

Original article

Myrmecia pilosula (Jack Jumper) ant venom: identification of allergens and revised nomenclature

Background: The 'Jack Jumper Ant' (JJA; *Myrmecia pilosula* species complex) is the major cause of ant sting anaphylaxis in Australia. Our aims were to determine the allergenicity of previously described venom peptides in their native forms, identify additional allergens and if necessary, update nomenclature used to describe the allergens according to International Union of Immunological Societies criteria.

Methods: Various polyacrylamide gel electrophoresis methods were used to separate JJA venom. Gel resolved venom was Western-blotted and probed with individual sera taken from patients with a history of JJA sting anaphylaxis and immunoglobulin E radioallergosorbent test (IgE RAST) tracer uptakes of > 1% to whole venom.

Results: Of 67 available sera, 54 had RAST uptakes > 1%. Thirteen IgE binding bands were identified using these sera. Pilosulin 3, [Ile⁵]pilosulin 1, and pilosulin 4.1 were recognized by 42 (78%), 18 (33%) and nine (17%) of the 54 sera that were tested. Immunoglobulin E-binding proteins with estimated molecular masses of 6.6, 22.8, 25.6, 30.4, 32.1, 34.4 and 89.8 kDa were each recognized by three or more individual sera. Two of these (25.6 and 89.8 kDa) were recognized by 46% and 37% of sera, respectively.

Conclusion: Nomenclature used to describe JJA venom allergens has been revised. Pilosulin 3 (Myr p 2) is the only major allergen, whilst [Ile⁵]pilosulin 1 (Myr p 1), and pilosulin 4.1 (Myr p 3) are minor allergens. There are an additional five IgE-binding proteins that require further characterization before they can be named as allergens. These findings provide a framework for standardizing venom extracts for diagnosis and immunotherapy.

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Ant sting anaphylaxis is common in Australia, with the majority of cases thought to be due to the *Myrmecia pilosula* species complex (Jack Jumper Ant, JJA; 1–3). Jack Jumper Ant are found in southern and eastern Australia, including Tasmania, Victoria, southern New South Wales, and cooler areas of South Australia and Western Australia (http://anic.ento.csiro.au/database/biota_details.aspx?BiotID=37534; accessed 5 January 2007). The population prevalence of JJA venom allergy amongst adults in rural Victorian has been reported to be 2.4% (2) and in Tasmania, the prevalence in the entire population has been estimated as 2.7% (1). Our group has demonstrated the effectiveness of venom immunotherapy (VIT) in preventing anaphylactic reactions to the sting of

the JJA (4). In our double-blind, placebo-controlled trial, of those who underwent a blinded sting challenge, 71% of participants in the placebo group compared with 0% in the active treatment group had an objective systemic reaction following sting challenge ($P < 0.0001$).

Jack Jumper Ant venom is composed of a number of peptides with molecular mass < 10 kDa and some higher molecular weight proteins (5). Early studies used sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to investigate JJA venom and identified several potential allergens with calculated molecular mass ranging from 2 to 8.5 kDa (6), although it should be noted that migration of venom peptides did not correspond with molecular weight (5). Two cDNA clones and their

proposed peptide products have been described as major allergens (7,8). The first of these is Myr p 1, which encodes a peptide that has been named pilosulin 1 (7,9). This peptide is only present in limited quantities in JJA venom, but its variant, [Ile⁵]pilosulin 1, a monomer peptide with a molecular mass of 6066 Da, has been shown by mass spectrometry analysis to be much more abundant (10). The second allergen to be described was Myr p 2, which was theorized to encode a peptide named pilosulin 2. Pilosulin 2 has not been observed in JJA venom, but exists as des-Gly²⁷-pilosulin 2 as part of a heterodimer. This heterodimer has been named pilosulin 3, comprising des-Gly²⁷-pilosulin 2 (renamed pilosulin 3a) and another peptide named pilosulin 3b (10). Mass spectrometry analysis indicated that the molecular mass of pilosulin 3 was 5608 Da (10). Inagaki et al. (11) have more recently described a novel peptide which they named pilosulin 4. This peptide was then shown to exist in JJA venom as [Glu³¹]pilosulin 4 and was present in an 8198 Da homodimer named pilosulin 4.1 (5). [Ile⁵]pilosulin 1 and pilosulin 3 account for approximately 80% of the total venom peptide content, but the frequency of allergic sera immunoglobulin (Ig) E binding these peptides is unknown.

