

Identification of jack-jumper ant (*Myrmecia pilosula*) venom allergens

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Summary

Jack-jumper ant venom proteins were electrophoretically separated on SDS–polyacrylamide gels, transferred to nitrocellulose and probed with sera from subjects who had experienced an allergic reaction after being bitten by a jack-jumper ant. Ant venom components that bound IgE antibodies were detected by addition of ¹²⁵I-anti-human IgE followed by autoradiography. Of the 17 polypeptides resolved by electrophoresis only three, of molecular weights approximately 14 kD, 12 kD and 10 kD, bound IgE antibodies from the panel of 50 sera examined. There was a marked similarity in the binding patterns by individual sera with almost all of the sera recognizing the 14 kD and 12 kD components. IgE-binding profiles of separated ant venoms from ants collected in different regions of Australia appeared to be very similar if not identical. Identification of the ant allergens is a necessary prelude to the preparation of standardized venom sac extracts suitable for safe and effective diagnostic and therapeutic use.

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Introduction

The jack-jumper ant (*Myrmecia pilosula*) (Fig. 1) is found widely in the sandy-soiled and highland areas of south-eastern Australia and Tasmania [1]. The precise incidence of anaphylaxis to jumper ant sting is unknown [3]; however, the incidence of severe allergy to ants, particularly in young children, is increasing [4]. More recently, the size of the clinical problem has again been emphasized. Eight consultant allergists in Victoria, contributing towards a register of patients allergic to the jumper ant sting, were able to collect 70 names in a 6-month period (J. Weiner, personal communication). Many of these patients had experienced a life-threatening anaphylactic reaction following the sting and thereafter needed to carry self-injectable adrenaline. Based on the earlier study of Clarke [5], and the more recent information from the jumper ant register, it is probable that several hundred patients are at risk of severe allergic reactions to these ants at any one time.

Currently, the only available extract for diagnosis and

immunotherapy is an extract of whole ants. The unsuitability of this extract for both purposes has recently been emphasized [3], making it clear that the preparation and standardization of jumper ant extracts urgently needs to be reviewed. The initial stages of such a task should involve an accurate and complete definition of the components of the source material and identification of all the allergens [6]. In order to do this, we used the procedure of protein blotting which involves the transfer of electrophoretically separated components to a membrane followed by probing with sera from allergic patients and anti-human IgE [7–9]. This approach allowed us to identify the IgE-binding polypeptides rapidly in a number of jumper ant venom extracts from different regions of south-east Australia, and provided information on the precise spectrum of components recognized by individual patients.

Materials and methods

Ant venom extracts

Jack-jumper ants were obtained from various sites in Victoria, South Australia and Tasmania. Generally they were collected using hand-held vacuum cleaners fitted

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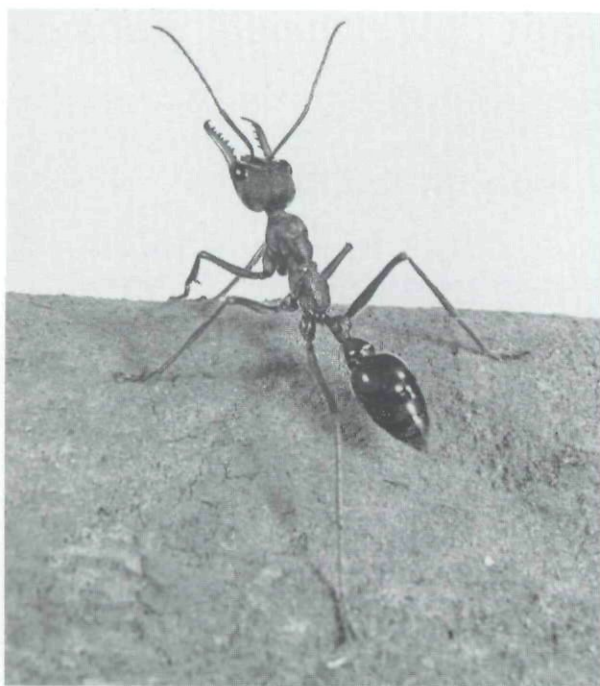


