

Imported fire ant immunotherapy: Effectiveness of whole body extracts

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The purpose of this study was to determine if whole body extract (WBE) immunotherapy for imported fire ant (IFA) hypersensitivity is effective. This evaluation was carried out by retrospectively interviewing 76 patients with a history of generalized allergic reactions to IFA stings and positive skin tests to IFA-WBE. The study groups consisted of 65 patients on immunotherapy and 11 similar patients who were not treated for various reasons. In addition, an IFA sting challenge was performed in 30 volunteers of the 65 patients on immunotherapy. The retrospective review showed that of the 65 patients on immunotherapy there had been 112 subsequent field-sting episodes in 47 patients. Only one sting episode in this group (2.1%) produced an anaphylactic reaction. Six of the 11 patients not on immunotherapy have had subsequent field re-sting episodes, and each has had a systemic reaction. Repeat skin testing on 31 of the 65 patients in the immunotherapy group showed persistent positive responses in five (16%), but each was at a lower dilution than initially. Responses of the other 26 of the 31 patients who had skin testing had become negative. The four untreated patients who were available for skin testing continued to have positive responses at comparable dilutions on skin testing. Sting challenges carried out on 30 volunteers from the 65 patients (all from the 31 who had repeat skin tests) on immunotherapy resulted in only local reactions. Therefore it appears IFA-WBE is effective in decreasing the incidence of anaphylaxis during subsequent field stings; reducing specific immunoglobulin E as demonstrated by skin testing; and protecting against systemic reactions provoked by a sting challenge with a single IFA. (J ALLERGY CLIN IMMUNOL 1992;90:210-5.)

Key words: Immunotherapy, imported fire ant, Hymenoptera, whole body extract

Since their importation through Mobile, Ala., in the late 1920s the imported fire ant (IFA) species have progressively invaded and migrated throughout the southeastern United States (Fig. 1). Humanity has been at odds with the IFA since that time, often over agricultural or livestock issues, but not infrequently over the reactions their stings cause in man. The most severe of these reactions, anaphylaxis, continues to be a problem in the southeastern United States. A survey conducted by the Fire Ant Subcommittee of the American Academy of Allergy and Immunology

Abbreviations used

IFA: Imported fire ant
WBE: Whole body extract

reported that IFA anaphylaxis has been responsible for more than 32 deaths in the southeastern United States.¹ Immunotherapy is recommended for individuals who have had a systemic allergic reaction to IFA stings, since IFA whole body extract (WBE) has been shown to contain significant amounts of venom antigen, and venom immunotherapy has been shown to be highly effective with other Hymenoptera.²⁻⁵ However, the efficacy of immunotherapy for fire ant hypersensitivity has not been studied in double-blind placebo-controlled trials as has been done for the winged Hymenoptera species.⁵ Also, because of difficulties in collecting venom from IFA (venom protein per fire ant is approximately 1000 times less than protein per insect for the other Hymenoptera [10 to 100 ng vs 50

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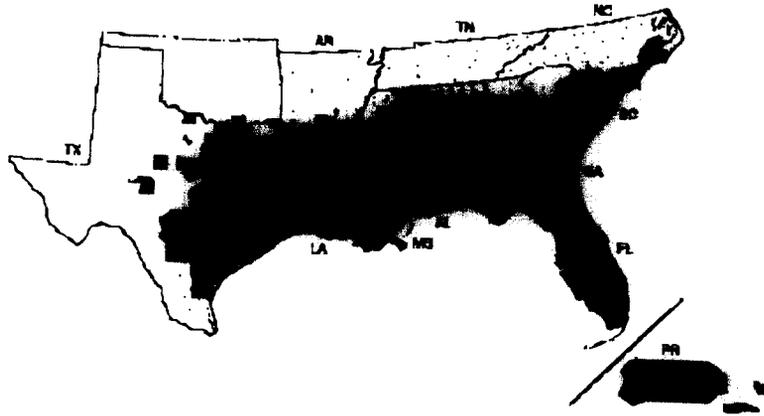


FIG. 1. U. S. Department of Agriculture map shows current areas of IFA infestation.