Following the death of a man who's serum recognized neither Myr p 1 nor Myr p 2, a review of JJA allergic sera found that approximately 20% had a similar lack of affinity for these peptides, yet clearly recognized whole venom (12). Wu subsequently performed individual patient sera immunoblots using SDS-PAGE resolved 2-mercaptoethanol-reduced venom and demonstrated four additional IgE-binding proteins with molecular mass of 11.7–43.5 kDa (13). Jack Jumper Ant venom is significantly altered by reduction and if it is not subsequently alkylated prior to electrophoresis, aberrant multimers have been shown to form (6). Therefore, the findings of Wu (13) require confirmation by immunoblotting using both nonreduced and reduced/alkylated venom.

All of the allergens in JJA venom should be identified so that venom preparations for routine diagnostic and therapeutic use can be standardized. Our aims were to utilize our recently developed PAGE methodologies (5) to determine the allergenicity of previously described JJA venom peptides in their native forms, to identify additional allergens and to update the nomenclature of JJA venom allergens according to International Union of Immunological Societies (IUIS) criteria (14).

Methods

Materials

Acrylamide and broad range biotinylated molecular weight markers were purchased from Bio-Rad (Hercules, CA, USA). Polyvinylidene difluoride (PVDF) membrane (Immobilon P[®]) was obtained from

Millipore (Billerica, MA, USA). Polyacrylamide gels were cast in a Hoefer Mighty Small gel caster and semi-dry and wet Western blot units were purchased from Amersham Biosciences (Uppsala, Sweden). Tris-buffered saline with Polysorbate 20 (TBS-T), containing 20 mM Tris (pH = 7.4), 0.9 M sodium chloride, and 0.05% Polysorbate 20, was freshly prepared from reagents purchased from Sigma-Aldrich (St Louis, MO, USA).

JJA venom

Jack Jumper Ant were collected from a variety of locations around Tasmania and venom was obtained by venom sac dissection. Reduced venom was alkylated prior to electrophoretic separation as per the method of Street et al. (8).

Acid urea PAGE

Acid urea PAGE (AU PAGE) was performed as per the method of Wiese et al. (5). About 5 µg of venom was added to each lane, resolved and transferred to PVDF membrane using a modified semi-dry method of Wang et al. (15) for 45 min at 1.5 mA/cm².

Gradient SDS-PAGE and tricine SDS-PAGE

Gradient SDS-PAGE mini-gels (10–20%) and 16.5% Tris tricine SDS-PAGE gels, referred to hereafter as 'gradient SDS-PAGE' and 'tricine SDS-PAGE', respectively, were run as previously described (5,16). Molecular weight markers were included with all experiments. About 5 µg of venom was added to each lane, resolved and transferred to PVDF membrane using a wet transfer method at constant current of 200 mA for 1 h as per the method of Towbin et al. (17).

Glycopeptide detection

Glycopeptides were visualized by staining nonreduced JJA venom which was resolved by gradient SDS-PAGE with Pro-Q[®] Emerald 300 Glycoprotein stain (Molecular Probes, Eugene, OR, USA), used according to the manufacturer's instructions. Imaging was performed with a Typhoon 9400 imager (Amersham Biosciences).

Protein staining

Proteins on PVDF membrane were stained with Coomassie blue R (0.1% in methanol : acetic acid : water 40 : 10 : 50) for 1 min, then destained with 50% methanol in water until the background was white. Whole protein staining of gel resolved peptides with Coomassie blue was performed as previously described (5).

