

Fire ant venom hypersensitivity

I. Comparison of fire ant venom and whole body extract in the diagnosis of fire ant allergy

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Levels of IgE antibody to fire ant whole body extract (FA-WBE) and fire ant venom (FAV) were evaluated in 60 patients with clinical histories of fire ant sting hypersensitivity and 11 frequently stung individuals without significant clinical reactivity to fire ant. An overlap of levels of IgE antibody to FA-WBE and FAV was seen between groups of patients with increasingly severe clinical reactivity; however, the group mean IgE antibody level of patients without significant clinical reactivity differed markedly from the group means of patients with more severe clinical reactivity ($p < 0.001$). In 31 patients a good correlation ($r = 0.6935$) was found comparing FA-WBE IgE antibody level with FA-WBE skin tests, and in 26 patients a good correlation ($r = 0.8564$) was obtained comparing FAV IgE antibody levels with FAV skin tests. An excellent Spearman rank correlation coefficient ($r = 0.9197$) was found on comparison of FAV and FA-WBE IgE antibody levels in 71 individuals. Cross-reactivity of FAV and FA-WBE was demonstrated by the ability of these reagents to competitively inhibit the binding of IgE antibody to solid-phase FA-WBE and FAV in the radioallergosorbent test (RAST). From these results we conclude that FAV and FA-WBE both contain relevant allergens important in fire ant-allergic individuals and that skin tests and RAST are both valid as diagnostic tests for fire ant allergy. Finally, there is a good correlation between RAST and skin tests using FA-WBE or FAV as test reagents, since these reagents are highly cross-reactive as demonstrated by their ability to inhibit each other in the RAST. (J ALLERGY CLIN IMMUNOL 71:448, 1983.)

Efficacy of whole body extract for diagnosis and treatment of Hymenoptera sting sensitivity has been placed in serious doubt¹⁻³; however, for one family of the order Hymenoptera, Formicidae (ants), whole body extract is presently being employed as the standard material for diagnosis and treatment for sting sensitivity. *Solenopsis invicta*, or the IFA, accounts for the majority of serious ant sting sensitivity. At present, 20 to 30 million people inhabit IFA-infested areas, and at a reported sting rate of 13.8% per month,⁴ 2.5 million individuals are stung on monthly

basis. Sixteen percent of stung individuals presenting for medical treatment for IFA sting display systemic symptoms, and it has been suggested that 0.61% of these individuals experience a generalized systemic anaphylaxis.⁵ IFA sting deaths, thought to be exceedingly rare, have also been reported with increasing frequency.⁶

FA-WBE for diagnosis and treatment of IFA hypersensitivity was empirically instituted in 1973 by Triplett,⁷ and Lockey and Rhodes⁸⁻¹⁰ found FA-WBE skin tests a useful procedure in the diagnosis of IFA allergy. Other authors, however, have found FA-WBE skin tests unreliable for the diagnosis of IFA hypersensitivity.¹¹ James et al.^{12, 13} found some mildly elevated RAST levels to FAV and FA-WBE in patients with positive skin tests to these respective reagents, while Baer¹⁴ showed high RAST levels to FAV and FA-WBE in a few IFA-sensitive individuals. These prior studies, however, have not adequately compared FAV and FA-WBE skin tests and RAST for efficacy in the diagnosis of IFA allergy.

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Abbreviations used

IFA:	Imported fire ant
FAV:	Fire ant venom
FA-WBE:	Fire ant whole body extract
RAST:	Radioallergosorbent test
RIU:	RAST inhibition units

Our studies indicate that FAV and FA-WBE are equally useful reagents in the diagnosis of IFA allergy, both by *in vivo* (skin tests) or *in vitro* (RAST) testing, and that FAV and FA-WBE contain similar allergens.