μg),⁶ the only commercially available preparation for IFA immunotherapy is WBE. There is reason for concern about the possible lack of protection from this treatment because of the proven ineffectiveness of WBE therapy for other vespid sting anaphylaxis. Hunt et al.⁵ compared the effectiveness of WBE with venom extract immunotherapy for other Hymenoptera (honey bee and yellow jacket). They demonstrated that WBE immunotherapy for Hymenoptera hypersensitivity was equivalent to placebo. The only therapy that showed protection was venom immunotherapy. Because of the work of Hunt et al.⁵ with other Hymenoptera, and because of reports of IFA-WBE immunotherapy failure by Paull and Coghlan,⁷ continued uncertainty exists over its usefulness. Nevertheless, it remains the only currently available and accepted form of therapy.

The purpose of this study was to examine the effectiveness of WBE immunotherapy for IFA hypersensitivity in two ways. The first was to perform a comparative analysis of the effect of WBE immunotherapy on the course of 65 patients on immunotherapy who are sensitive to IFA with the course of 11 sensitive patients who were not treated with immunotherapy at the patients' requests. The second method used was a deliberate sting challenge with a single IFA in 30 of the patients on immunotherapy.

PATIENTS IN THE STUDY AND METHODS

Subjects

The initial survey was carried out through a retrospective chart review of all patients currently followed in our clinic who had been diagnosed as having systemic allergic reactions to IFA. All patients with histories of anaphylaxis to IFA sting and positive skin tests to IFA were contacted and had clinic interviews. In this group of 76 patients, 65 had agreed to the recommendations to start immunotherapy, whereas 11 had declined. Thirty-five (31 on maintenance

immunotherapy, 4 not on immunotherapy) agreed to additional testing after their interview. Follow-up skin tests with Hollister-Stier (Hollister-Stier Laboratories, Spokane, Wash.) IFA-WBE was carried out in these 35 patients by following a standard format as described below. Also, 30 of the 31 patients on IFA-WBE immunotherapy who agreed to repeat skin tests consented to undergo a controlled sting challenge. The four patients not on immunotherapy did not receive a controlled sting challenge.

Skin testing

Progressive skin testing was carried out on 35 patients. Positive and negative controls plus 1:1000 wt/vol WBE skin tests were performed with a prick method. If negative, this was followed by intradermal testing starting with 1:10,000,000 wt/vol dilution progressing in 10-fold increments to 1:1000 wt/vol. A positive response is a wheal greater than 5×5 mm, with surrounding erythema. Patients selected for immunotherapy were treated with Hollister-Stier extracts. Therapy was generally started at 0.05 ml of 1:100,000 wt/vol and advanced to a maintenance of 0.5 ml of 1:100 wt/vol.

Sting challenge

After signing a consent form as approved by our institutional review board, each patient had an intravenous line placed. A live sting challenge by one *Solenopsis invicta* IFA was carried out on the forearm opposite the skin test arm on the day of the skin test. Vital signs were obtained immediately before the challenge and then every 15 minutes over the next 60 minutes after the challenge. All sting challenges were performed in an intensive care-like room within our hospital, fully staffed by physicians, and equipped with monitoring and resuscitation equipment. Each patient returned to the clinic 24 hours after the sting to verify the occurrence of the sterile pustule, which is characteristic of a *Solenopsis invicta* sting.

IFAs were obtained from local natural sources. Each was identified by behavioral characteristics by one of the authors (T.F.) as being *Solenopsis invicta*. Each ant used in the sting

TABLE I. Demographics of treated and untreated patients

	Immunotherapy group	Untreated group
No. of patients	65	11
Age range	2-76 (mean: 37)	23-61 (mean: 42)
Sex (M:F)	22:43	7:4
Initial IFA sting history		
Reaction type		
Generalized cutaneous	24 (37%)	1 (9%)
Generalized life-threatening	41 (63%)	10 (91%)
No. multiple anaphylaxis	9 (14%)	1 (9%)
Study evaluation		
No. without subsequent stings	18	5
No. with repeat stings	47 (112 events)	6 (11 events)
Reaction type*		
Generalized cutaneous	0	2
Generalized life-threatening	1 (2.1%)	4

**p* value comparing 1/47 to 6/6 is <0.00001.

challenge was preserved in 70% isopropyl alcohol. These were later evaluated by an entomologist and confirmed as *Solenopsis invicta* in each case.

Statistics

A Fisher's Exact Test was used to compare the retrospective patient populations.

