test with the wheal being at least twice the diameter of a control test done with diluent only. The wheal had to be at least 5 mm or greater in diameter to be considered positive. The synthesized alkaloid fractions were diluted and tested in a similar manner. All of the solutions were sterilized by passing them through a millipore filter and all were checked for sterility before use. The dilutions of extracts were prepared

TABLE II. Clinical features of control group

Patients	Age (yr)	Sex	Inhalant allergy	Other Hymenoptera sensitivity
L. A.	18	M	No	No
H. B.	39	M	No	No
P. B.	40	F	No	No
K. B.	25	M	No	No
D. D.	30	M	No	No
W. H.	22	M	No	No
D. H.	26	M	Yes	No
W. J.	29	M	Yes	No
H. P.	33	M	No	No
A. P.	20	M	Yes	No
A. S.	38	M	No	No
A. W.	27	F	Yes	No
C. W.	29	M	No	No
Mean	28.92	11 M 2 F	9 No 4 Yes	13 No

a few days prior to testing the first patients in the study. The extracts were stored at 6° C with no apparent loss of potency over the 6-mo period the study was performed.

Skin test with whole body extract

Mixed fire ant WBE, WBE of *S. invicta* and *S. richteri* separately, and MSI extract were obtained from Greer Laboratories, Lenoir, N. C. These extracts were all diluted with aqueous allergy diluent to the desired concentrations. Testing with these extracts was started at 1:100,000 weight volume (W/V) concentration by scratch technique. Subsequently, ID tests were performed with 10-fold increasing concentrations until a positive skin test was obtained or until the 1:100 W/V concentration was used for MSI and mixed fire ant or a 1:1,000 W/V concentration for *S. invicta* and *S. richteri*. In all cases, when a positive test was obtained at a given concentration, no stronger concentrations were tested for that preparation on any given patient.

Sera were collected from three very sensitive patients (W. S., G. W., and R. P.) who had negative histories for systemic disease and were carefully screened for serology and hepatitis-associated antigens. The sera were also cultured for sterility. They were then divided into equal aliquots, and one aliquot was heated at 56° C for 4 hr to destroy skin fixing or reagenic antibody activity. Informed consent was previously obtained from three control patients who had negative skin tests. Then, 0.1 cc aliquots of the unheated sera was injected ID into three different locations on the volar surface of the right forearm of the control patient. The same procedure was followed with the heated sera on the left forearm. Forty-eight hours later, each skin test site was challenged with 0.05 cc of the 1:40,000 V/V concentration of venom from *S. invicta* and *S. richteri*. In addition, the *trans* stereoisomer of the synthesized alkaloid was similarly tested. These results were read at 20 minutes after challenge using the same criteria for the previously described positive tests.

RESULTS

The 18 patients with fire ant hypersensitivity are listed in Table I along with certain other clinical features. The 13 control patients are listed in Table II. It is interesting to note that 5 of the sensitive patients (28%) had histories suggestive of atopy with symptoms of hay fever or asthma. Four of the control subjects (31%) had similar histories. Three (17%) of the sensitive patients also had a history of generalized or systemic reactions to other stinging insects, whereas none of the control patients had a similar history.

TABLE III. Results of skin testing with venom preparations, whole body extracts, and synthesized alkaloids in fire ant-sensitive patients