MATERIALS AND METHODS

Patients

Sixty IFA-allergic patients from our area were identified at our clinic or by referral over a 2 yr period. Patients ranged in age from 18 mo to 76 yr and all patients had typical IFA pustular lesions and histories of large local or systemic reactions after IFA stings. None of these patients had received prior whole body extract immunotherapy. Additionally, 11 frequently stung nonallergic individuals were identified from our patient population and served as controls. Patient reactions to IFA stings were assigned to one of six categories (local, large local, urticarial, angioedema, asthmatic, or shock) on the basis of the most severe symptom found in the history. Large local reactions were defined as swelling extending over two contiguous joint spaces. Shock in all instances was documented as occurring with hypotensive blood pressures obtained at the office or emergency room in association with loss of, or altered, consciousness.

FAV

FAV was obtained by the method of Blum et al.¹⁵ In brief, venom was obtained by stroking the IFA abdomen with a 2 μ l capillary tube and was stored at -70° C prior to use. Venom was reconstituted by crushing five (2 μ l) capillary tubes in 1 ml of sterile saline with 0.5% human serum albumin (1:100 v/v). The extract was then passed through a 0.22 μ m millipore filter and tested for sterility prior to use.

FA-WBE

FA-WBE (1:10 v/v dilution) was purchased commercially from Hollister-Stier Laboratories, Spokane, Wash.

Skin tests

Skin tests were performed with FA-WBE or FAV by injecting 0.02 ml intracutaneously in 10-fold dilutions from a 10³ to a 10⁷ v/v dilution in sterile buffered isotonic saline (Travenol Laboratories, Deerfield, Ill.) using 0.5% human serum albumin (Sigma Chemical Co., St. Louis, Mo.) as a macromolecular stabilizing agent. Skin tests with FA-WBE and FAV were first performed in 10 nonallergic individuals to determine irritant potential. Six of 10 nonallergic individuals reacted with a positive skin-test response to FAV at

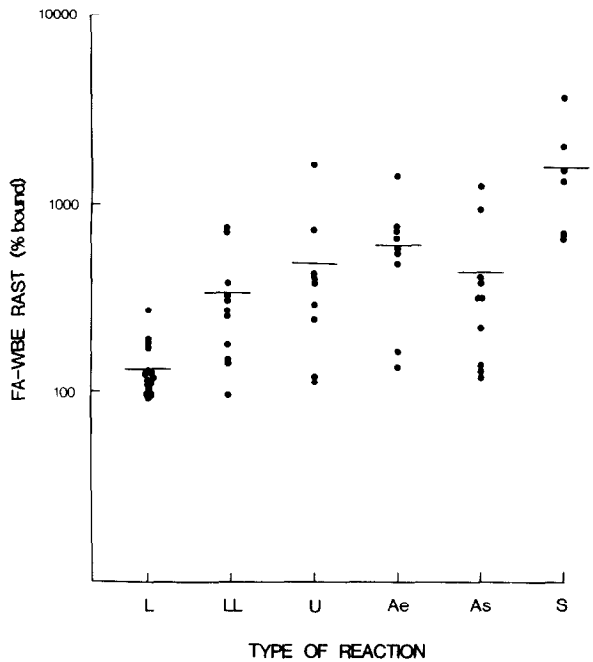


FIG. 1. Type of reaction vs FA-WBE RAST (% bound; n = 63). L, Local; LL, large local; U, urticaria; Ae, angioedema; As, asthmatic; S, shock. FA-WBE RAST on the ordinate is indicated on a log scale as percent of a negative control patient:

$$\frac{\text{Patient RAST level}}{\text{Negative control RAST level}} \times 100 = \% \text{ bound}$$