RESULTS

All 76 patients had histories of systemic reactions to IFA stings and positive skin tests to the WBE. The results of the initial cutaneous skin testing are the following: 14 patients were positive at prick 1:1000 wt/vol, 19 were positive at 1:10⁷ wt/vol intradermal, 26 at 1:10⁶ wt/vol, 16 at 1:10⁵ wt/vol, 8 at 1:10⁴ wt/vol, and 5 at 1:1000 wt/vol intradermal.

A wide range for positive responses occurred ranging from 1:1000 wt/vol by a prick method to 1:1000 wt/vol by the intradermal method. Immunotherapy to IFA was recommended to each patient. Sixty-five of the 76 patients accepted this recommendation, and 11 patients declined. A comparison of these two groups is listed in Table I. The mean ages of those receiving immunotherapy and those refusing immunotherapy were 37 and 42 years, respectively. The male-to-female ratio in the therapy group was 1:2 and in the untreated group was 2:1. These groups were comparable in the severity of their systemic allergic reactions to stings. Sixty-three percent of the immunotherapy group and 91% of the untreated group had histories of severe life-threatening reactions with cardiorespiratory signs and symptoms such as wheezing and hypotension. Approximately 10% (9 of 65 [14%] immunotherapy group and 1 of 11 [9%] untreated group) of both groups had histories of multiple epi-

sodes of anaphylaxis on exposure to IFA stings before their evaluation.

Sixty-five patients received immunotherapy. They had been on immunotherapy from 6 months to 18 years. Each had reached a maintenance dose of 0.2 to 0.5 ml of 1:20 to 1:100 wt/vol of Hollister-Stier IFA-WBE. The variability of maintenance doses reflects the absence of a recommended standard therapeutic dose and the management styles of the many physicians that have rotated through Wilford Hall Medical Center in the last 18 years. Each patient was receiving an injection every 1 to 4 weeks. None of these individuals had more than local reactions at the site of injection.

On subsequent accidental (in-field) stings only one patient out of 47 (2.1%) on immunotherapy reported anaphylaxis. One hundred twelve stinging episodes occurred in these 47 patients. Six of the 11 patients in the untreated group suffered repeat accidental stings. Although some of these events produced only local reactions, each of the untreated patients who had in-field stings had either a generalized cutaneous or systemic life-threatening anaphylactic event on resting at least once. Thirty-five of the 76 patients in the retrospective review were willing to return for subsequent evaluation. Thirty-one of these individuals were in the treated group, and four were in the untreated group. Fig. 2 demonstrates the results of their follow-up skin tests. Individuals on immunotherapy had a decrease in specific immunoglobulin E as measured by skin testing, whereas those in the untreated group were essentially unchanged.

Thirty of the 31 immunotherapy patients consented to a sting challenge. The four untreated patients did not undergo a sting challenge because each continued