Patients	MSI	Fire ant mix (WBE)	<i>S. invicta</i> (WBE)	<i>S. richteri</i> (WBE)	<i>S. invicta</i> (venom)	<i>S. richteri</i> (venom)	Cis-2	Trans-2
J. N. C.	100,000	10,000	100,000	10,000	400,000	4 million	Neg	4,000
J. C.	1,000	10,000	1,000	1,000	40,000	400,000	4,000	4,000
M. C.	100	1,000	Neg	Neg	Neg	Neg	Neg	Neg
W. G.	Neg	100,000	100,000	10,000	400,000	40,000	Neg	Neg
S. G.	10,000	100,000	100,000	100,000	400,000	400,000	Neg	4,000
R. J.	1,000	10,000	1,000	1,000	40,000	40,000	Neg	Neg
R. K.	1,000	100,000	1,000	1,000	40,000	4 million	Neg	Neg
T. K.	Neg	100,000	100,000	10,000	400,000	4 million	Neg	4 million
K. L.	1,000	10,000	1,000	Neg	4,000	400,000	ND	ND
M. M.	100,000	ND	Neg	Neg	4,000	4 million	Neg	40,000
J. M.	1,000	1,000	1,000	1,000	40,000	40,000	Neg	40,000
E. M.	Neg	ND	10,000	10,000	400,000	400,000	ND	ND
B. O.	100	1,000	Neg	1,000	40,000	400,000	4,000	40,000
R. P.	Neg	ND	100,000	100,000	40,000	4 million	Neg	40,000
T. P.	Neg	1,000	Neg	Neg	40,000	40,000	Neg	Neg
W. S.	1,000	ND	1,000	1,000	40,000	40,000	Neg	Neg
N. W.	1,000	10,000	10,000	10,000	40,000	400,000	4,000	4,000
G. W.	1,000	100,000	100,000	10,000	40,000	400,000	Neg	400,000
18	11/18	14/14	14/18	14/18	15/18	17/18	0/16	6/16
% positive	61	100	78	78	83	94	0	38

Results are expressed as the reciprocal of either W/V concentrations in WBE or V/V dilutions of venom which gave positive test: a positive response at 1:100,000 W/V would be listed as 100,000. Negative listing given to those with no response at strongest concentration tested. Ratios at bottom of columns represent ratio of positives to total tested. Positives for MSI and WBE are those 1,000 or greater, while for the venoms, 40,000 or greater.

ND: not done.

Table III lists skin reactivity of sensitive individuals to WBE, the two venom preparations, and the synthesized alkaloids. Table IV does the same for the control group. When the reactions in the control group were used as a guide, it appeared that a positive response to WBE of 1:1,00 W/V concentration or less represented a significant skin test response as no more than two of the control subjects had a positive skin test at these dilutions to any of the different WBE. For the venom preparations, it appeared that a positive response at 1:40,000 V/V or less represented a significant response as only one control patient reacted to any of the venom preparations at these concentrations. These results are summarized in Tables V and VI. Using these criteria for both positive and negative responses, one can see that both the whole body extract and separate venom preparations of fire ants were, for the most part, reliable indicators of clinical sensitivity. Every sensitive patient had a positive response to the mixed fire ant WBE, and 78% of the patients reacted to each of the WBE of the two individual fire ant species. Over 83% of sensitive patients responded to the venom of *S. invicta* and 94% to the venom of *S. richteri*, again confirming the sensitivity elicited by the separate fire ant venoms. These results with WBE of fire ants are surprising in view of the poor reliability of skin testing materials with WBE of winged Hymenoptera.^{23, 24} In addition, these results suggest shared or cross-reactive antigens in both the WBE and specific venoms.

Even though only 3 of the sensitive patients had a history of generalized reactions to the stinging insects, 11 of 18 (61%) had positive skin testing re-

TABLE IV. Results of skin testing with venom preparations, whole body extracts, and synthesized alkaloids in nonallergic control patients with no history of fire ant sensitivity

Patients	MSI	Fire ant mix (WBE)	<i>S. invicta</i> (WBE)	<i>S. richteri</i> (WBE)	<i>S. invicta</i> (venom)	<i>S. richteri</i> (venom)	Cis-2	Trans-2
L. A.	1,000	1,000	1,000	Neg	Neg	Neg	Neg	Neg
H. B.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
P. B.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
K. B.	100	100	100	100	Neg	40,000	Neg	Neg
D. D.	1,000	100	1,000	Neg	Neg	Neg	Neg	Neg
W. H.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
D. H.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
W. J.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
H. P.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
A. P.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
A. S.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
A. W.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
C. W.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Positives	2/13	1/13	2/13	0/13	0/13	1/13	0/13	0/13
% positive	15	8	15	0	0	8	0	0

