
Effect of imported fire ant extract on the degradation of mountain cedar pollen allergen

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Background: Dust mite, cockroach, and mold extracts have been shown to contain proteases capable of degrading the proteins in other extracts. Loss of potency of allergens has been reported in mixtures containing cockroach and fungal extracts. Fire ant venoms consist of 90% to 95% n-alkyl and n-alkenyl piperidine alkaloids, which are not allergenic. No studies are available addressing the mixture of imported fire ant (IFA) whole-body extract with other allergens or the presence of proteolytic activity in the venom extract.

Objectives: To evaluate the stability of mountain cedar pollen extract mixed with IFA whole-body extract and to qualitatively analyze the extract mixture for degradation of mountain cedar protein.

Methods: One milliliter each of mountain cedar and IFA whole-body extracts at a concentration of 500 $\mu\text{g/mL}$ were combined and stored at 4°C for 1, 3, 6, 15, 30, 60, 90, and 180 days. Separate mixtures of 1 mL of mountain cedar and IFA with 1 mL of human serum albumin were used as controls. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed, and protein bands were qualitatively analyzed for degradation.

Results: We detected 3 distinct IFA protein bands and 1 mountain cedar protein band. With respect to these bands, no protein degradation was observed during 6 months of study in the extract mixture compared with the controls.

Conclusions: Imported fire ant whole-body extract does not seem to degrade mountain cedar protein. Mixtures of allergenic extracts may be able to include IFA whole-body extract.

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INTRODUCTION

Immunotherapy is effective in treating IgE-mediated conditions, including Hymenoptera sensitivity, allergic rhinitis, and allergic bronchial asthma. The criteria for starting immunotherapy include a history of significant allergen exposure, sensitivity to the allergen as demonstrated by the results of skin prick testing or in vitro testing for specific IgE, and a pattern of exposure to allergen that correlates with the pattern of symptoms.¹ Atopic individuals are commonly sensitized to several allergens, and under these circumstances, immunotherapy containing all the relevant allergens is indicated.²

Allergen extracts used for specific immunotherapy should have known composition, potency, and stability. Allergens are usually available as certified single extracts, and many are now standardized for potency and batch-to-batch reproducibility. However, allergen extracts are composed of very heterogeneous mixtures of biological substances, which may contribute to their rapid degradation.³ In addition to degradation, allergen extracts are subject to loss of potency due to several factors. More dilute concentrations lose potency more rapidly than more concentrated extracts largely owing to adsorption of protein onto the surface of the vial. This loss can be retarded by the addition of more protein in the form of allergen extract or human serum albumin. On the other hand, preservatives that have enzyme-inhibiting properties, such as

glycerin, can effectively retard the loss of potency caused by proteolysis.^{1,3–5} Loss of potency is also accelerated by higher temperatures, necessitating the refrigeration of allergen extracts.^{6,7} Finally, loss of potency has been demonstrated when mixing different allergen extracts.^{4,8}

It is common practice in the United States to use immunotherapy vaccines containing a mixture of relevant allergens.² Some of these extracts have been shown to contain proteases that lead to the degradation of proteins in the mixture. Protease-containing extracts include dust mite, cockroach, and mold.^{4,8,9} Mold and cockroach extracts have been shown to cause loss of potency of certain pollen allergens when mixed together.^{4,8} No specific data are available addressing the loss of potency of allergens when combining imported fire ant (IFA) whole-body extract with pollen or other allergens. The objectives of this study were to evaluate the stability of mountain cedar pollen extract mixed with IFA whole-body extract and to qualitatively analyze the extract mixture for degradation of mountain cedar protein.