Fig. 1. A *Myrmecia* ant in a state of alertness. Ants from this genus are known by a variety of names across Australia including jack-jumpers, bullants, bulldog ants, inch ants, inchmen and sergeant ants. The worker caste of the more than 100 species of the genus range in length from 10 to 40 mm. Each year, stings from *Myrmecia* ants are the cause of many adverse reactions including anaphylaxis. Specimens housed in museum collections under the name *M. pilosula* are sufficiently variable to suggest that several sibling species occur and there appears to be at least seven such species. Six of these sibling species are yet to receive scientific names [2]. A *Myrmecia* ant stings by grasping the victim with its mandibles, arching its body and envenomating via the sting mechanism which emerges from the tip of the abdomen. Photograph courtesy of CSIRO Division of Entomology, Canberra, Australia.

with collecting tubes which were placed in dry ice. The ants were later thawed at room temperature and their venom sacs quickly dissected out, placed in iced water and then frozen. All samples were later thawed and emulsified by two 10-sec bursts in a Silverson heavy duty emulsifier followed by centrifugation at 1000 *g* for 10 min at 5°C in a Beckman GPR centrifuge. Supernatants were frozen at -25°C and stored at -70°C until required for use. Using the Pierce BCA* Protein Assay Reagent [10], protein concentrations of the supernatants were found to be in the range 254–646 µg ml⁻¹.

Sera

Sera were obtained from patients with a clinical history of allergy to jumper ants and from atopic patients attending

the Allergy Clinic at Royal North Shore Hospital who had no clinical history of jumper ant allergy. Cord serum was obtained from the Department of Obstetrics, Royal North Shore Hospital and used as an IgE-'free' control.

Ant venom RAST

All sera were tested for jack-jumper ant venom-specific IgE antibodies in a RAST assay using 6-mm nitrocellulose discs impregnated with 2.5 µg of venom proteins/disc [11]. Bound IgE antibodies were detected by the addition of ¹²⁵I-labelled anti-human IgE (Pharmacia).

PAGE and electrotransfer to nitrocellulose

SDS-PAGE [12] and gradient PAGE under non-reducing conditions were carried out as previously described [9,13]. Proteins in polyacrylamide gels were stained with Coomassie brilliant blue G-250 (CBB) [14]. For electroblotting of the venom extracts collected from different regions, 8.2 µg of protein/µl sample buffer/well were applied and the blotted proteins were probed using a pool of 20 sera from subjects allergic to ant venom. For electroblotting and subsequent probing with individual atopic sera, a total volume of 150 µl sample containing 30 µg protein (from venom from ants collected at Templestowe, Victoria) was loaded onto each gel. 2-Mercaptoethanol (3%) was included in all samples. To ensure reliability of the MW estimations, low MW markers (Pharmacia) were included in each gel. Materials and methods for electrotransfer of separated proteins to nitrocellulose were as previously described [9,15].

Probing of nitrocellulose membrane strips and autoradiography

The materials and methods for the identification by autoradiography of the IgE-binding jack-jumper ant venom polypeptides have been described [16].

Results

Gradient PAGE under non-reducing conditions proved inadequate in resolving the ant venom proteins. However, when an extract of jack-jumper ant venom (from ants collected at Templestowe, Victoria) was subjected to SDS-PAGE, 17 bands were visible following CBB staining (Fig. 2a, b). The bands occurred in the MW range of 5–200 kD. The heaviest staining components were those in the MW region of <15 kD and were poorly resolved. The remaining polypeptides of higher MW, though lightly stained, were well resolved.

