Preventing anaphylaxis to venom of the jack jumper ant (*Myrmecia pilosula*)

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Dedication

To my wife Danielle who has somehow managed to cope with two daughters under the age of three as well as my seemingly endless commitment to work and study, and who has kept me feeling loved, fed, and watered. To my daughters Stella and Felicity who's earliest memories will lead them to think that a laptop computer was somehow grafted to my body; their interruptions were always welcome and kept me more-or-less sane. Moreover, to our unborn child Michelle who has provided the inspiration to ensure this thesis is submitted before her birth so that I can return my whole attention to the family.

Contents

Summary

Background *Myrmecia pilosula* (the "jack jumper" ant, JJA) is the principal cause of ant venom anaphylaxis in Australia. Whereas honeybee and wasp venom allergy can be treated by venom immunotherapy (VIT), no such treatment is available for ant sting allergy. In addition, information on the natural history of JJA sting allergy is required to identify those most likely to benefit from immunotherapy. The main objectives of this research were to establish: (i) the prevalence, natural history and determinants of reaction severity for JJA allergy, and; (ii) the efficacy and tolerability of JJA VIT.

Methods A search of the Royal Hobart Hospital (RHH) forensic register, a random telephone survey, and a review of emergency department (ED) presentations were performed. Three hundred eighty-eight JJA allergic volunteers were assessed, including serum venom-specific IgE RAST, and then followed up for accidental stings over a 4-year period. Finally, a randomised double-blind, placebo-controlled, crossover trial of JJA VIT was performed. Laboratory parameters measured during the trial were; leukocyte stimulation index (SI), IL-4 production, IgE RAST, histamine release test (HRT), leukotriene release test (LRT) and basophil activation test (BAT). Intradermal venom skin testing (VST) was also performed at trial entry.

Findings The prevalence of JJA sting allergy was 2.7% in the Tasmanian population, compared to 1.4% for honeybee. People aged ≥35 had a greater risk of both sting allergy and hypotensive reactions. Four deaths were identified, all in adults with significant comorbidities. During follow-up, 79 (70%) of 113 accidental jack jumper stings caused systemic reactions. Only prior worst reaction severity predicted the severity of follow-up reactions, with the majority of people experiencing similar or less severe reactions when stung again.

Sixty-eight otherwise healthy JJA allergic adult volunteers were enrolled in the clinical trial. Systemic reactions to therapy were recorded in 34% during VIT. Objectively defined systemic reactions to sting challenges arose in 1/35 after VIT (mild self-limiting urticaria only) versus 21/29 in the placebo group. Treatment with oxygen, intravenous adrenaline infusion and volume resuscitation was effective and well tolerated. Hypotension was always accompanied by a relative bradycardia, which was severe and treated with atropine in two patients.

In the placebo group, only VST and HRT were predictive of sting challenge results. Although IgE RAST, leukocyte SI and IL-4 production, LRT and BAT all correlated well with VST, they did not predict sting challenge outcome. After successful VIT,

venom-induced leukocyte IL-4 production tended to fall, whereas IgE RAST increased and a natural decline in HRT reactivity was reversed.

Interpretation VIT is highly effective in prevention of JJA sting anaphylaxis and is likely to be of most benefit to people with a history of severe systemic reactions, which usually occur in people aged over 35. Neurocardiogenic mechanisms &/or direct cardiac effects may be important factors in some anaphylaxis deaths. Systemic reactions to immunotherapy are common and require immediate access to resuscitation facilities. The HRT warrants further investigation as a test for selecting those most likely to benefit from VIT. None of the tests evaluated appear to be reliable markers of successful VIT.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree of diploma with any university; and that to the best of my knowledge and belief does not contain any material written by another person except where due reference is made in the text.

Simon G. A. Brown, 15 October 2003

Acknowledgements

The development of an effective venom immunotherapy for people allergic to the sting of the “jack jumper” began in Tasmania in the 1980’s thanks largely to the late Dr Paul Clarke who brought the extent of the problem to the attention of doctors, media and local politicians. Dr Clarke initiated the process leading to this trial by involving the Royal Hobart Hospital Department of Emergency Medicine though my predecessor, Dr Bryan Walpole.

A milestone for our team was establishing a link with the Flinders Medical Centre. My decision to pursue PhD studies with Flinders University was determined by early interactions with Dr Bob Heddle. It was immediately apparent that his enthusiastic supervision would make a world of difference. Our research has benefited enormously from Bob’s keen clinical and analytical skills, unparalleled experience of venom immunotherapy for bee sting allergy, generous provision of time, and not least of all his Australian Rules football-oriented humour. I am also grateful for the academic guidance provided by Professor Peter Roberts-Thomson.

