

**EXPRESSION OF A TICK TOXIN FOR THE
DEVELOPMENT OF A CANINE VACCINE**

BY

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of the degree of Master in the Faculty of Science,
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DECLARATION

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I certify that this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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TABLE OF CONTENTS

ABBREVIATIONS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1	PREVIEW	01
1.2	SCORPIONS	02
1.2.1	The structure of scorpion toxins	02
1.2.2	Scorpion toxin expressions	03
1.2.3	Monoclonal antibodies against scorpion toxins	07
1.3	SPIDERS	10
1.3.1	Spider toxin structures	10
1.3.2	Expression of spider fusion toxins	11
1.3.3	Monoclonal antibodies to spider toxins	12
1.4	TICKS	14
1.4.1	Cattle tick <i>Boophilus microplus</i>	14
1.4.2	Fusion protein expression for vaccine studies	15
1.4.3	Characterisation of antigens from <i>Boophilus microplus</i>	17
1.5	THE AUSTRALIAN PARALYSIS TICK, <i>IXODES HOLOCYCLUS</i>	18
1.5.1	Parasitic life cycle	19

1.5.2	Tick attachment and feeding	20
1.5.3	Features of tick paralysis vs current treatments paralysis	22
1.5.4	Isolation and purification of paralysis toxin	23
1.6	AIMS OF THIS PROJECT	28

CHAPTER 2: PRODUCTION AND CHARACTERISATION OF MONOCLONAL ANTIBODIES AGAINST MALTOSE BINDING PROTEIN-HT-1 FUSION PROTEIN

2.1	INTRODUCTION	29
2.2	MATERIALS AND METHODS	30
2.2.1	Production of monoclonal antibodies	30
2.2.1.1	Mouse immunisation protocol	30
2.2.1.2	Preparation of myeloma cells	30
2.2.1.3	Fusion	30
2.2.1.4	HAT selection	31
2.2.1.5	Enzyme linked immunoassay	31
2.2.1.6	Cloning	32
2.2.1.7	Cryopreservation	32
2.2.1.8	Thawing cells	32
2.2.2	Characterisation of monoclonal antibodies	33
2.2.2.1	SDS gel electrophoresis	33
2.2.2.2	Western blots	33
2.2.2.3	Immunodetection	33
2.2.2.4	Isotyping	34
2.2.2.5	Inhibition ELISA	34

2.3	RESULTS	35
2.3.1	Production of monoclonal antibodies	35
2.3.2	Characterisation of the monoclonal antibodies	40
2.4	DISCUSSION	50

CHAPTER 3: UBIQUITIN FUSION PROTEIN EXPRESSION AND TOXICITY ASSAYS

3.1	INTRODUCTION	55
3.2	MATERIALS AND METHODS	56
3.2.1	Ubiquitin HT-1 fusion protein expression	56
3.2.1.1	Primer design for pMal-p2 vector	56
3.2.1.2	PCR amplification	56
3.2.1.3	Agarose gel electrophoresis	56
3.2.1.4	Ligating HT-1 gene into pGEM-T vector	57
3.2.1.5	Electroporation	57
3.2.1.6	Plasmid restriction	57
3.2.1.7	Sequencing	58
3.2.1.8	Insertion of HT-1 gene into ubiquitin vector	58
3.2.1.9	Transformation	59
3.2.1.10	Expression protocol	59
3.2.1.11	Affinity purification	59
3.2.1.12	Enzyme expression and Ni-NTA purification	60
3.2.1.13	Concentration of fusion protein	60
3.2.1.14	Protein estimation	61
3.2.1.15	Deubiquitin enzyme cleavage	61
3.2.1.16	Reverse Phase HPLC	61

3.2.1.17	Tricine SDS-PAGE	61
3.2.1.18	Silver staining	62
3.2.1.19	Western blotting	62
3.2.1.20	Protein A column purification	62
3.2.2	Protection assay	63
3.2.2.1	Crude tick homogenate preparation	63
3.2.2.2	Toxicity assay	63
3.2.2.3	Rabbit immunization and protection assay	63
3.2.2.4	Mouse immunization and protection assay	64
3.3	RESULTS	65
3.3.1	Ubiquitin fusion protein expression / purification	65
3.3.2	Cleavage	72
3.3.3	Immunogenicity	76
3.3.4	Protection assays	82
3.4	DISCUSSION	88
3.4.1	Fusion protein expression	88
3.4.2	Protein expression and purification	90
3.4.3	Ubiquitin cleavage	91
3.4.4	Immunological characterization	92
3.4.5	Future direction	95
	BIBLIOGRAPHY	97
	APPENDIX	112