TABLE V. Skin test reactions of fire ant-hypersensitive patients (18) to various concentrations of WBE, venoms, and synthesized alkaloids

Whole body extract (W/V)	Neg	1:100	1:1,000	1:10,000	1:100,000
MSI	5	2	8	1	2
Mixed fire ant	0	0	4	5	5
<i>S. invicta</i>	4	0	6	2	6
<i>S. richteri</i>	4	0	6	6	2
Venoms (V/V)	Neg	1:4,000	1:40,000	1:400,000	1:4 million
<i>S. invicta</i>	1	2	10	5	0
<i>S. richteri</i>	1	0	5	7	5
Alkaloids	Neg	1:4,000	1:40,000	1:400,000	1:4 million
Trans-2	6	4	4	1	1
Cis-2	13	3	0	0	0
Diluent	12	4	2	0	0

sponses to the commercial MSI preparation commonly used to test and treat patients with this specific problem. Further evaluation of the data, however, shows that 8 of the 11 had a positive response at the 1:1,000 W/V concentration which was the borderline value considered positive. Therefore, although the responses to MSI were present, they were relatively weak. It is difficult to make a definite conclusion concerning cross-reactivity based on this data. We do feel justified, however, in saying that there appears to be a suggestion of some degree of cross-reactivity between winged Hymenoptera and the imported fire ant species tested.

Skin testing with the synthesized alkaloids was indeed interesting. Six of the 16 patients tested (38%) had positive tests to the *trans* isomer of the alkaloid, while no one in the control group or sensitive group reacted to the *cis* isomer.

Passive transfer of skin reactivity to the skin of negative control subjects was

TABLE VI. Skin test reactions of control patients (13) to various concentrations of WBE, venoms, and synthesized alkaloids

Whole body extract (W/V)	Neg	1:100	1:1,000	1:10,000	1:100,000
MSI	10	1	2	0	0
Mixed fire ant	10	2	1	0	0
<i>S. invicta</i>	10	1	2	0	0
<i>S. richteri</i>	12	1	0	0	0
Venoms (V/V)	Neg	1:4,000	1:40,000	1:400,000	1:4 million
<i>S. invicta</i>	13	0	0	0	0
<i>S. richteri</i>	12	0	1	0	0
Alkaloids	Neg	1:4,000	1:40,000	1:400,000	1:4 million
<i>Trans</i> -2	13	0	0	0	0
<i>Cis</i> -2	13	0	0	0	0
Diluent	13	0	0	0	0

accomplished with two of the three sera with which it was attempted. This appears to be specific as one sera was from a patient sensitive to the *trans* isomer, and it produced a positive response in the control patients when subsequently challenged with this isomer. The other sera were from patients negative to skin tests with the *trans* isomer and these did not convey a positive response to the control patients. Both sera transferred reactivity to the venoms of the two species. The heated sera failed to transfer reactivities to the control subjects. This is evidence in favor of IgE being responsible for transfer of reactivity and for the positive skin tests in the sensitive group.

DISCUSSION

Fire ants constitute a serious economic and medical problem, particularly in the Gulf Coast area of the United States. There are at least five species of true fire ants in this country: *S. geminata*, *S. xyloni*, *S. aurea*, and the two imported species *S. richteri* and *S. invicta*.²⁵ The two imported species have been by far the most successful and are thought to elicit the most painful stings. *S. invicta* has rapidly spread throughout the Gulf Coast area into extreme south Florida and south central Texas during the past 20 years (Fig 1).²