Sera from 50 patients allergic to jumper ant venom

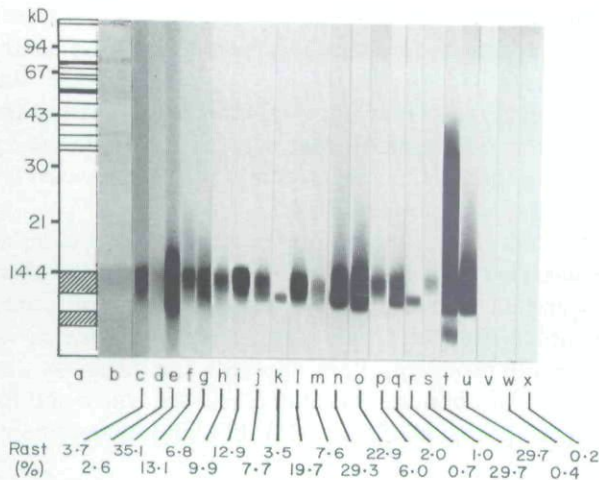


Fig. 2. Identification by autoradiography of the IgE-binding jack-jumper ant venom components transferred to nitrocellulose after SDS-PAGE. The corresponding RAST result is shown underneath each strip. a, Schematic representation of strip b; b, CBB stain showing the proteins present in a jack-jumper ant venom extract following SDS-PAGE; c-u, strips incubated with individual human sera from jumper-ant-allergic patients; c-p, u-x, 19-hr autoradiography; q-t, 4-days autoradiography; t, u, incubated with the same serum; v, incubated directly with ^{125}I -anti-human IgE; w, incubated with cord serum; x, incubated with human serum from a subject not allergic to jack-jumper ant but highly allergic to house dust mite.

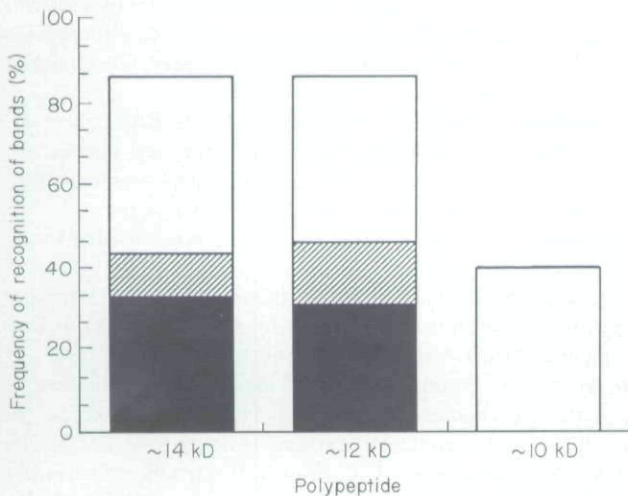


Fig. 3. Histogram showing the frequency of binding of subjects' sera ($n=50$) to the SDS-PAGE-separated, IgE-binding components of ant venom. Blank, hatched and solid areas of the bars represent the proportions of the serum panel that showed weak, medium and strong binding, respectively.

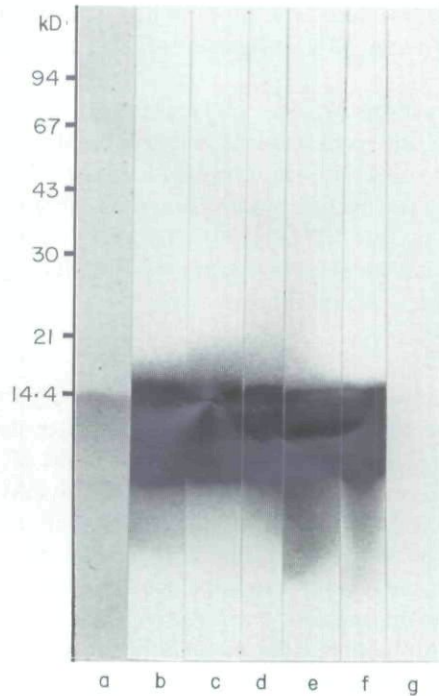


Fig. 4. Identification and comparison by autoradiography of the IgE-binding components transferred to nitrocellulose strips after SDS-PAGE of five different jumper ant venom extracts and a bullant venom extract. In each case, $8.2 \mu\text{g}$ protein were loaded per well a, CBB protein stain of jack-jumper ant venom; b, Jack-jumper ant from Templestowe (Victoria); c, jack-jumper ant from Gisborne (Victoria); d, jack-jumper ant from Langwarrin (Victoria); e, jack-jumper ant from Hobart (Tasmania); f, jack-jumper ant from Millicent (S. Australia); g, bullant from Gisborne (Victoria). Strips were probed with a serum pool formed from mixing equal volumes of sera from 20 different subjects allergic to jumper ant venom. The sera showed RAST uptakes against jumper ant venom ranging from 1.3 to 48.2%.

