

A Proteomic Study of Innate Immune Protection in the
Tammar wallaby (*Macropus eugenii*)

Kiran S Ambatipudi
BVSc & A. H. (2002)
MSc (2003)

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Department of Biological Sciences
Division of Environmental and Life Sciences
Macquarie University
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Proteomic analysis of neutrophil proteins in the tammar wallaby (*Macropus eugenii*).

Comparative Biochemistry and Physiology, Part D: (2006) 1, 283- 291.

Kiran S. Ambatipudi, Julie M. Old, Michael Guilhaus, Mark Raftery, Lyn Hinds and

Elizabeth M. Deane

Chapter 3

In search of neutrophil granule proteins of the tammar wallaby (*Macropus eugenii*).

Molecular Immunology: (2008) 45, 690-700

Kiran S. Ambatipudi and Elizabeth M. Deane.

Chapter 4

A proteomic approach to analysis of antimicrobial activity in marsupial pouch secretions.

Developmental and Comparative Immunology: (2008) 32, 108-120

Kiran S. Ambatipudi, Janice Joss, Mark Raftery and Elizabeth M. Deane

Chapter 5

A comparative proteomic analysis of skin secretions of the tammar wallaby (*Macropus eugenii*) and the wombat (*Vombatus ursinus*).

Comparative Biochemistry and Physiology, Part D: (2007) 2, 322-331

Kiran S. Ambatipudi, Janice Joss and Elizabeth M. Deane.

Chapter 6

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Summary

This study has taken a proteomics approach to investigating two aspects of innate immune protection in a model marsupial the tammar wallaby, *Macropus eugenii*. The proteins of neutrophils and their granules have been documented using two-dimensional gel electrophoresis (2DE) and mass spectrometry (MS). The first step in this project required development of protocols for the effective isolation of neutrophils and their granules. Fifty three abundant proteins were initially identified from neutrophils and subsequently a range of protocols including stimulation with PMA, Ionomycin and calcium as well as differential centrifugation and cell lysis were used to isolate granule proteins. Five antimicrobial proteins of granule origin were identified along with a number of proteins associated with the process of exocytosis. The identification of these proteins from the neutrophils in the tammar wallaby clearly shows the degree of conservation of such proteins across different mammal species.

The second portion of this project was aimed at examining the unique nature of the marsupial pouch in protecting the young immunologically incompetent animal. Pouch secretions collected at major stages of the reproductive cycle showed varying levels of antimicrobial activity primarily against Gram negative *E. coli* but not against the Gram positive *S. aureus*. Greatest antimicrobial activity was observed in samples collected at oestrus, the anticipated time of birth of the young animal. Subsequent proteomic analysis, using 2DE and LC-MS/MS, led to confident identification of a range of peptides matched to β -lactoglobulin. As the likely origin of β -lactoglobulin could be mammary gland or

digested products from the gut of the pouch young, samples from these sources were also analyzed. In parallel with this portion of the study the changes in the skin proteome (the secretome) of the pouch were investigated through proteomic analysis of secretions from pouch skin of immature, mature reproductively active and post-reproductive females and from a non-pouch skin site. A limited number of proteins could be reliably identified although clear differences in the patterns of secretion were observed at these different life stages. Of the proteins that could be identified, globins were present at all stages with dermcidin, a known potent antimicrobial identified in an opportunistic sample collected from a common wombat, *Vombatus ursinus*. Limited though the successful identification of proteins secreted into the pouch has been, this project has clearly shown that (i) secretions from this site are unique (ii) the types of proteins secreted vary dependent on the reproductive maturity of the female and (iii) there is demonstrable antimicrobial activity against Gram negative organisms although the active component could not be specifically identified.

Declaration

The work presented in this thesis, to the best of my knowledge, is original except where acknowledged in the text. I hereby declare that I have not submitted this material, in whole or in part, for a higher degree at this or any other University or institution.

Kiran S Ambatipudi
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Contribution of Authors

Ambatipudi is the lead author and undertook all the experimental protocols and extensive planning. Training in use of the instrumentation and advice on analysis of MS data was provided by Raftery and Guilhaus. Hinds provided help, access and advice on marsupials as did supervisors to this project, Old and Deane. For the two papers on which Joss is an author Paper 3, Joss undertook the milk and gut proteomics (Gels +MS) and for Paper 4 – the wombat skin gel.

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