The venom of the fire ant is truly unique and different from venoms of other Hymenoptera or other animal species known to cause systemic reactions. Fire ant venom is bactericidal,²² hemolytic,²⁶ cytotoxic,^{27, 28} and insecticidal.²² In normal individuals, there is a predictable sequence of dermatologic events following a fire ant sting, culminating in a typical sterile pustule. This usually takes days to heal, sometimes with resultant pigmentation, scar formation, and on occasion severe secondary infections.²¹ The venom is an aqueous suspension of insoluble alkaloids in which the alkaloids are thought to possess almost all of the biologic activity. There are felt to be trace amounts of polypeptides and proteins in fire ant venom constituting less than 1% of the total weight.²⁸ The alkaloids consist of a series of 2 to 6 disubstituted piperidines with variation in each species, and on occasion, within the species. The chemical structure of the alkaloids has

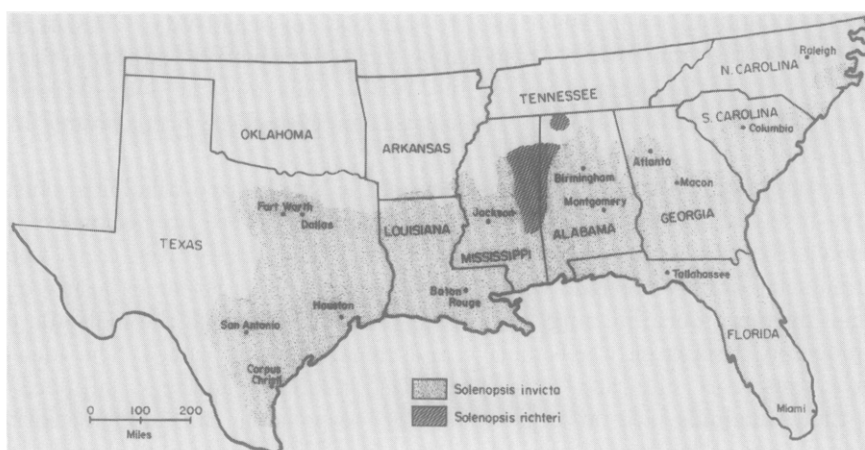


FIG. 1. Distribution of imported fire ants in the Gulf Coast area of the United States.

been studied at length by MacConnell,^{20,29} Blum,²⁵ and Brand³⁰ and their associates. The alkaloid structure consists of a piperidine nucleus with a methyl group at the 2 position and an 11 to 16 member carbon aliphatic chain attached to the 6 position on the ring. The difference in the various structures of the alkaloids reside mainly in the aliphatic side chains and relates to the number of carbons in the chain, the arrangement of double bonds and stereoisomers. The more toxic and apparently allergenic venoms of the imported species, also thought to represent the more highly evolved species, contain higher proportions of alkaloids with more carbons (C-13 and C-15) and more double bonds in the side chain, and the *trans* configuration for the stereoisomers.

Skin testing with the venom appears to be a highly useful technique in separating sensitive from nonsensitive individuals. Only one patient in our group had a history of a generalized reaction and yet failed to give a positive skin test result to one of the venoms. On the other hand, only one control patient had a positive test and that was to *S. richteri* only. Therefore, the venoms gave only one false positive response and one false negative response in all the patients tested in this study. Of interest is the fact that everyone except this patient had a positive test to the venom of *S. richteri*. Since only *S. invicta* is found to naturally occur in San Antonio, patients presumably had not been exposed to *S. richteri* which strongly suggests common or cross-reactive antigens between the venoms of these two imported species.