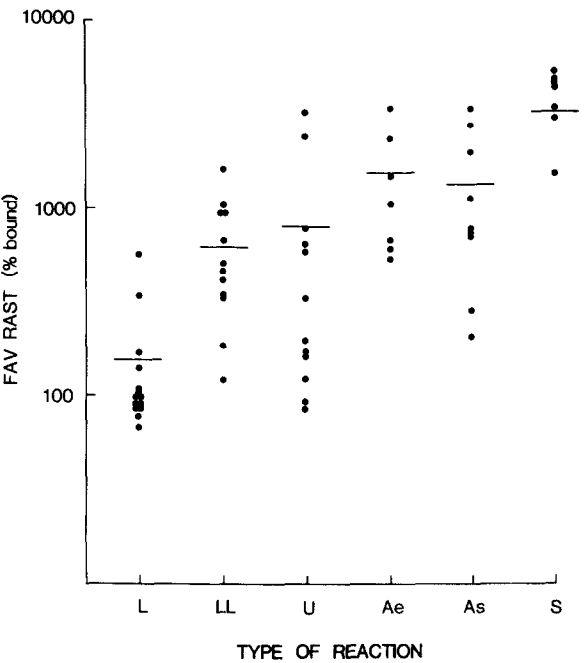


FIG. 2. Type of reaction vs FAV RAST (% bound; n = 61). Abbreviations, abscissa, and ordinate are as in Fig. 1.

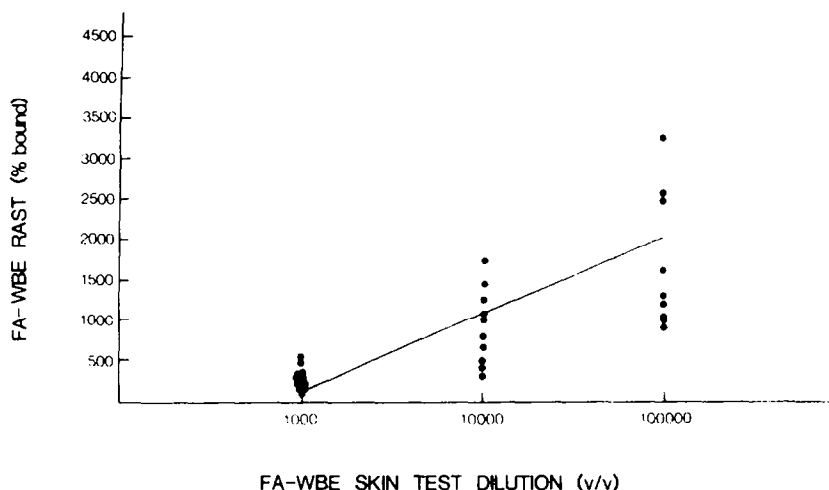


FIG. 3. FA-WBE skin-test dilution (v/v) vs FA-WBE RAST (% bound; $n = 31$). Abscissa shows on a log scale skin-test dilution (v/v). Ordinate shows FA-WBE RAST (% bound) as in Fig. 1 on a linear scale.

a 10^3 v/v dilution and one of 10 reacted at a 10^4 v/v dilution. With FA-WBE, two of 10 nonallergic individuals reacted with a positive skin-test response at a 10^3 v/v dilution. Reactions were measured at 15 min. Serial 10-fold dilutions were performed and skin-test reactivity was determined as the lowest concentration of FA-WBE or FAV to give a 7 to 9 mm wheal. Appropriate histamine and saline control tests were performed in each patient.

RAST

IgE antibodies were measured by the RAST.¹⁶ Solid-phase antigens were prepared by covalently linking FAV or FA-WBE to 6.3 mm filter paper discs (Schleicher and Schuell, Keene, N.H.) as described previously,¹⁷ with the following modifications: (1) 4 ml of a 1:100 dilution of FAV in 0.1M K_2HPO_4 - KH_2PO_4 , pH 8.0, were coupled to 100 activated filter paper discs; (2) 5 ml of FA-WBE were coupled to 100 activated filter paper discs. Direct RAST and RAST inhibitions were performed as previously described,^{17, 18} with the following modifications: Inhibition of the binding of IgE antibody from a high-binding IFA-allergic patient to FAV or FA-WBE cellulose discs was accomplished with a fluid-phase FAV or FA-WBE. To compare the degree of inhibition of FAV or FA-WBE, inhibitor added was expressed in terms of RIU, with 1 RIU equal to the amount of fluid-phase FAV (FA-WBE) to cause 50% inhibition of the binding of the IgE antibody from a high-binding IFA-allergic patient to disc FAV (FA-WBE).