METHODS

Separate vials containing 1 mL each of aqueous mountain cedar (*Juniperus ashei*) and IFA whole-body extracts (*Solenopsis invicta* and *Solenopsis richteri* mixture) at a concentration of 500 $\mu\text{g/mL}$ were combined and subsequently stored at 4°C for 1, 3, 6, 15, 30, 60, 90, and 180 days. Separate mixtures of 1 mL of mountain cedar and IFA with 1 mL of human serum albumin were used as controls. The controls served to account for loss of potency due to other causes, specifically, time. Each period corresponds to the amount of

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time that the allergens were in mixture at 4°C. To halt any further degradation at the end of each period, the vials were frozen at -70°C. This enabled us to evaluate all the samples at the same time.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed on 10% to 20% gradient gels (PAGEr Gold Precast Gel; Cambrex, Rockland, ME) along with low-molecular-weight controls (Cambrex). The gels were run for 90 minutes at 125 V and were subsequently stained with 0.025% Coomassie brilliant blue R-250. The molecular weight of the stained proteins was calculated by comparing the protein's position on the gel with a standard curve constructed from the molecular weight standard bands. Mountain cedar and IFA protein bands were qualitatively analyzed for degradation. For each period, we compared the protein bands obtained from the mixture of the 2 allergens with the bands from the control specimens containing a single extract.

RESULTS

The sodium dodecyl sulfate–polyacrylamide gel electrophoresis data obtained for the 6-month study are shown in Figure 1. Each of the 8 periods contains columns for the allergen mixture and the 2 controls. We detected 3 distinct IFA protein bands of approximately 12,000, 14,000, and 26,000 Da and 1 mountain cedar band corresponding to a protein of approximately 44,000 Da. There was no evidence of degradation of these proteins in the mixture of extracts during the 6-month period compared with the mountain cedar and IFA whole-body extract controls.

DISCUSSION

Proteases are present in extracts of fungi, cockroaches, and dust mites, and the avoidance of mixtures containing molds and cockroach has been recommended.^{1,8–10} *Alternaria* extract has been shown to reduce the potency of Timothy and Bermuda grasses, white oak, box elder, and cat, and cockroach extract has a deleterious effect on Timothy grass, Russian thistle, and box elder.⁴ However, no recommendations regarding mixtures with IFA extract are available, and IFA immunotherapy is usually administered as a single-extract vaccine. Ninety percent to 95% of the IFA venom consists of nonprotein, n-alkyl and n-alkenyl piperidine alkaloids. These alkaloids are not allergenic but have hemolytic, bactericidal, insecticidal, and cytotoxic properties. They are responsible for the pain and subsequent sterile pustule associated with fire ant stings.^{11,12} A total of 21 different proteins have been identified in fire ant venom, among which are phospholipase A and hyaluronidase. Allergic activity has been established for all of these proteins.^{11,13} Despite the extensive data available addressing the components of venom, there is no commercially available IFA venom vaccine. Instead, whole-body extract has been used, which contains allergenic and nonallergenic components. Data indicate that it is effective for use in immunotherapy because it contains sufficient venom allergens to protect against subsequent stings.^{11,14}