Patient sera

Individual patient sera with known IgE reactivity to JJA venom, determined by radioallergosorbent test [RAST; performed as previously described in Ref. (3)], taken prior to VIT in our trial were available (4). Sera with RAST tracer uptake > 1.0% to whole JJA venom were used. Prior to immunoblotting, sera were diluted 1 : 10 with blocking solution supplied in the ECL Advance kit (Amersham Biosciences). The VIT trial and use of patient sera were approved by both the Royal Hobart Hospital and Flinders Medical Centre ethics committees.

Immunoblotting

Immunoblots with AU PAGE and gradient SDS-PAGE resolved venom were performed using nonreduced venom. Immunoblots on tricine SDS-PAGE resolved venom were performed using both reduced/alkylated and nonreduced venom.

To fix the venom peptides to the membrane, the blotted PVDF membrane was placed in approximately 300 ml of water and heated in a microwave oven for 10 min (18,19). Membranes were then blocked for 1 h with 3% skim milk powder in TBS-T and sera were incubated with the membranes overnight at room temperature. Immunoglobulin E-binding proteins were identified by incubation with antihuman IgE antibody followed by avidin-peroxidase (both obtained from Bioclone, Sydney, Australia). The membranes were developed using an ECL advance kit (Amersham Biosciences) and IgE-binding bands visualized using both an Image Master VDS-CL (Amersham Biosciences) and radiograph film (Hyperfilm[®], Amersham Biosciences). Immunoblot data were analyzed with Quantity One software (Bio-Rad), which calculated the molecular weight of SDS-PAGE resolved bands and provided densitometry data. A band was considered positive if its signal was 25% or more greater than background. Negative controls, consisting of non-JJA allergic sera, were included in all experiments.

Statistical analysis

Confidence intervals for proportions were determined by the binomial method, and cross-tabulations of allergen recognition were assessed using the Fisher exact test using Analyse-It[™] (Analyse-It Software Ltd, Leeds, UK) for Microsoft Excel[®]. Statistical significance was defined by a type I error value of 0.01 or less because multiple analysis were performed.

Results

Of the 68 patients included in the VIT trial, 13 had a RAST value of <1.0% and sera from one patient was unavailable, leaving 54 available for analysis. Baseline clinical characteristics of these patients are provided in Table 1.

Coomassie blue stain of PVDF membranes indicated that transfer of AU and SDS-PAGE resolved nonreduced venom was successful, but transfer of reduced/alkylated venom was only reliable following SDS-PAGE (data not shown). Examples of AU PAGE, gradient SDS-PAGE,

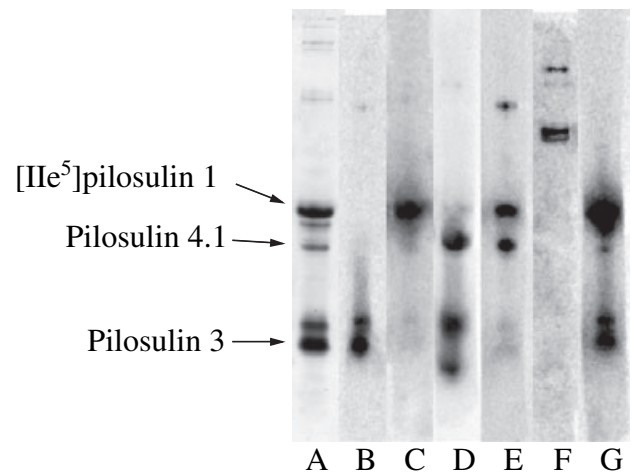


Figure 1. Acid urea polyacrylamide gel electrophoresis (AU PAGE) immunoblots to identify low-molecular weight allergens. About 5 µg of nonreduced Jack Jumper Ant (JJA) venom was resolved via AU PAGE and stained with Coomassie blue (lane A) or transferred to PVDF membrane. Transferred venom was probed with individual patient sera to identify immunoglobulin E-binding proteins. Lanes B–G, the results obtained from different sera; lane A, Coomassie blue-stained nonreduced JJA venom. Arrows demonstrate the identity of the major bands, as previously determined by mass spectrometry (5); lane B, exclusive pilosulin 3 recognition; lane C, exclusive recognition of [Ile⁵]pilosulin 1; lane D, recognition of pilosulin 3 and pilosulin 4.1; lane E, recognition of [Ile⁵]pilosulin 1, pilosulin 4.1 and another undefined protein; lane F, no recognition of low-molecular weight allergens, but recognition of other undefined proteins; lane G, recognition of [Ile⁵]pilosulin 1, pilosulin 3, and pilosulin 4.1.

and tricine SDS-PAGE immunoblots are illustrated in Figs 1–3, respectively.