Our results with WBE are consistent with those of Triplett,¹⁹ Rhoades and associates,³ and Lockey² who reported in almost all of their sensitive patients positive skin tests with WBE. Seventeen of the 18 sensitive patients in our study had a positive test to one of the WBE. In the nonsensitive control subjects we had two false positive reactions, both of which reacted at the 1:1,000 W/V concentration, suggesting that positive tests at weaker concentrations are indeed highly significant. The reliability of skin testing with WBE of fire ant is markedly different than the experience of those using the WBE of *Vespida* and *Apida*. Many investigators have found that the WBE of the winged Hymenoptera are of

very little diagnostic value when used to skin test for sensitivity.^{23, 24} Our study would suggest that the antigens responsible for hypersensitivity states to fire ants are preserved in the preparation of the WBE. This may help to explain the excellent results obtained from hyposensitization with these preparations in the limited studies done to date.^{3, 19}

It is interesting that the *trans* isomer of the synthesized alkaloids caused a positive response in 6 of the 16 patients tested. There were no responses to the *cis* isomer. Brand and associates³¹ have suggested that unsaturated *trans* isomers favor polarity of the molecule which might in turn favor better exposure of the nitrogen on the piperidine ring. Conceivably, this could make the molecule more immunogenic. This is only speculative and remains to be proved. The fact that positive tests were obtained to a synthesized alkaloid, however, supports the view that alkaloids are at least partially responsible for hypersensitivity, perhaps acting as haptens. Since 10 of the sensitive patients had negative reactions to the two alkaloids used for the testing, we may speculate that they were sensitive to some of the other alkaloids present in the venom or that there may be other antigens in the venom, in addition to the alkaloids.

Triplett¹⁹ reported on skin testing of 7 fire ant-sensitive patients with WBE of other Hymenoptera and noted that all 7 had positive response. In addition, he reported that 2 of his 18 patients (11%) had histories of systemic reactions to other Hymenoptera.

Lockey's² studies of 4 fire ant-sensitive patients with a history of a systemic reaction following stings show positive skin tests to WBE and MSI in 3 of the 4 patients. One of these patients appears to have developed systemic reactions to both the wasp and imported fire ant.

Rhoades and colleagues³ reported positive skin tests to other (winged) Hymenoptera in 17 of 39 (44%) of fire ant-sensitive patients and noted that 8 of 104 patients (7.7%) had histories of other insect sensitivity besides fire ant sensitivity. In our study, 11 of 18 (61%) had positive skin tests to MSI and 3 of 18 (16.7%) had histories of systemic reactions to other winged Hymenoptera. The frequency of positive histories of hypersensitivity to other Hymenoptera in fire ant-sensitive patients of the four studies combined is 14 of 144 patients or 10%. This figure is much higher than the estimated incidence in the general population.^{32, 33} This, along with the combined incidence of positive skin tests to other Hymenoptera in the four studies (30 of 68 tested = 57%), suggests the presence of shared or cross-reactive antigens between fire ant and other (winged) Hymenoptera species.

This finding is unexpected in view of the marked difference in Hymenoptera venoms. Fire ant venom, as described above, consists mainly of alkaloids in aqueous suspension with only trace amounts of protein, whereas Vespida, Apida, and other ant species (Myrmicine) have vasoactive amines, polypeptides, and proteins in high concentrations. The protein components are thought to contain the most significant antigens in these venoms.³⁴ Because the WBE of fire ants appear to be good antigens, and because of the recent availability of pure venoms, the apparent successful use of hyposensitization with WBE in treatment, the increasing number of systemic reactions to fire ants, and the ability to synthesize

reactive venom components, it would appear that interest in fire ant hypersensitivity will increase and that fire ant hypersensitivity may be a useful model in studying mechanisms for all types of Hymenoptera hypersensitivity.

CONCLUSIONS

Venom, as well as WBE, of the imported fire ant appears to be a useful tool in evaluating hypersensitivity by skin testing. The WBE appeared to be as useful as the venom for skin testing. Available evidence suggests cross-reactivity or shared antigens between fire ant venom, WBE, and WBE of other Hymenoptera species. This is true even though the venoms of fire ants and other Hymenoptera are vastly different. Passive transfer studies suggest the positive skin tests are mediated by an IgE mechanism. Fire ant hypersensitivity may be a useful model for future studies of mechanisms and treatment of Hymenoptera hypersensitivity, since fire ant venom is relatively simple when compared to venoms of other species and active components of the venom can be synthesized. In vitro and immunologic studies are needed to better define the components of the venom responsible for hypersensitivity.