Statistical analyses

Geometric mean IgE antibody levels between groups of patients were compared by the *t* test. Correlation coefficients of least-squares regression lines were obtained to compare skin-test and RAST results and a Spearman rank correlation coefficient was used to compare FAV and FA-WBE RAST results.

RESULTS

Levels of IgE antibody to FA-WBE and FAV in the serum on nontreated IFA-allergic and frequently stung nonallergic control patients were compared with severity of reaction (Figs. 1 and 2). As a group, patients with large local or systemic reactions (urticaria, angioedema, asthma, or shock) had statistically higher levels of IgE antibody as measured by RAST to FA-WBE (Fig. 1) and FAV (Fig. 2) than our control patients who experienced only local reactions ($p < 0.005$); however, as in other insect-sensitive patients, an overlap of IgE levels was seen between frequently stung nonallergic and allergic patients. No statistical difference of IgE level to FA-WBE or FAV was found in patients with increasingly severe reactivity (large local or systemic reactions).

As is seen in Figs. 3 and 4, excellent correlations were found between IgE antibody level and serial skin-test dilution level with FA-WBE ($r = 0.6935$) and FAV ($r = 0.8569$) as test reagents in our patients. Because of the irritant potential of FAV and to a lesser degree FA-WBE at a 10^3 v/v dilution, no attempt was made to distinguish between individuals with positive skin tests at a 10^3 v/v dilution of FA-WBE or FAV from those who did not react with a positive skin-test response at this level. These individuals were all thought to be negative skin-test reactors and grouped at 10^3 v/v (Figs. 3 and 4).

In Fig. 5, FAV RAST was compared with FA-WBE RAST in 60 IFA-allergic and 11 nonallergic frequently stung control patients. An excellent Spearman rank correlation coefficient of 0.9197 was obtained.

Cross-reactivity of FAV and FA-WBE were com-

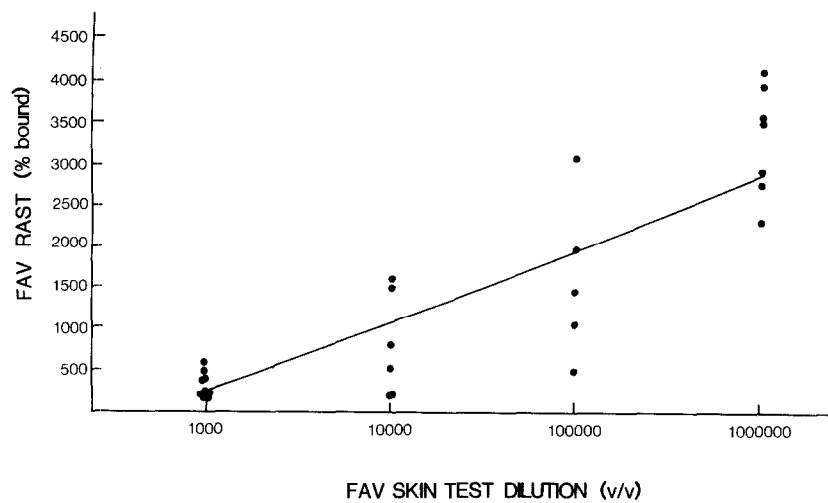


FIG. 4. FAV skin-test dilution (v/v) vs FAV RAST (% bound; n = 26). Abscissa and ordinate are as in Fig. 3.