IgE-binding bands

Thirteen distinct IgE-binding bands were identified in nonreduced venom. Ten of these were recognized by three or more sera, whilst proteins with molecular mass of 42.8, 43.8 and 77.6 kDa were each recognized once. The number of bands recognized by individual sera ranged from 1 to 6 (median 3). Fifteen patients recognized only one band; these exclusively recognized bands were [Ile⁵]pilosulin 1 ($n = 3$), pilosulin 3 ($n = 10$), and the 6.6 and 34.4 kDa proteins ($n = 1$ each).

[Ile⁵]pilosulin 1 and pilosulin 3 were recognized by 18 of 54 (33.3%, 95% CI: 21.1–47.5%) and 42 of 54 (77.8%, 95% CI: 64.4–88%) of patient sera, respectively. Of all, 87% (95% CI: 75.1–94.6%) of patients recognized [Ile⁵]pilosulin 1 and/or pilosulin 3. Pilosulin 4.1 was recognized by nine of 54 (16.7%, 95% CI: 7.9–29.3%). Bands at 6.6, 22.8, 25.6, 30.4, 32.1, 34.4 and 89.8 kDa were each recognized by at least three sera (Table 2). Seven patients did not recognize [Ile⁵]pilosulin 1 and/or pilosulin 3, and examples of these immunoblots

Table 1. Baseline data from subjects included for analysis ($n = 54$)

Median age	46 (range: 18–63)
Males	64.8%
Previous worst grade of reaction (%) [*]	
Grade 2	11.1 ($n = 6$)
Grade 3	54.4 ($n = 31$)
Grade 4	31.5 ($n = 17$)
Median time since last reaction (years)	2.5 (range: 0–35)
Median <i>Myrmecia pilosula</i> venom-specific IgE (RAST tracer uptake)	4.15% (range: 1–32.8)

^{*}As per the system of Muller (25).

IgE, immunoglobulin E; RAST, radioallergosorbent test.

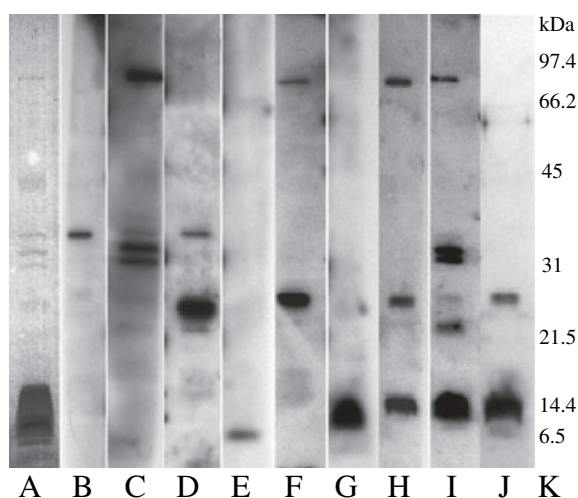


Figure 2. Gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblots. About 5 μ g of nonreduced Jack Jumper Ant venom was resolved via gradient SDS-PAGE and stained with Coomassie blue (lane A) or transferred to PVDF membrane. Transferred venom was probed with individual patient sera to identify immunoglobulin E-binding proteins. Lanes B–F, immunoblots where bands other than pilosulin 3 or [Ile⁵]pilosulin 1 are recognized; lanes G, typical immunoblot where pilosulin 3 and/or [Ile⁵]pilosulin 1 are recognized; lanes H–J, immunoblots where pilosulin 3 and/or [Ile⁵]pilosulin 1 are recognized, in addition to other high-molecular weight bands; lane K, molecular weight markers.

are illustrated in Fig. 2 (lanes B–F). The immunoblots from three patients were similar to lane F and the pattern of the bands recognized in lanes B–E was unique.