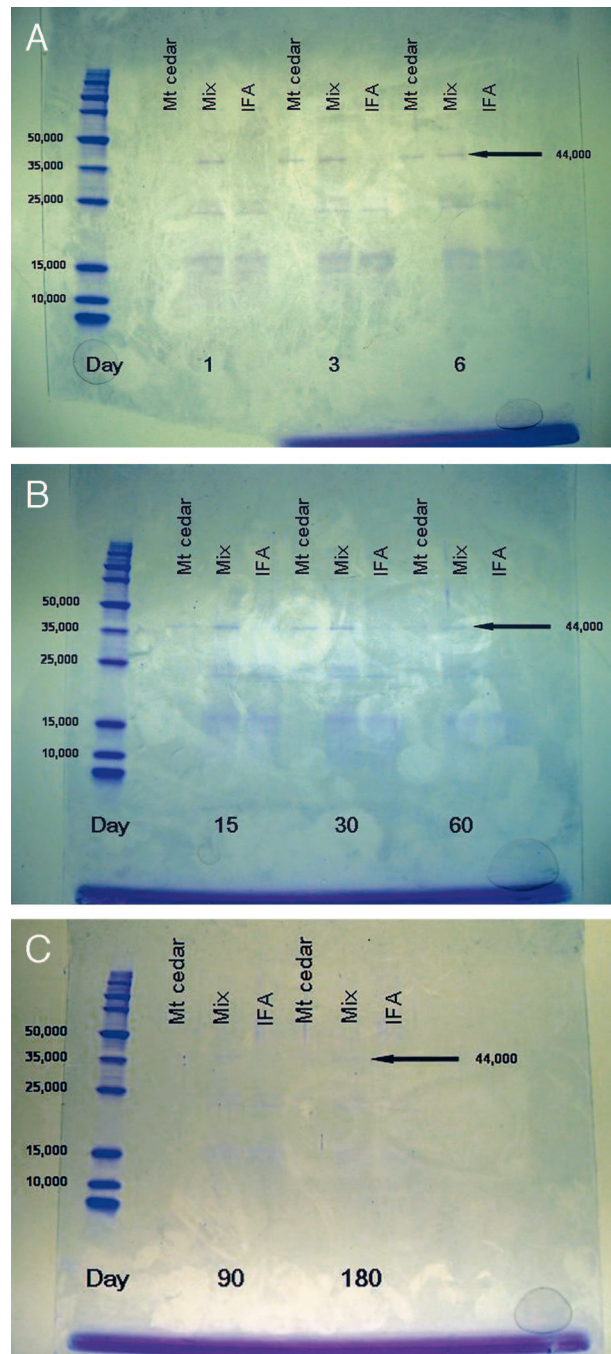


Figure 1. Results of sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the allergen mixture and controls on days 1, 3, and 6 (A); days 15, 30, and 60 (B); and days 90 and 180 (C). Molecular weight standards are shown on the left, and individual columns are labeled at the top. Arrow indicates the mountain (Mt) cedar band of approximately 44,000 Da; IFA, imported fire ant.

We detected 1 mountain cedar and 3 distinct IFA protein bands. With respect to these bands, no protein degradation

was observed in the extract mixture compared with the controls during the 6 months of study. Although no increased degradation of protein was qualitatively observed, no comment can be made about the possible changes in allergenicity because IgE binding was not investigated. The bands fade in proportion to the incubation time, but no significant differences between the mixture and controls were seen. The fading of bands was expected because the passage of time is a known cause of protein degradation. Hypersensitivity to IFA is the most common venom allergy in endemic areas such as San Antonio, TX, and mountain cedar sensitivity is one of the more relevant pollen allergens in the region. The rationale for mixing these 2 extracts is the possibility of patients monosensitized to mountain cedar also becoming sensitized to IFA. Immunotherapy with IFA is administered by the same schedule as aeroallergens, which would make it feasible to mix with aeroallergens. Based on our results, IFA could be mixed with mountain cedar without concern for degradation of the extracts. An argument against mixing a venom with an aeroallergen is the difficulty with adjustment of the immunotherapy schedule after a systemic reaction. Although venom immunotherapy can be lifesaving, aeroallergen immunotherapy is an elective treatment modality. The reaction may have been to the aeroallergen. Decreasing the dose or discontinuing immunotherapy as a result of a systemic reaction could place a venom-sensitive patient at unnecessary risk.

In conclusion, the efficacy of IFA whole-body extract for use in specific immunotherapy has been established. Many atopic patients, though, are polysensitized and require vaccines for all of their relevant allergens. In such cases, several injections may be required, which may be more painful and could increase the incidence of administration errors. We showed that IFA whole-body extract does not seem to degrade mountain cedar protein. Further quantitative studies would be beneficial to establish the potency and stability of other pollen extracts used in combination with IFA extract. If this stability can be confirmed, then mixtures of allergenic extracts could include IFA whole-body extract.

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