

RAST-inhibition studies of the imported fire ant *Solenopsis invicta* with whole body extracts and venom preparations

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Whole body extracts of imported fire ants (IFAWBE) are the only reagents currently available for diagnosis and immunotherapy of patients with anaphylaxis to these Hymenoptera. To characterize better IFAWBE of the species Solenopsis invicta, we evaluated the sera of 29 patients with systemic or large local reactions to imported fire ant (IFA) stings. Forty-eight percent (14/29) of these sting-sensitive patients were IFAWBE RAST positive ($\geq 6\%$ binding of total radioactivity added). With a pool of sera with an initial IFAWBE-RAST value of 16.2% binding, we evaluated RAST inhibition by IFA venom (IFAV), IFAWBE, and the venom component, transpiperidine. Maximum RAST inhibition obtained was 84% with 300 $\mu\text{g/ml}$ of IFAV, 95% with 5 mg/ml of protein IFAWBE, and insignificant with undiluted transpiperidine. We conclude that IFAWBE contains large quantities of immunoreactive venom components other than transpiperidine and that the allergenicity of IFAWBE and venom resides in the small amount of protein present in IFAV. (J ALLERGY CLIN IMMUNOL 1988;81:1096-1100.)

Allergic reactions to stings of the two IFA species, *Solenopsis invicta* and *S. richteri*, are the predominant form of insect sting anaphylaxis in the southeastern area of the United States. As many as 58% of the population in areas like New Orleans are stung each year, and up to 5% of subjects stung require medical attention.¹⁻⁴ Anaphylactic reactions may occur in up to 0.6% of IFA sting victims.⁵

Little is known about the antigenic and allergenic properties of IFAV. Unlike venom from other Hymenoptera, which is 70% protein, IFAV appears to contain only 2% to 5% protein.⁶ The major constituents are 2,6-disubstituted-piperidines, pri-

Abbreviations used

IFA:	Imported fire ant
IFAV:	Imported fire ant venom
IFAWBE:	Imported fire ant whole body extract
PBS:	Phosphate-buffered saline

marily trans-2-methyl-6-*N*-undecylpiperidine or its enantiomer.⁷⁻⁹

IFAWBEs continue to be used for clinical purposes since it is difficult and expensive to obtain pure venom.^{10, 11} In studies in which IFA-allergic subjects were skin tested with IFAV and IFAWBE, 91% of the participants had immediate dermal reactions to IFAV, but only 53% reacted to IFAWBE.¹² These data are in concordance with our crossed immunoelectrophoresis studies that demonstrated that allergenicity of IFAWBE results from the IFAV and that not all IFAV components are present in IFAWBE.¹³

In this investigation, we have further characterized the components of IFAWBE with the technique of RAST inhibition. These studies demonstrate that IFAWBE contains immunoreactive venom components other than transpiperidine and establish the central position of venom proteins in human hypersensitivity reactions to IFA. This finding emphasizes the importance of the protein constituents of both IFAWBE and IFAV in future studies.

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In conducting the research described in this article, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

MATERIAL AND METHODS

IFA antigens

A standard reference IFAWBE was prepared by a method developed in our laboratory.¹³ Briefly, 12 IFA colonies were collected from the New Orleans area into separate plastic bags and were stored at 4° C. With a suction apparatus, live IFA were obtained from these colonies and stored at -70° C until venom was extracted. IFAs collected from each colony were identified by an entomologist, Mr. M. Andis, New Orleans Mosquito Control, New Orleans, La., before pooling for extraction. All 12 colonies were *S. invicta*, the most common species of IFA.

IFAWBE was prepared by extracting 25 gm of IFA in 250 ml of PBS (pH 7.4) in a Waring (Waring Products Div., New Hartford, Conn.) blender with three 1-minute bursts. The mixture was extracted overnight at 4° C, centrifuged at 16,000 g for 20 minutes to remove particulate material, and concentrated on an Amicon (Amicon Div./W.R. Grace & Co., Danvers, Mass.) UM-05 ultrafilter (exclusion limit 500 daltons) to a final volume of approximately 30 ml. The final concentration of 54.5 mg of dry weight per milliliter contained 28.93 mg/ml of protein. This material was sterile filtered, aliquoted, and stored at -70° C until use.