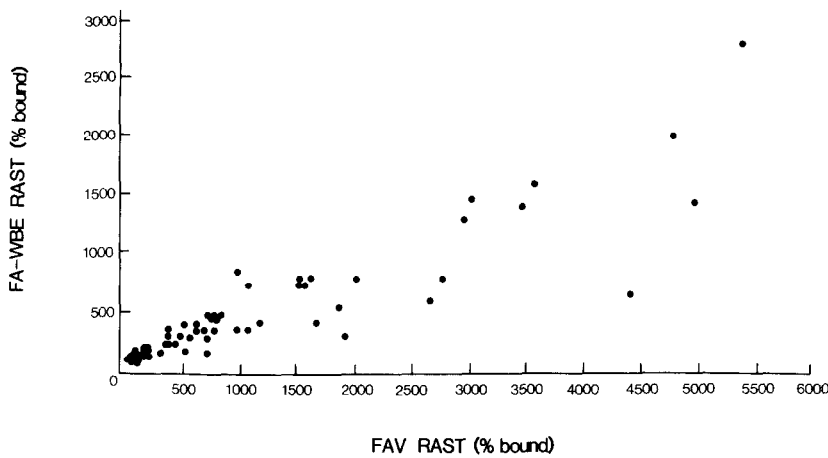


FIG. 5. FAV RAST (% bound) vs FA-WBE RAST (% bound; n = 71). Abscissa and ordinate on a linear scale with % bound as in Fig. 1.

pared by RAST inhibition (Figs. 6 and 7). In the situation with disc FAV inhibited with fluid-phase FA-WBE, 90 RIU were required for 50% inhibition of the binding of IgE antibody from a high-binding IFA-allergic patient, while only 8 RIU were required for 50% inhibition in the same situation with disc FA-WBE inhibited with fluid FAV.

DISCUSSION

The role of FAV and FA-WBE in the diagnosis of FA allergy has not been clearly elucidated. Several investigators⁷⁻¹³ have noted the usefulness of FA-WBE in the diagnosis of IFA allergy. Triplett⁷ found “positive” reactivity to FA-WBE in all but one of 18 FA allergic patients, while Rhodes et al.,¹⁰ in a study of 104 IFA-allergic patients, found FA-WBE skin testing a “useful procedure.” Other authors^{11, 12} have

shown IFA-WBE to be a poor material as compared with FAV for the diagnosis of IFA allergy. Only 33% of patients with anaphylactic reactions to IFA had positive IFA-WBE skin tests, while 92% had positive skin tests with FAV in a study by Strom et al.¹¹ Of IFA-allergic patients studied by James et al.,¹² 78% reacted to skin tests with mixed FA-WBE and 94.% reacted to FAV. However, it is disconcerting to note that nine of 18 patients (50%) who had minimal or no reactivity to FA-WBE (i.e., 10³ v/v dilution or less) had positive skin tests to FAV. Furthermore, in a RAST procedure (the methods of which were not described), only 17% of these patients had a level of IgE antibody to FA-WBE, which was greater than four times negative control binding, and 39% reacted in a similar fashion to FAV.¹³