were examined for IgE binding to the venom polypeptides electroblotted onto nitrocellulose. Six of the sera showed no IgE binding. Of the 17 resolved polypeptides, only three (MW about 14 kD, 12 kD and 10 kD) bound IgE antibodies (Fig. 2 c-u). The intensity of IgE-binding as detected on the autoradiographs closely correlated with the RAST results (Fig. 2). With the exception of two patients (Fig. 2 p, r) all patients exhibited IgE antibody binding to both the 14 kD and 12 kD components. Thirty-nine per cent of the sera showed binding to the 10 kD component, but whenever there was recognition of this component, it was weak compared with the other two IgE-binding polypeptides. The percentages of subjects' sera showing strong, moderate or weak radiostaining with the three reactive polypeptides are shown in the histogram (Fig. 3).

Sera from subjects not allergic to jack-jumper ant venom did not react with any of the transferred polypep-

tides (Fig 2x). Similarly, no binding was seen with cord serum, or when ^{125}I -anti-human IgE was used alone (Fig. 2v, w).

When venom extracts of jack-jumper ants collected from five different regions were separated by SDS-PAGE, electroblotted and probed with the allergic serum pool, very strong IgE-binding activities were seen in the MW region < 15 kD (Fig. 4). This activity was approximately equal for all extracts, and no IgE binding to other polypeptides was detectable.

Discussion

In this study, employment of protein blotting and RAST led to the identification of the allergenic polypeptides present in the venom of the jack-jumper ant *M. pilosula*. Of the 17 components separated by SDS-PAGE, only three reacted with IgE antibodies in the sera of subjects allergic to ant venom. The IgE-binding polypeptides present in the venom also appear to be the polypeptides present in the greatest quantity. With the exception of only two of the 44 reactive sera, both the 14 kD and 12 kD components were recognized by serum IgE antibodies. Weaker binding of IgE antibodies to the 10 kD component occurred with 39% of the sera. This simplicity and similarity in the patients' allergen recognition patterns is in marked contrast to the heterogeneous IgE antibody responses seen in subjects allergic to a variety of inhalant allergens [17,18].

Good correlations were found between the IgE antibody binding detected on protein blots and the corresponding RAST results (Fig. 2). The simple RAST described here should prove to be a valuable supplement to the skin-prick test in the diagnosis of immediate hypersensitivity to *M. pilosula* venom. In fact, in some highly sensitive subjects where even minute amounts of venom may provoke a reaction, some clinicians may think it prudent to employ the RAST rather than risk a skin-prick test-induced response. In addition, an *in-vitro* test for IgE antibodies to ant venom will be useful for following immunological changes after immunotherapy.

Qualitative gel electrophoretic analysis of *M. pilosula* venom obtained from ants collected at different geographical locations did not reveal any clear differences and, likewise, no differences were seen in the IgE antibody binding characteristics of the different venoms.

It is now important to assess the allergenic relationship, if any, between ant venoms from different species and in particular between the two most reactive *M. pilosula* polypeptides of MW 14 kD and 12 kD. Studies currently being undertaken (unpublished) indicate that there is little or no allergenic crossreactivity between *M. pilosula* venom and the venoms from the bullant (*M. pyriformis*) and the honey bee (*Apis mellifera*).

Jack-jumper ant stings are a major cause of severe allergic reactions to arthropod envenomation in south-east Australia. Identification of the jack-jumper ant allergenic proteins is an important initial step in efforts to produce a standardized venom preparation for use by allergists and for the preparation of purified material for diagnosis and immunotherapy.

Because of the simple allergen recognition profiles of the allergic subjects, the clear identification of the allergens and their relatively low MWs, application of recombinant DNA methods would appear to be the strategy of choice for producing pure allergens for diagnosis and therapy, for obtaining the amino acid sequences and for identifying the allergenic (IgE-binding) determinants [19,20].

Acknowledgments

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