Glycosylation stain

Pro Q[®] Emerald 300 stain revealed a glycosylated band with approximate molecular mass of 45 kDa, but the IgE-binding bands did not contain carbohydrate moieties (data not shown), confirming that the allergic sera IgE were binding to primary amino acid sequences.

Effect of reducing venom

Thirteen immunoblots of sera that recognized pilosulin 3 on AU PAGE were investigated by tricine SDS-PAGE immunoblot and all were shown to bind pilosulin 3. After reduction/alkylation, all 13 recognized pilosulin 3a and four recognized both pilosulin 3a and pilosulin 3b. Immunoblots with pooled positive sera showed that IgE binding to [Ile⁵]pilosulin 1 and the 89.8 kDa band was unaffected by the reduction process, but pilosulin 4.1 and the 6.6, 22.8, 25.6, 30.4, 32.1 and 34.4 kDa bands disappeared and were not replaced by any new IgE-binding bands. This was confirmed with tricine SDS-PAGE immunoblots using selected individual sera from those who recognized these proteins. Examples are illustrated in lanes C–H of Fig. 3.

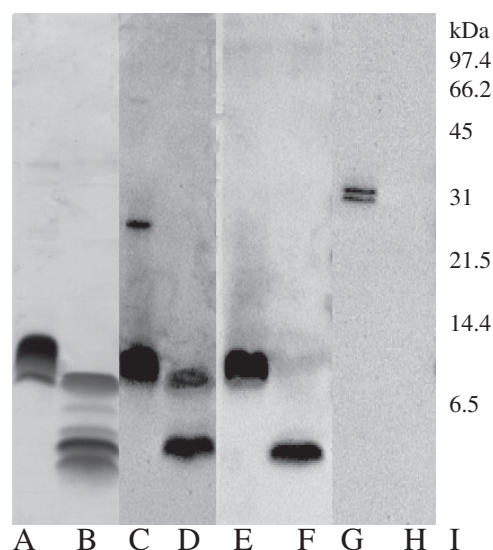


Figure 3. Tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblots. About 5 μ g of both nonreduced and reduced/alkylated Jack Jumper Ant venom was resolved via tricine SDS-PAGE and stained with Coomassie blue (lanes A and B, respectively) or transferred to PVDF membrane. Venom was probed with individual patient sera to identify immunoglobulin (Ig) E-binding bands. Lanes C–H are examples of results obtained from different sera; lanes C and D, recognition of 25.6 kDa protein and low-molecular weight species in nonreduced venom (lane C). Following reduction/alkylation (lane D), the 25.6 kDa band disappears and is not replaced, but recognition of pilosulin 3a and [Ile⁵]pilosulin 1 is apparent; lanes E and F, low-molecular weight species are identified in nonreduced venom (lane E). Following reduction/alkylation (lane F), only pilosulin 3a is recognized. Lanes G and H, recognition of 30.4 and 32.1 kDa band in nonreduced venom (lane G). Upon reduction/alkylation (lane H), no IgE-binding bands are apparent; lane I, molecular weight markers.

Potential cross-reactivity between IgE-binding bands

All three patients that recognized the 30.4 kDa protein also recognized the 32.1-kDa protein ($P < 0.0001$) and a correlation was found between the recognition of the 25.6- and 89.8-kDa proteins ($P = 0.0027$). There were no other clear patterns of potential cross-reactivity.