In our studies we contend that both FAV and

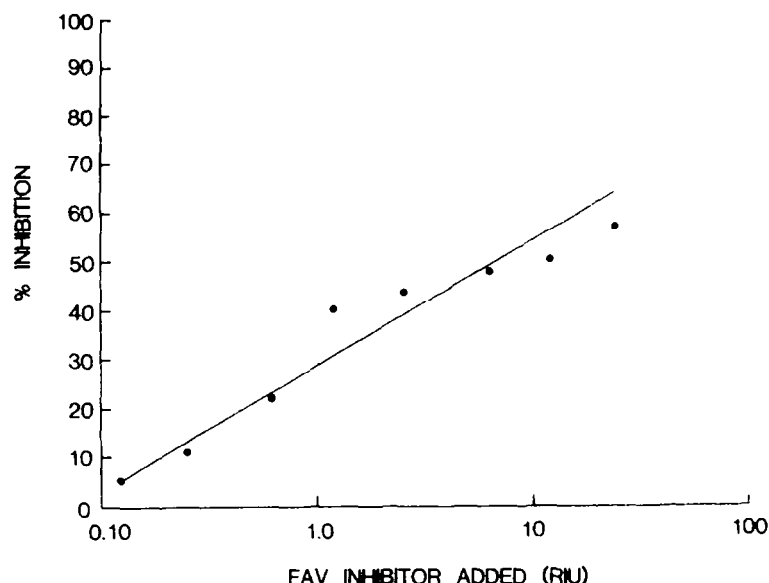


FIG. 6. FAV inhibitor added (RIU) vs percent inhibition. IgE antibody to IFA from a high-binding IFA-allergic donor is inhibited from binding to a cellulose disc covalently linked to FA-WBE, by the addition of fluid-phase FAV in the first stage of the RAST. Abscissa shows FAV added; ordinate shows percent RAST inhibition.

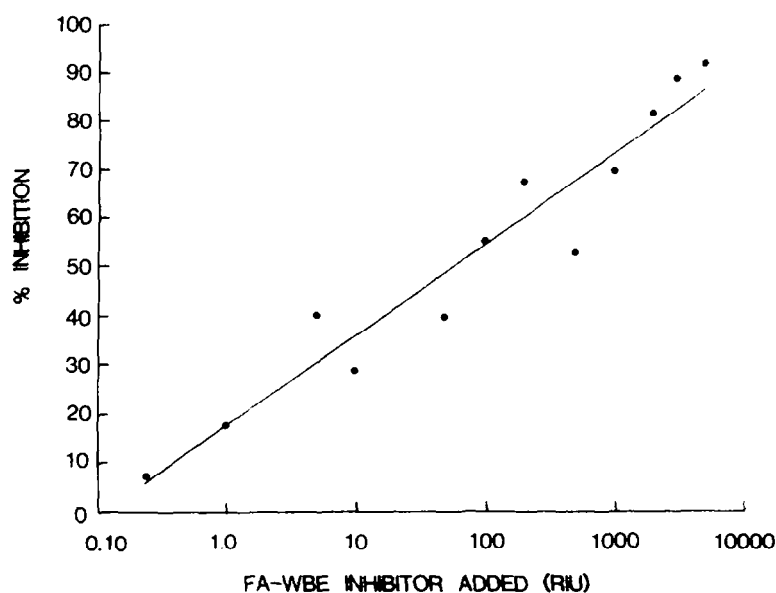


FIG. 7. FA-WBE inhibitor added (RIU) vs percent inhibition. IgE antibody to FA from a high-binding IFA-allergic donor is inhibited from binding to a cellulose disc covalently linked to FAV by the addition of fluid-phase FA-WBE in the first stage of the RAST. Abscissa shows FA-WBE added (as in Fig. 7); ordinate shows percent RAST inhibition.

FA-WBE contain relevant allergens important in IFA-allergic individuals and that skin tests and RAST are both valid diagnostic tests for IFA allergy. As in other insect-allergic individuals, there is an overlap of RAST levels in frequently stung nonallergic and allergic individuals, and the clinical severity of an IFA

sting reaction cannot be predicted by the RAST level. Skin tests with FA-WBE or FAV at a 10^3 v/v dilution are of little value because irritant positive reactions are common and an occasional irritant response is seen in patients with FAV at a 10^4 v/v dilution. As with other extracts, standardization of FA-WBE is

important and variability of positive skin-test results with FA-WBE as seen by other authors may reflect variability of allergenicity of different FA-WBEs.

In summary, there is a good correlation between skin tests and RAST results using FA-WBE or FAV as test reagents and there was a very high degree of similarity when comparing IgE levels to FAV and FA-WBE in frequently stung IFA-nonallergic and IFA-allergic individuals. Finally, FAV and FA-WBE share cross-reactive allergens as demonstrated by their ability to inhibit each other in the RAST; however, FAV appears approximately 10 times more potent than FA-WBE in allergenic potential.

Addendum

Since submission for publication, serum from five other high-binding IFA-allergic patients have shown inhibition curves similar to those of our initial patient (Figs. 6 and 7).

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