The Royal Hobart Hospital Research Foundation, Department of Health and Human Services (via the Dick Buttfield Memorial Scholarship) and Cosy Cabins Tasmania (in memory of Mr Arthur F. Park who died following a jack jumper sting in 1999) all provided generous financial support. NSL Health Ltd (Melbourne), at the instigation of Dr Brian Baldo, then provided a substantial funding boost enabling us to expand the scope of our project. I am also indebted to many staff in the Emergency Departments and Pathology Departments of the Royal Hobart Hospital and North West Regional Hospital Burnie, for their assistance during all stages of our work.

The University of Tasmania School of Medicine and the Royal Hobart Hospital provided laboratory space and clinical facilities. I am particularly grateful for the support of Dr Greg Woods and Professor Konrad Muller, both from the Discipline of Pathology at the Hobart Clinical School, who provided a great deal of encouragement and training in laboratory methods.

Numerous co-investigators, students and research assistants helped during various stages of the project. These are listed as authors &/or acknowledgments in our published papers included in the Appendices. In particular I thank pharmacist Michael Wiese without whom the clinical trial could not have occurred, Dr Konrad Blackman, Vikki Stenlake (Research Nurse), Sandra Ahokas (Administrative Assistant), Matilda

Haas (Research Assistant and Science Honours Student), Andrew Black and Anand Parameswaran (Medical Honours Students), and David Spiers (Research Assistant).

Medical research requires strong collaborations. Therefore, this thesis necessarily includes some data that has been described in theses of my collaborators. Immunological studies reported in brief for one case from our series of deaths (Chapter 3) also forms part of the PhD thesis of Qi Xuan Wu (University of Sydney). Three honours students performed a wide range of laboratory studies under my supervision for the project (Matilda Haas- cellular proliferation and cytokine production studies; Andrew Black- histamine/leukotriene release and basophil activation studies; Anand Parameswaran- serum IgE analysis). Their theses report detailed laboratory methods and preliminary data. However, the analysis of all results (including clinical correlation) is reported here for the first time (Chapters 10 and 11).

The 68 Tasmanians who enrolled in the clinical trial deserve the highest praise. Without them, all our endeavours would have been in vain. Each had the choice of waiting until a proven treatment was available thus leaving it for others to take the risk. To each of them I extend my thanks on behalf of at least 10,000 Australians who stand to benefit immediately and many more who will benefit in the future.

Finally, Dr Bryan Walpole will not be forgotten. His vision and moral support have been inspirational throughout my career at the Royal Hobart Hospital, and his masterstroke can be summed up in his own words: “Mate, would you mind taking on this research for me after I leave? It’ll just be a matter of giving some injections to a few people, a couple of hours each week for a few months…”

Abbreviations

BAT	Basophil activation test
ED	Emergency department
HRT	Histamine release test
IFA	Imported fire ant, *solenopsis* spp.
IFN-γ	Interferon gamma
IL-4	Interleukin 4
JJA	Jack jumper ant, *Myrmecia pilosula* species complex
LRT	Leukotriene release test
Myr p 1	*Myrmecia pilosula* venom peptide allergen 1
Myr p 2	*Myrmecia pilosula* venom peptide allergen 2
RAST	Radioallergosorbent test
sIgE	Allergen-specific Immunoglobulin E
sIgG	Allergen-specific Immunoglobulin G
SI	Stimulation index
SIT	Specific immunotherapy (aeroallergen and venom)
sp., spp.	Species (sp.- singular, spp.- pleural)
VIT	Venom immunotherapy
VST	Intradermal venom skin testing
WBE	Whole body extract

Foreword

In 1964, an analysis of 5-years of enquiries to Commonwealth Serum Laboratories for medical advice found that allergy to the sting of *Myrmecia pilosula* (the jack jumper ant, JJA) was a problem mainly in Tasmania and Victoria.[1] The potential extent and severity of this problem was outlined in 1986 by Dr Paul Clarke, who also questioned the contemporary practice of desensitisation using ant whole body extracts (WBE) and suggested further research into the use of pure venom (venom immunotherapy, VIT) to treat ant venom allergy.[2] *Myrmecia* WBE preparations were withdrawn in the early 1990s and at a scientific meeting in 1995 were reported to be ineffective.[3]

This thesis describes clinical research conducted in Tasmania to develop a venom immunotherapy for JJA sting allergy. Behind the scenes, development of a method for extracting large amounts of JJA venom in the field, further investigation of the native venom allergens, development of improved analytical techniques, and venom stability studies have been underway. That work has been expertly managed by my pharmacist colleague Michael Wiese, and will be reported elsewhere.