Discussion

This study has demonstrated that JJA venom contains one major and two minor allergens that have been well characterized and five other allergens that have yet to be named. Pilosulin 3, [Ile⁵]pilosulin 1, and pilosulin 4.1 were clearly shown to bind allergic sera IgE. Pilosulin 3 is the only major allergen in JJA venom and within it, pilosulin 3a is the primary antigenic determinant. Immunoglobulin E-binding proteins with molecular masses of 25.6 and

Table 2. Identity and description of IgE-binding proteins in nonreduced JJA venom that were recognized by three or more individual sera

Molecular mass (name*)	Number of patients (n = 54)	Recognition (95% confidence interval)	Allergen name
6066 Da ([Ile ⁵]pilosulin 1)	18	33.3% (21.1–47.5%)	Myr p 1.0102
5608 Da (pilosulin 3)	42	77.8% (64.4–88%)	Myr p 2.0101
8198 Da (pilosulin 4.1)	9	16.7% (7.9–29.3%)	Myr p 3.0101
6.6 kDa	10	18.5% (9.3–31.4%)	NA†
22.8 kDa	7	13% (5.4–24.9%)	NA†
25.6 kDa	25	46.3% (32.6–60.4%)	NA†
30.4 kDa	3	5.6% (1.2–15.4%)	NA†‡
32.1 kDa	3	5.6% (1.2–15.4%)	NA†‡
34.4 kDa	6	11.1% (4.2–22.6%)	NA†
89.8 kDa	20	37% (24.3–51.3%)	NA†

*If a name has been allocated.

†Unable to be named according to IUIS criteria, as amino acid or cDNA sequence information is unavailable.

‡Cannot yet be designated as an allergen according to IUIS criteria, as less than five patients recognized the protein.

IgE, immunoglobulin E; IUIS, International Union of Immunological Societies; JJA, Jack Jumper Ant.

89.8 kDa were recognized by at least 37% of individuals and as the upper 95% confidence limit crossed 50%, these may be designated major allergens as more sera are tested. Our analysis suggested significant antigenic cross-reactivity between these two proteins. Glycoprotein stains did not identify carbohydrate moieties on any allergens, indicating that IgE binding was to amino acid sequences and carbohydrate moieties were not responsible for cross-reactivity.

Higher molecular weight bands (i.e. > 20 kDa) that were affected by the reduction process did not form any new IgE-binding bands, suggesting that these represent unique allergens rather than different forms or multimers of other allergens. This is further supported by the fact that sera exclusively recognized some of these bands and there was no demonstrated antigenic cross-reactivity between the newly discovered and previously known allergens. Further investigation of the composition of these bands is required.

Multiple PAGE methods were required to separate the various peptides and proteins in JJA venom – each type of PAGE was utilized to identify a specific group of allergens. By utilizing all of these methods we were able to

identify the main IgE-binding proteins, as each sera recognized one or more bands. Peptides in JJA venom with a molecular mass under 10 kDa were well separated by AU PAGE and the resolved peptides have previously been characterized (5). Because of inconsistent transfer to nitrocellulose membrane, previous investigators had difficulty performing immunoblots with AU PAGE resolved peptides (20), but the semi-dry blotting method we employed for AU PAGE resolved venom (15) was effective for nonreduced venom, but transfer of reduced venom proved troublesome. Therefore, tricine SDS-PAGE was used to assess the IgE binding to reduced venom peptides. Gradient SDS-PAGE was particularly useful to determine the IgE-binding capacity of proteins with a molecular mass over 20 kDa and the 6.6 kDa band (Fig. 2, lane E), which was located well below the diffuse band that was typical for patients who recognized other lower molecular weight peptides (Fig. 2, lanes G–J).

Presumably because of difficulties in the SDS-PAGE separation of nonreduced JJA venom, most prior investigations into venom allergens have utilized reduced venom. Electrophoretic separation of reduced but not alkylated venom may lead to aberrant multimer formation (6), so immunoblots using reduced and not alkylated JJA venom must be interpreted with caution, as IgE may bind to aberrant multimers instead of natural peptides. We reduced and alkylated venom to prevent re-formation of aberrant multimers during electrophoresis, which may explain the lack of correlation between the IgE-binding components described here and those described by Wu (13).

With the exception of two minor allergens in honey bee venom [mellitin (21) and Api m 6 (22)], hymenoptera venom allergens identified thus far almost exclusively have a molecular weight of 10–50 kDa (23,24). Jack Jumper Ant venom allergy appears to be unique in that the most important allergen is pilosulin 3, which has a low molecular mass (5608 Da). The significance of having such a large contribution by mass of this highly allergenic small peptide to JJA venom (10) is unclear.