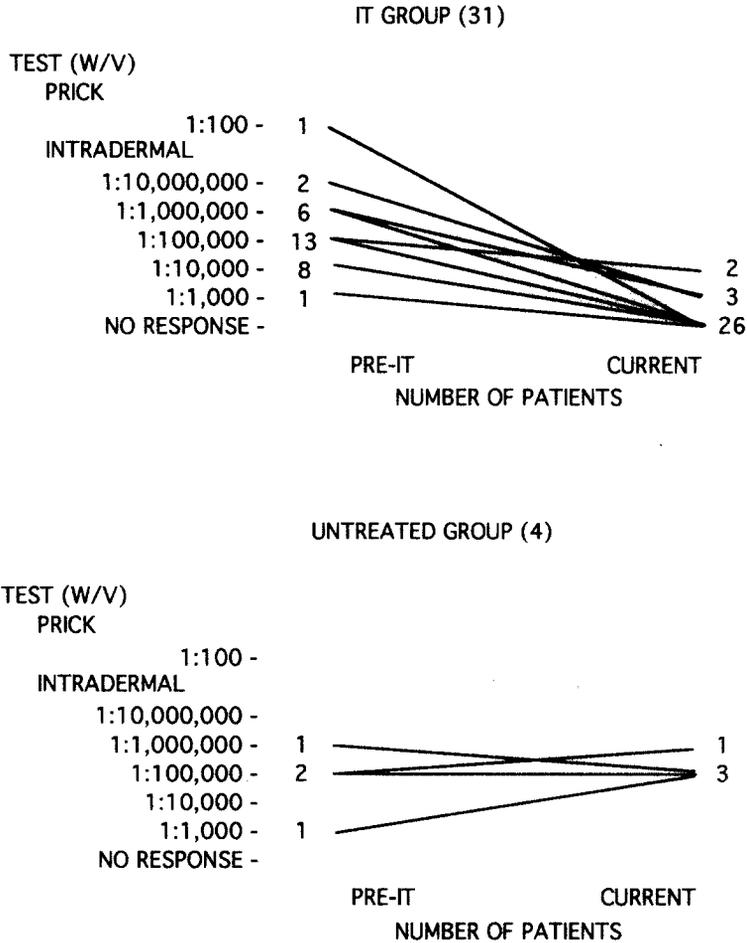


FIG. 2. Comparison of skin test results before and after immunotherapy from treated group and initially and at follow-up for untreated group. *IT*, Immunotherapy.

to have substantiated anaphylaxis after accidental natural fire ant stings. Each patient received one sting from the IFA, *Solenopsis invicta*. None of the 30 patients had more than a local reaction at the site of the sting. On the other hand, the typical pustule developed in each, which is seen approximately 24 hours after the sting and is highly characteristic of the IFA.

The summary of our results are as follows: Seventy-six patients with histories of anaphylaxis to IFA stings and positive skin tests were evaluated retrospectively. Sixty-five patients had been placed on WBE immunotherapy, and 11 refused therapy. Out of the 112 subsequent stings received by 47 patients on immunotherapy, only one (0.9% [sting] or 2.1% [patient]) had an anaphylactic reaction. In contrast, six of the 11 patients not on immunotherapy have had subsequent re-stings, and each has had a systemic reaction. Repeat skin testing of patients on immunotherapy showed a consistent decline in skin test responsiveness with persistent positive responses in only five (16%)

cases. However, the four untreated patients continued to have positive responses of a comparable severity. Sting challenges carried out on 30 of the patients on immunotherapy resulted in only local reactions.

DISCUSSION

Many questions remain to be answered in relation to our current approach to patients sensitive to IFAs. An important one is whether or not WBE is truly effective in preventing anaphylaxis in IFA-sensitive patients with a prior history of anaphylaxis. Only two previous series on the effectiveness of WBE in patients with systemic reactions to IFA have been reported. The summation of these two studies is that 110 patients were given immunotherapy with WBE. Of these patients, 27 were re-stung accidentally, and two suffered recurrence of a systemic reaction.^{8,9} Information concerning the dosing and source of the immunotherapy WBE were not reported in these articles. These two articles then provide us with a reaction rate

on immunotherapy of at least 1.8% (2 of 110), with the maximum rate being 7.4% (2 of 27) of the patients. This agrees closely with our recurrent reaction rate of 2.1% on subsequent accidental stings in our group of patients on immunotherapy. It is also in agreement with current effectiveness data for other winged Hymenoptera venom therapy.

One of the difficulties with studies that rely on natural field stings to ascertain effectiveness of immunotherapy, is in determining that the insect responsible for the sting is actually the insect to which the patient is receiving immunotherapy. This is much less of a problem with IFA because of the characteristic pustule. However, to overcome this potential problem and to objectively document the absence of an allergic response to an IFA sting, sting challenges with confirmed IFAs were performed. This experimental sting challenge reproduced the typical response seen in a natural IFA sting; that is, the sterile pustule. However, in none of the 30 patients tested did anaphylaxis recur after challenge. Therefore administration of immunotherapy with WBE of IFA to patients with a history of anaphylaxis to IFA sting appears to be protective against systemic reactions provoked by these sting challenges.

For this protocol it was decided to perform a single IFA sting challenge. However, surveys of individuals who react to IFA stings have shown that patients stung by an IFA are more likely to receive multiple stings. Since no information on an appropriate dose was available, it was decided to err on the side of safety and initially attempt a sting challenge with a single IFA. It is possible therefore that the results demonstrated only reflect an inadequate testing dose. Nevertheless, a recent report by the Fire Ant Subcommittee of the American Academy of Allergy and Immunology showed that of the 32 deaths attributed to anaphylaxis caused by IFA stings most of these individuals had received fewer than five stings.¹ This suggests that larger numbers of stings (>5) are not needed to produce a severe reaction. The exact dose in terms of number of stings that is appropriate for an adequate challenge remains in question.