Venom, which had been collected from *S. invicta* by an electric-shock method, was a kind gift of Mr. Miles Guralnick, Vespa Laboratories, Spring Mills, Pa. Transpiperidine was prepared by Dr. T. Jones, William and Mary College, Va., with previously reported methods.¹⁴

Study subjects

Clinical data and sera, from 29 patients who attended Tulane Clinical Immunology Service or other allergy clinics in the New Orleans area or the allergy clinic, Brooke Army Medical Center, Fort Sam Houston, Texas, for evaluation of reactions to IFA, were used for this study. All these patients had experienced systemic allergic reactions (urticaria, asthma, and/or vasomotor collapse) or large local reactions¹ after IFA sting. Skin testing was performed in most patients, but reagents from different commercial suppliers were used and the data were not suitable for analysis. Some of the individuals, although they had a strong history of IFA allergy, were skin test negative. Sera from five normal individuals with no history of reactions to IFA stings were used for control purposes.

Total serum IgE determinations

Levels of total serum IgE in patients' sera were determined with a commercially available radioimmunoassay for IgE (Kallestad Laboratories, Austin, Texas). This test is a double-antibody assay with a sensitivity of 5 IU/ml. The normal range of IgE values for our laboratory is 20 to 300 IU/ml.

RAST for IFAWBE-specific IgE antibodies

Sera were tested by RAST to determine levels of specific IgE antibodies to IFAWBE. For the RAST, our reference

standard IFAWBE was dialyzed (molecular weight cutoff, 3500) overnight in pH 8.0, 0.1 mol/L of borate buffer, and, based on preliminary experiments to determine optimal binding, 10 mg/ml of protein was coupled to cyanogen bromide-activated paper disks for 6 hours at room temperature on a clinical rotator, washed three times with 1.0 mol/L of ethanolamine, pH 8.0, stored overnight at 4° C in ethanolamine, and then washed three times with RAST assay buffer containing 0.2% of bovine serum albumin.¹⁵ For the test, 50 µl of test serum plus 50 µl of PBS were incubated overnight with an IFAWBE disk, in duplicate. After three washes with 2.5 ml of physiologic saline to remove unreacted serum, disks were incubated overnight with 100 µl of ¹²⁵I-labeled anti-IgE antiserum, washed with saline to remove reacted material, and counted in a gamma counter to determine radioactivity in counts per minute bound to the disks. The sample counting time was determined by counting 100 µl of radiolabeled material (total activity) for a time necessary to reach 25,000 counts.

For evaluation of results, a RAST percent binding (percent of total radioactivity added) was calculated.¹⁶ Based on results from studies with control sera, RAST percent binding of 3% to 6% was considered borderline and RAST percent binding ≥6% was considered definite positive.

RAST-inhibition testing with IFAWBE, IFAV, and transpiperidine

For RAST-inhibition testing, a pool of 18 serum samples from individuals with IFA-specific IgE antibodies was prepared. Samples were from individuals enrolled in this study and from other individuals on whom insufficient information was available for inclusion in the study. Fifty microliters of the pool (IFAWBE RAST percent binding, 16.2%) was coin-cubated overnight at 22° C with 50 µl of a range of dilutions of IFAWBE, IFAV, transpiperidine, or PBS, and with IFAWBE-coupled disks, in duplicate. For IFAV, concentrations were 3, 6, 30, 60, 300, and 600 µg/ml; for IFAWBE, the concentrations were 0.0032, 0.016, 0.08, 0.4, 2.0, 5.0, and 10 mg/ml; for transpiperidine, the dilutions were 1:10, 1:5, and undiluted. After three washes with 2.5 ml of physiologic saline to remove unreacted serum or inhibitor, disks were incubated overnight with 100 µl of ¹²⁵I-labeled anti-IgE antiserum, washed with saline to remove unreacted material, and counted in a gamma counter to determine radioactivity bound to disks. The time of counting of the samples was determined by counting 100 µl of radiolabeled material (total activity) for a time necessary to reach 25,000 counts. After log₁₀ transformation, percent inhibition of IFAWBE RAST by the different dilutions of IFAWBE, IFAV, and transpiperidine was calculated, and the results were plotted. The maximal inhibiting concentration and 50% inhibition concentration were calculated from the graph.