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REFERENCES

- 1 Barnard, J. H.: Studies of 400 Hymenoptera sting deaths in the United States, *J. ALLERGY CLIN. IMMUNOL.* 52: 259, 1973.
- 2 Lockey, R. F.: Systemic reactions to stinging ants, *J. ALLERGY CLIN. IMMUNOL.* 54: 132, 1974.
- 3 Rhoades, R. B., Schafer, W. L., Schmid, W. H., Wubben, P. F., Dozier, R. M., Townes, A. W., and Wittig, H. J.: Hypersensitivity to the imported fire ant: A report of 49 cases, *J. ALLERGY CLIN. IMMUNOL.* 56: 84, 1975.
- 4 Lofgren, C. S., Banks, W. A., and Glancey, B. M.: Biology and control of imported fire ants, *Annu. Rev. Entomol.* 20: 1, 1975.
- 5 Reisman, R. E.: Stinging insect allergy—Treatment failures, *J. ALLERGY CLIN. IMMUNOL.* 52: 257, 1973.
- 6 Sobotka, A. K., Valentine, M. D., Benton, A. W., and Lichtenstein, L. M.: Allergy to insect stings. I. Diagnosis of IgE-mediated Hymenoptera sensitivity by venom-induced histamine release, *J. ALLERGY CLIN. IMMUNOL.* 53: 170, 1974.
- 7 Sobotka, A., Franklin, R., Valentine, M., Adkinson, N. F., and Lichtenstein, L.: Honeybee venom: Phospholipase A as the major allergen, *J. ALLERGY CLIN. IMMUNOL.* 53: 103, 1974. (Abst.)
- 8 Elliott, W. B., Shepherd, G. W., and Arbesman, C. E.: Stinging insect hypersensitivity. I. Preparation of single antigens from bee venom, *J. ALLERGY CLIN. IMMUNOL.* 53: 103, 1974. (Abst.)
- 9 Charavejasarn, C. C., Wypych, J. I., Reisman, R. E., and Arbesman, C. E.: Stinging insect hypersensitivity. II. Studies of bee venom antigenicity, *J. ALLERGY CLIN. IMMUNOL.* 53: 104, 1974. (Abst.)
- 10 Reisman, R. E., Wypych, J. I., Yeagle, N., and Arbesman, C. E.: Stinging insect hypersensitivity. III. Detection and clinical significance of IgE antibodies to insect venom in man, *J. ALLERGY CLIN. IMMUNOL.* 53: 110, 1974. (Abst.)
- 11 Hoffman, D. R., and Shipman, W. H.: Allergenic analysis of bee venom fractions, *J. ALLERGY CLIN. IMMUNOL.* 55: 73, 1975. (Abst.)