ABBREVIATIONS

(Abu) ₈ AahII	<i>Androctonus australis hector</i> toxin analog
AaHIT	<i>Androctonus australis hector</i> insect toxin
AaIT	<i>Androctonus australis hector</i> insect selective toxin
AcNPV	<i>Autographa californica Nuclear Polyhedrosis Virus</i>
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BM	<i>Boophilus microplus</i>
BmK	<i>Buthus martensill Karsch</i>
bp	base pair
BSA	bovine serum albumin
cDNA	complementary DNA
Da	Dalton
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
ED ₅₀	paralytic unit
EDTA	ethylene diaminetetra-acetic acid
ELISA	enzyme linked immunoassay
Fab	fragment antigen binding
FBS	fetal bovine serum
Fc	fragment constant
GM	midgut membrane
GST	glutathione S-transferase
HAT	hypoxanthine, aminopterin, thymidine
HPLC	high pressure liquid chromatography
HPRT	hypoxanthine phosphoribosyl transferase enzyme
HT	holocyclus toxin
HWTX	Huwentoxin
Ig	immunoglobulin
IMAC	immobilized metal affinity column

IPTG	isopropylthio- β -D-galactopyranoside
kbp	kilobasepairs
kDa	kiloDalton
KTX2	kaliotoxin2
LB	Luria broth
LD ₅₀	dose causing 50% death
Lqh α IT	<i>Leiurus quinquestriatus hebraeus</i> alpha insect toxin
mA	milliamps
mAb	monoclonal antibody
MBP	maltose binding protein
mM	millimolar
MW	molecular weight
MWCO	molecular weight cut off
NaAz	sodium azide
NBT	nitro blue tetrazolium
Ni-NTA	nickel-nitrilotriacetic acid
OD	optical density
PCR	polymerase chain reaction
pmol	picomole
p-NPP	p-nitrophenyl phosphate
PVDF	polyvinylidenedifluoride
rpm	revolutions per minute
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SSNAP	sparingly soluble non-antigenic protein
%T	total acrylamide concentration (w/v)
TBE	tris buffered ethylene diaminetetra-acetic acid
TEMED	N,N,N',N'-tetramethylenediamine
TFA	trifluoroacetic acid
TsNTxP	<i>Tityus serrulatus</i> nontoxic protein
U	unit

UCH-L1	ubiquitin carboxyl terminal hydrolase-L1
USP	ubiquitin specific protease
UV	ultraviolet
V	volts
YUH1	yeast deubiquitinating hydrolase 1

LIST OF FIGURES

Figure 1.1	<i>Ixodes holocyclus</i> distribution	18
Figure 1.2	<i>I. holocyclus</i> life cycle	19
Figure 1.3	Electron micrograph of the mouthparts of <i>Ixodes holocyclus</i>	20
Figure 1.4	Level of toxicity (Tick activity scale) at each stage of life cycle	21
Figure 1.5	Dog antibody responses against ticks by feeding ticks on dogs	25
Figure 2.1	Schematic diagram for the production of monoclonal antibodies	36
Figure 2.2	Polyclonal antibodies binding response to HT-1 fusion protein	37
Figure 2.3	Photomicrograph of a large fusion cell colony growing in HAT media at seven day post fusion	38
Figure 2.4	ELISA result for the positive hybrids	39
Figure 2.5	Absorbance readings for the monoclonal antibody secreting clones	41
Figure 2.6	Western blot of mAb 1, mAb 15, mAb 16A9 and mAb 16F11 binding to HT-1 fusion protein under reduced and non-reduced conditions	42
Figure 2.7	Schematic diagram of recombinant toxin	43