The nomenclature of JJA venom allergens, cDNA clones, peptides, and their processed forms has proved a source of confusion for a number of reasons, including ambiguous allocation of names and discrepancies between the peptides predicted by cDNA (7,8) and what was observed following mass spectrometric analysis (5,10). As such, the nomenclature of JJA venom allergens requires clarification. We propose the following revision,

Table 3. Revised nomenclature of named allergens in Jack Jumper Ant (JJA) venom

Allergen	Isoallergens	Isoforms/variants	Trivial names
Myr p 1	Myr p 1.01	Myr p 1.0101 Myr p 1.0102	Pilosulin 1 (7,9) [Ile ⁵]pilosulin 1 (10)
Myr p 2	Myr p 2.01	Myr p 2.0101	Pilosulin 3, a heterodimer of des-Gly ²⁷ -pilosulin 2 (also named pilosulin 3a) and pilosulin 3b (10)
Myr p 3	Myr p 3.01	Myr p 3.0101	Pilosulin 4.1 (5)

according to IUIS criteria (14) which has been summarised in Table 3:

- 1 Myr p 1 is a minor allergen. Pilosulin 1 and its predominant isoform/variant [Ile⁵]pilosulin 1 should be named **Myr p 1.0101** and **Myr p 1.0102**, respectively.
- 2 Myr p 2 is a major allergen. The previous Myr p 2 has been shown to form a monomer chain within pilosulin 3, and as such, the pilosulin 3 heterodimer should be viewed as a revised definition of Myr p 2. This pilosulin 3 isoform should be referred to as **Myr p 2.0101**.
- 3 Myr p 3 is a minor allergen. In native venom it exists only as a homodimer, which has previously been given the trivial name pilosulin 4.1. This isoform should be referred to as **Myr p 3.0101**.
- 4 Because only three patients recognized the 30.4- and 32.1-kDa proteins and only four recognized pilosulin 3b, these cannot yet be defined as allergens. The 6.6, 22.8, 25.6, 34.4 and 89.8 kDa proteins appear to be allergens, but as no cDNA or amino acid sequence data are available, they cannot be named according to IUIS criteria.

We have identified eight JJA venom allergens and updated the nomenclature to define three of these allergens, Myr p 1, Myr p 2, and Myr p 3. Further characterization of unnamed IgE-binding bands should be performed prior to naming these proteins as allergens. Radioallergosorbent test inhibition with specific inhibi-

tors or probing the expressed products of a cDNA library with allergic patient sera can then more accurately determine the importance of each species. Construction of recombinant or synthetic peptides may; however, pose some challenges, as many of the IgE-binding epitopes appear to be dependant upon the tertiary and/or quaternary protein structure.

By identifying the main allergens in JJA venom, we have provided a framework for standardizing venom extracts for diagnosis and immunotherapy. Furthermore, we are now able to undertake laboratory studies investigating the mechanisms of successful VIT. Characterization of the newly identified allergens may help to precisely define the nature of each of these species and further define their significance in JJA sting anaphylaxis.