Another concern, not addressed by this study, is that the natural history of IFA hypersensitivity has not been determined. It is possible that the 30 patients who received sting challenges would not have reacted whether they were on immunotherapy or not. However, the fact that the six individuals who refused immunotherapy continued to suffer from recurrent anaphylaxis after accidental stinging episodes argues strongly against this point. Of course, the small number of individuals in this group and the fact that these data are retrospective makes future prospective studies

important. Specific immunoglobulin E to the IFA as measured by cutaneous reactivity does appear to be diminished through the use of WBEs for immunotherapy. In this respect IFA-WBE is similar to other Hymenoptera venoms.¹¹⁻¹³

Many studies have shown that WBE contains relevant venom allergens.^{14, 15} A recent study published by Hoffman et al.¹⁰ indicated that there may be sufficient venom protein (Sol i III) in WBE to be effective in immunotherapy. This article reports that 0.5 ml of 1:100 wt/vol extract contains 1500 to 2200 ng of Sol i III venom protein. A single sting with IFA contains less than 100 ng of Sol i III venom protein. Therefore a maintenance dose of 0.5 ml of 1:100 wt/vol WBE gives approximately 10 times the natural dose (single sting) and should be effective. Other Hymenoptera venom treatment usually gives 100 µg (two times the natural sting dose) of venom protein.

In conclusion, it appears that immunotherapy with IFA-WBE in patients with a history of anaphylaxis to IFA is protective against systemic reactions provoked by a sting challenge with a single IFA. The retrospective analysis of 65 patients with systemic allergic reactions to IFA stings who received immunotherapy showed a recent reaction rate on field sting of 2.1% per patient. This contrasts sharply and significantly ($p < 0.00001$) with a 100% repeat reaction rate in the six patients who did not receive immunotherapy and were re-stung. A controlled prospective trial of WBE versus placebo is needed to confirm these conclusions and to help to define the natural history of IFA hypersensitivity.

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Inhibitory effect of cetirizine on the bronchial eosinophil recruitment induced by allergen inhalation challenge in allergic patients with asthma

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In patients with asthma there is a recruitment of eosinophils in bronchoalveolar lavage fluid (BALF) after the late asthmatic reaction (LAR). Cetirizine is a selective H₁ antagonist that inhibits the eosinophil recruitment induced by allergen in the skin. The aim of this study was to evaluate whether cetirizine was able to inhibit the LAR-induced inflammatory reaction. Twelve allergic asymptomatic subjects with asthma (aged 18 to 58 years) without any treatment were enrolled in the study; FEV₁ was >83% predicted in each case. An allergen inhalation-challenge test was performed to assess the presence of an LAR. In a double-blind, randomized, placebo-controlled study, the patients were treated for 8 days with either cetirizine, 15 mg twice a day (six patients, group 1), or placebo (six patients, group 2). On day 8, a second allergen inhalation-challenge test with the same allergen was performed, and BAL was realized 24 hours later; as usual 250 ml of saline was instilled by 50 ml aliquots, and the first recovery was analyzed separately. In each case, the LAR observed after treatment was similar to the first one. In placebo-treated patients, an increased number of cells, mainly eosinophils, was observed in the first recovery of BALF compared with the number in subsequent recoveries. These numbers were significantly higher than numbers observed in cetirizine-treated patients. Cetirizine did not modify significantly the allergen inhalation-challenge test, but it inhibited the recruitment of inflammatory cells, mainly eosinophils. (J ALLERGY CLIN IMMUNOL 1992;90:215-24.)

Key words: Asthma, eosinophils, bronchoalveolar lavage, allergen inhalation challenge, cetirizine

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Eosinophils are now considered to be one of the main cell types involved in inflammation of the asthmatic lung. Eosinophils can generate a wide range of mediators and play an important role in the pathogenesis of bronchial asthma, including bronchial hyperreactivity,^{1,3} bronchial epithelial damage,^{4,5} and probably ageing of the bronchi.⁶ Eosinophils are observed even in mild asthma in the BALF,⁷⁻¹³ as well