Statistics

Before analysis, log₁₀ transformation was performed to normalize values of IFAWBE RAST percent binding, total

TABLE I. RAST results with IFAWBE*

Patient	RAST	Total IgE
1	6.3	61.0
2	15.0	445.0
3	1.2	13.0
4	2.0	10.0
5	4.6	205.0
6	33.7	270.0
7	18.5	91.0
8	3.8	200.0
9	19.2	305.0
10	4.3	147.0
11	0.9	5.0
12	16.0	285.0
13	11.5	93.0
14	21.5	127.0
15	6.7	39.0
16	13.5	796.0
17	0.8	86.0
18	2.3	9.0
19	16.4	182.0
20	3.4	72.0
21	10.3	194.0
22	4.3	31.0
23	1.9	95.0
24	1.6	144.0
25	5.1	124.0
26	10.4	105.0
27	3.0	136.0
28	5.7	82.0
29	22.9	184.0

*RAST values are presented as percent binding of total radioactivity added. Total IgE values are presented in international units per milliliter.

IgE, and concentration of inhibiting IFA antigens. Linear regression analysis was performed with the Statpro statistical computer program.¹⁷ Tests for significant slopes were performed according to Kleinbaum and Kupper.¹⁸ Tests for parallel slopes in RAST-inhibition assays used the Pharmacological Calculations computer program.¹⁹

RESULTS

Clinical data on study participants

The study population consisted of 13 male (mean age of 44.2 years) and 16 female (mean age 29.2 years) subjects. Twenty-two subjects were white, two were black, one was Hispanic, and four were Oriental or other races. The mean time of residence in the gulf south area when subjects were observed by the study physician, was 11.6 years. Eleven patients had histories of atopic disease (three, asthma and eight, hay fever), but the remaining 18 patients had no history of allergic disease. Twenty-four subjects had experienced systemic reactions, and five had had large local

reactions. All 29 subjects had experienced previous IFA stings. One individual reported a previous IFA-related systemic reaction, two reported previous large local reactions, 11 reported previous stings with pustules only, three were unable to classify reactions, and 12 subjects reported no previous reactions.

Total IgE levels and RAST with IFAWBE

Total IgE levels of the 29 patients sera ranged from 5 to 796 IU/ml. The geometric mean value was 94.3 ± 1.25 SEM IU/ml. Levels in control sera ranged from 5 to 214 IU/ml with a geometric mean of 54.7 ± 1.95 . Total IgE levels in the patient group were not significantly greater than control levels ($0.05 < p < 0.10$).

Percent serum IFAWBE RAST binding of patients' sera ranged from a low of 0.8% to a high of 33.7% (Table I). Fourteen of the 29 sera (48%) elicited a RAST percent binding $\geq 6\%$. Percent serum IFAWBE RAST percent binding of the five control sera ranged from 0.7% to 1.6%. Linear regression analysis demonstrated that there was a statistically significant correlation ($r = 0.65$; $p < 0.001$) between log total IgE levels and log IFAWBE RAST percent binding (Fig. 1), indicating a relationship between IFA sensitivity and total IgE in this population.