Figure 2.8	Binding responses of mAbs to maltose binding protein and irrelevant His-6-tagged protein	44
Figure 2.9	Inhibition ELISA for the binding responses of mAbs to maltose binding protein, irrelevant His-6-tagged protein and fusion protein	47
Figure 2.10	Monoclonal antibodies' reactivities to native toxin	49
Figure 3.1	Confirmation of HT-1 insert that was cloned into the ubiquitin vector in TG1 bacterial cells	66
Figure 3.2	Western blot of the ubiquitin fusion protein expressed (soluble fraction) under various IPTG concentrations and induction periods	67
Figure 3.3	Western blot of the ubiquitin fusion protein expressed (insoluble fraction) under various IPTG concentrations and induction periods	68
Figure 3.4	Expression and purification of ubiquitin fusion protein from soluble and insoluble fractions using native and denaturing conditions	71
Figure 3.5	Deubiquitin enzyme expression and purification	73
Figure 3.6	Silver stain of HPLC purified fusion protein, enzyme and cleavage product	75

Figure 3.7	Western blot of commercial dog anti-tick serum binding to ubiquitin fusion protein	77
Figure 3.8	Western blot of protein A column purified immune rabbit serum binding to HT-1 fusion protein	79
Figure 3.9	Western blot of immune mouse sera binding to HT-1 fusion protein	80
Figure 3.10	Western blot of commercial dog anti-tick serum, immune rabbit and mouse serum binding to crude tick homogenate	81

LIST OF TABLES

Table 2.1	Immunoglobulin class determination	48
Table 3.1	Toxicity assay	84
Table 3.2	Protection assays using purified immune rabbits serum	85
Table 3.3	Protection assays using immune mouse serum	86
Table 3.4	Protection assays using commercial dog anti-tick serum	87

ABSTRACT

Acute, ascending, flaccid motor paralysis of forelimbs and death due to respiratory failure is the dominant characteristic of tick toxicosis by the Australian paralysis tick, *Ixodes holocyclus*. A tick toxin vaccine has the potential to be an effective preventative measure against tick toxicosis that affects thousands of domestic and companion animals each year. In previous research for the development of an anti-tick vaccine, Masina (1999) used a maltose-binding protein (MBP) holocyclus toxin (HT-1) fusion protein as immunogen. It was partially protective against challenges with crude tick extract in neonatal mice but unable to protect dogs against paralysis caused by direct tick attachment.

Subsequently, four monoclonal antibodies generated in this study against the fusion protein were found to be incapable of binding to the native toxin in crude tick extract in Western blots. These results indicated that the HT-1 component of the MBP fusion protein was incorrectly folded compared to the native toxin.

An expression system using ubiquitin fusion protein was investigated. Ubiquitin is a 10 kDa carrier protein which is smaller than the 43 kDa MBP. A smaller fusion partner was considered as it is less likely to interfere with HT-1 folding. The ubiquitin-HT-1 fusion protein was expressed in both soluble and insoluble forms, corresponding to the cytoplasmic fraction and inclusion bodies. The soluble form could be purified under non-denaturing conditions utilising the incorporated His-6-tag and a Ni affinity column. The insoluble form, like the periplasmically expressed MBP-HT-1 fusion protein, could only be purified using denaturing conditions.

The purified soluble ubiquitin-HT-1 fusion protein appeared to have the correct conformational folding as it was recognized by commercial dog anti-tick serum in Western blots. The purified soluble ubiquitin-HT-1 fusion protein was used to immunise rabbits and mice for protection experiments, but was found to be unprotective. However, serum from immunized animals was able to detect a 5 kDa protein from crude tick extract in a Western blot. This 5 kDa protein was also recognized by the commercial dog anti-tick serum and

has the molecular weight corresponding to the HT-1 neurotoxin. Creation of monoclonal antibodies to this ubiquitin-HT-1 fusion protein may therefore aid future development towards a tick vaccine. These antibodies could in turn be used to isolate native HT-1 from the crude tick extract instead of a combination of conventional chromatography methods. This approach would allow isolation of HT-1 in more significant quantities than is currently possible to enable confirmation of the HT-1 sequence. Suspected problems associated with incorrect sequence or interference by the carrier protein to cause a non-protective response could also be resolved.