- 12 Hunt, K. J., Sobotka, A., Valentine, M. D., Zeleznick, L. D., and Lichtenstein, L. M.: Diagnosis of Hymenoptera hypersensitivity by skin testing with Hymenoptera venoms. *J. ALLERGY CLIN. IMMUNOL.* 55: 74, 1975. (Abst.)
- 13 Ilea, V., Okazaki, T., Wypych, J. I., Reisman, R. E., and Arbesman, C. E.: Comparison of the antigenic properties of bee venom and phospholipase A, *J. ALLERGY CLIN. IMMUNOL.* 55: 74, 1975. (Abst.)
- 14 Yunginger, J. W., Jones, R. T., Leiferman, K. M., and Gleich, G. J.: Measurement of IgE antibody to honeybee components in beekeepers and their family members, *J. ALLERGY CLIN. IMMUNOL.* 55: 74, 1975. (Abst.)
- 15 Reisman, R. E., Light, W. C., Okazaki, T., Ilea, V., and Arbesman, C. E.: Stinging insect allergy. IV Comparison of bee venom and whole bee body extract in human insect hypersensitivity, *J. ALLERGY CLIN. IMMUNOL.* 55: 79, 1975. (Abst.)
- 16 Lichtenstein, L. M., Valentine, M. D., and Sobotka, A. K.: A case for venom treatment in anaphylactic sensitivity to Hymenoptera sting, *N. Engl. J. Med.* 290: 1223, 1974.
- 17 Busse, W. W., Reed, C. E., Lichtenstein, L. M., and Reisman, R. E.: Immunotherapy in bee-sting anaphylaxis. Use of honeybee venom, *J.A.M.A.* 231: 1154, 1975.
- 18 Blum, M. S.: Comparative exocrinology of the Formicidae, *Proceedings of VII Congress of IUSSI, London, 1973*, p. 23.
- 19 Triplett, R. F.: Sensitivity to the imported fire ant: Successful treatment with immunotherapy, *South. Med. J.* 66: 477, 1973.
- 20 MacConnell, J. G., Blum, M. S., and Fales, H. M.: Alkaloid from fire ant venom: Identification and synthesis, *Science* 168: 840, 1970.
- 21 Caro, M. R., Derbes, V. J., and Jung, R.: Skin responses to the sting of the imported fire ant (*Solenopsis saevissima*), *Arch. Dermatol.* 75: 475, 1957.
- 22 Blum, M. S., Walker, J. R., Callahan, P. S., and Novak, A. F.: Chemical, insecticidal, and antibiotic properties of fire ant venom, *Science* 128: 306, 1958.
- 23 Schwartz, H. J.: Skin sensitivity in insect hypersensitivity, *J.A.M.A.* 194: 703, 1965.
- 24 Bernton, H. S., and Brown, H.: Studies on the Hymenoptera. I. Skin reactions of normal persons to honeybee extract, *J. ALLERGY* 36: 315, 1965.
- 25 Blum, M. S., Brand, J. M., Duffield, R. M., and Snelling, R. R.: Chemistry of the venom of *Solenopsis aurea*, *Ann. Entomol. Soc. Am.* 66: 702, 1973.
- 26 Adrouny, G. A., Derbes, V. J., and Jung, R. C.: Isolation of a hemolytic component of fire ant venom, *Science* 130: 449, 1959.
- 27 Jung, R. C., Derbes, V. J., and Burch, A. D.: Skin response to solenamine, a hemolytic component of fire ant venom, *Dermatol. Trop.* 2: 241, 1963.
- 28 Buffkin, D. C., and Russell, F. E.: Some chemical and pharmacological properties of the venom of the imported fire ant, *Solenopsis saevissima richteri*, *Toxicon* 10: 526, 1972.
- 29 MacConnell, J. G., Blum, M. S., and Fales, H. M.: The chemistry of fire ant venom, *Tetrahedron* 26: 1129, 1971.
- 30 Brand, J. M., Blum, M. S., Fales, H. M., and MacConnell, J. G.: Fire ant venoms: Comparative analyses of alkaloidal components, *Toxicon* 10: 259, 1972.
- 31 Brand, J. M., Blum, M. S., and Ross, H. H.: Biochemical evolution in fire ant venoms, *Insect Biochem.* 3: 45, 1973.
- 32 Chafee, F. H.: The prevalence of bee sting allergy in an allergic population, *Acta Allergol.* 25: 292, 1970.
- 33 Settignano, G. A., and Boyd, G. K.: Prevalence of bee sting allergy in 4,992 Boy Scouts, *Acta Allergol.* 25: 286, 1970.
- 34 Habermann, E.: Bee and wasp venoms. The biochemistry and pharmacology of their peptides and enzymes are reviewed, *Science* 177: 314, 1972.