RAST-inhibition studies with IFAWBE, IFAY, and transpiperidine

The results of inhibition studies of IFAWBE RAST by IFAWBE, IFAY, and transpiperidine are illustrated in Fig. 2. IFAWBE elicited maximal inhibition (95%) when 5 mg of protein per milliliter was added and 50% inhibition with 0.068 mg of protein per milliliter. IFAY elicited maximal inhibition (84%) when 300 $\mu\text{g/ml}$ was added and 50% inhibition with 6 $\mu\text{g/ml}$. The slopes of the inhibition lines for IFAWBE and IFAY were not different (r for IFAWBE, 0.953; r for IFAY, 0.969; $p > 0.05$) and therefore were considered parallel. The intercepts of both preparations were also not significantly different ($p > 0.05$). Transpiperidine had no inhibitory effect on IFAWBE RAST levels (no significant regression, $p >> 0.05$).

DISCUSSION

The results of this study demonstrate that only 48% of individuals with clinical history of an allergic reaction after IFA sting had circulating IgE antibodies to IFAWBE detectably by RAST. Results of RAST-inhibition studies demonstrated specificity of these IgE antibodies for IFAY. A synthetic venom alkaloid, transpiperidine, did not inhibit the IFAWBE RAST, indicating that the specificity of the IgE antibodies was to some component of IFAY other than piperidine.

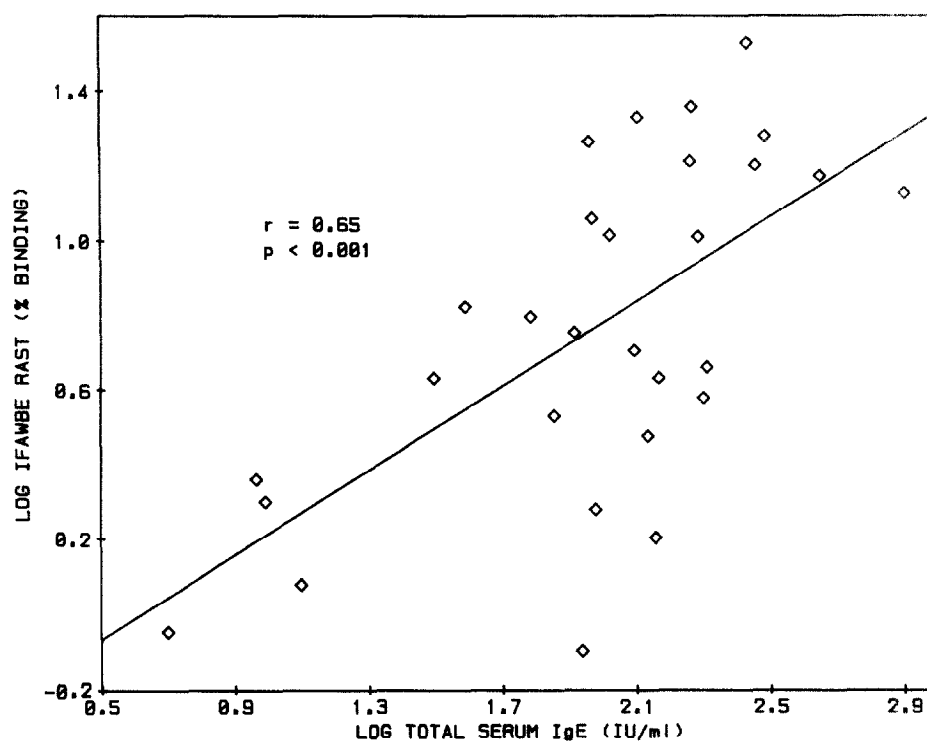


FIG. 1. Linear regression of log total serum IgE and log RAST percent binding with IFAWBE.