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References

1. Brown SG, Franks RW, Baldo BA, Heddle RJ. Prevalence, severity, and natural history of Jack Jumper ant venom allergy in Tasmania. *J Allergy Clin Immunol* 2003;**111**:187–192.
2. Douglas RG, Weiner JM, Abramson MJ, O'Hehir RE. Prevalence of severe ant-venom allergy in southeastern Australia. *J Allergy Clin Immunol* 1998;**101**:129–131.
3. Street MD, Donovan GR, Baldo BA, Sutherland S. Immediate allergic reactions to *Myrmecia* ant stings: immunological analysis of *Myrmecia* venoms. *Clin Exp Allergy* 1994;**24**:590–597.
4. Brown SG, Wiese MD, Blackman KE, Heddle RJ. Ant venom immunotherapy: a double-blind, placebo-controlled, crossover trial. *Lancet* 2003;**361**:1001–1006.
5. Wiese MD, Chataway TK, Davies NW, Milne RW, Brown SG, Gai WP et al. Proteomic analysis of *Myrmecia pilosula* (Jack Jumper) ant venom. *Toxicon* 2006;**47**:208–217.
6. Donovan GR, Street MD, Tetaz T, Smith AL, Alewood D, Alewood P et al. Expression of jumper ant (*Myrmecia pilosula*) venom allergens: post-translational processing of allergen gene products. *Biochem Mol Biol Int* 1996;**39**:877–885.
7. Donovan GR, Baldo BA, Sutherland S. Molecular cloning and characterization of a major allergen (Myr p I) from the venom of the Australian jumper ant, *Myrmecia pilosula*. *Biochim Biophys Acta* 1993;**1171**:272–280.
8. Street MD, Donovan GR, Baldo BA. Molecular cloning and characterization of the major allergen Myr p II from the venom of the jumper ant *Myrmecia pilosula*: Myr p I and Myr p II share a common protein leader sequence. *Biochim Biophys Acta* 1996;**1305**:87–97.
9. Wu QX, King MA, Donovan GR, Alewood D, Alewood P, Sawyer WH et al. Cytotoxicity of pilosulin 1, a peptide from the venom of the jumper ant *Myrmecia pilosula*. *Biochim Biophys Acta* 1998;**1425**:74–80.
10. Davies NW, Wiese MD, Brown SG. Characterisation of major peptides in 'Jack Jumper' ant venom by mass spectrometry. *Toxicon* 2004;**43**:173–183.
11. Inagaki H, Akagi M, Imai HT, Taylor RW, Kubo T. Molecular cloning and biological characterization of novel antimicrobial peptides, pilosulin 3 and pilosulin 4, from a species of the Australian ant genus *Myrmecia*. *Arch Biochem Biophys* 2004;**428**:170–178.
12. Brown SG, Wu QX, Kelsall GR, Heddle RJ, Baldo BA. Fatal anaphylaxis following jack jumper ant sting in southern Tasmania. *Med J Aust* 2001;**175**:644–647.
13. Wu QX. Immunobiology of peptides of the Jumper Ant *Myrmecia pilosula*. PhD thesis, University of Sydney, Sydney, 2000.
14. King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W. Allergen nomenclature. *Allergy* 1995;**50**:765–774.
15. Wang MS, Pang JS, Selsted ME. Semi-dry electroblotting of peptides and proteins from acid-urea polyacrylamide gels. *Anal Biochem* 1997;**253**:225–230.

16. Schagger H, von Jagow G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 1987;**166**:368–379.
17. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1979;**76**:4350–4354.
18. Swerdlow PS, Finley D, Varshavsky A. Enhancement of immunoblot sensitivity by heating of hydrated filters. *Anal Biochem* 1986;**156**:147–153.
19. Ida N, Hartmann T, Pantel J, Schroder J, Zerfass R, Forstl H et al. Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J Biol Chem* 1996;**271**:22908–22914.
20. Donovan GR, Street MD, Baldo BA. Separation of jumper ant (*Myrmecia pilosula*) venom allergens: a novel group of highly basic proteins. *Electrophoresis* 1995;**16**:804–810.
21. Paull BR, Yunginger JW, Gleich GJ. Melittin: an allergen of honeybee venom. *J Allergy Clin Immunol* 1977;**59**:334–338.
22. Kettner A, Hughes GJ, Frutiger S, Astori M, Roggero M, Spertini F et al. Api m 6: a new bee venom allergen. *J Allergy Clin Immunol* 2001;**107**:914–920.
23. King T, Guralnick M. Hymenoptera allergens. In: Lockey R, Bukantz S, Bousquet J, editors. *Allergens and allergen immunotherapy*, 3rd edn. New York: Marcel Dekker, 2004:339–354.
24. Muller UR. Recombinant Hymenoptera venom allergens. *Allergy* 2002;**57**:570–576.
25. Muller U. *Insect sting allergy: clinical picture, diagnosis, and treatment*. New York: Gustav Fischer Verlag, 1990.