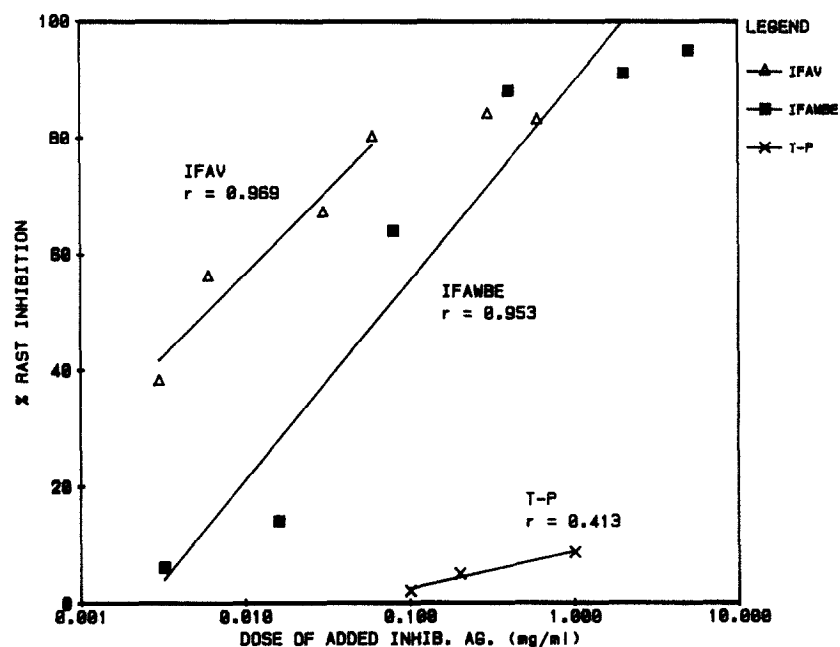


FIG. 2. RAST inhibition of IFAWBE RAST by IFAV, IFAWBE, and transpiperidine.

A statistically significant correlation was obtained between IFAWBE RAST values and total circulating IgE.

Previous studies have suggested that IFAWBEs are less than optimal reagents for the detection of IgE

responsible for IFA sting reactions. Strom et al.¹² reported that, when strict criteria were applied, only 53% of sting-sensitive patients skin tested with IFAWBE had positive immediate hypersensitivity skin tests, whereas 91% of the same group reacted to IFAV.

Our in vitro data demonstrating that only 48% of sting-sensitive patients had positive IFAWBE RAST are in concordance with the previous findings. The RAST-inhibition data presented here help clarify these observations by demonstrating that the allergenic activity of IFAWBE resides in IFAV component(s). Differences in the levels of RAST inhibition by IFA preparations probably reflect the concentration and purity of IFAV in the inhibiting preparations. The specificity of patient IgE for the venom component of IFA suggests that IFAV may be a better reagent for either in vivo or in vitro diagnostic testing. Furthermore, the data in this study support our previous findings, with crossed immunoelectrophoresis techniques, that the primary allergenicity of IFAV resides in the nonalkaloidal component(s) of IFAV.¹³

Our finding that transpiperidine does not inhibit the IFAWBE RAST supports similar findings with crossed immunoelectrophoresis techniques that suggest that allergenicity to IFA resides in the <5% nonpiperidine protein component(s) of IFAV. A recent study by Hannan et al.²⁰ indicates that the specificity of IgE antibodies to IFAV is to phospholipase A, which is present in trace amounts of IFAV. Specificity of IgE antibodies in other Hymenoptera venom has also been demonstrated to be to phospholipase A components of their venom,² although the chemical is present in larger amounts in the venom of these species.

These findings indicate that IFAV may be the most appropriate reagent for clinical and laboratory diagnosis of IFA allergy. Although it is not addressed here, these data also suggest that IFAV may be optimal for use in individuals requiring immunotherapy for IFA sensitivity. Currently, because of the difficulty in obtaining IFAV, only IFAWBE is available for general use. Until techniques are developed to obtain large enough quantities of IFAV for clinical use, it would appear appropriate to confirm the presence of IFAV in commercial IFAWBE by in vitro methods before use. This would decrease the possibility for false negative skin testing and RAST results that now exists with IFAWBE. Further studies are necessary to identify and characterize the specific allergenic components in IFAV and to confirm the efficacy of IFAV for diagnosis and treatment of IFA sensitivity.

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