Effects of Abomasal Nematode Parasites *In Vivo* and *In Vitro*

A thesis presented
in partial fulfilment of the requirements
for the degree of
DOCTOR OF PHILOSOPHY
In Animal Science

at Massey University, Palmerston North
New Zealand

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2003

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Abstract

During abomasal parasitism, parietal cells are inhibited and may be lost, although the exact mechanism is not known. The present *in vivo* and *in vitro* experiments investigated whether the hypoacidity was linked to the host's inflammatory response and whether they are directly targeted by parasites.

Pathophysiological consequences of abomasal parasitism, particularly the effects on parietal cells were studied in different immunological settings in high-fleece weight lambs (HFW) and their unselected controls (C). Lambs were infected with a single dose of *Ostertagia circumcincta* L_3 at the age of 6.5 months (Experiment 1) or 4.5 months (Experiment 2). The older lambs also received further doses of L_3 weekly following primary infection. Lambs were killed on Day 94 or Day 8 or 28 respectively. Serum gastrin and pepsinogen concentrations, blood eosinophils, abomasal pH, paracortical cells in wool follicles (wool quality), FEC and worm burden were monitored. Antibody levels were measured in Experiment 1 and lymphocyte types in abomasal lymph nodes and food intake in Experiment 2.

Tissue eosinophil and parietal cell counts, mucosal thickness and general histopathological changes in abomasal tissues from lambs in Experiment 2 were studied along with tissues collected from randomly bred lambs between five and 30 days after infection with *O. circumcincta* L_3 or six to 72 hours after transplantation of adult *O. circumcincta*.

HFW lambs exhibited resilience, but its expression differed with age. Paracortical cells in wool follicles were lower and increased later in both experiments, yet FEC was higher in older HFW lambs. The rise in abomasal pH was delayed and serum pepsinogen concentrations were higher in older HFW lambs. Generally, blood eosinophils tended to be higher in controls and cytokine responses suggested a lower Th2 response in HFW lambs. In Experiment 2, tissue eosinophils were lower in HFW sheep on Day 8. Resilience in HFW lambs appeared to be based on a down-regulated

inflammatory response and the expression of resilience, which was dependent on full immune responsiveness with age. Additional bases for resilience may be the ability to delay gastric hypoacidity and a higher food intake before and during parasitism as seen in the younger HFW lambs.

No conclusive answer could be given to the question of hypoacidity being secondary to inflammation, although there was a strong link between inflammation and raised abomasal pH associated with vacuolation and loss of parietal cells. Tissue eosinophils were correlated with abomasal pH in all lambs. Parietal cell loss coincided with hypoacidity and inflammation. Blood eosinophil levels did not correlate well with abomasal mucosal eosinohils. In tissues collected 30 hours after larval infection, eosinophils appeared chiefly in the tips of folds, but parietal cell numbers were not reduced.

Evidence for a direct effect of parasite excretory/secretory products on epithelial cells was obtained *in vitro* using the HeLa cell test system for vacuolating activity (neutral red uptake). Adult products were more potent than L_3 chemicals.

These experiments support roles for both the host inflammatory response and parasite chemicals in inhibiting and damaging parietal cells.

Acknowledgements

I wish to thank the Institute of Food, Nutrition and Human Health and Institute of Veterinary, Animal and Biomedical Sciences for allowing me to use their facilities and for their administrative support throughout my Ph.D. I am also indebted to the latter for granting me the Joan Berry Fellowship in 1999, 2000 and 2001 as well as the Phyllis Irene Grey Fellowship in 2000, which helped considerably with my living costs during the duration of my studies. Generous funding from Meat New Zealand, C. Alma Baker Trust and E. & C. Thoms Bequest enabled the work to be undertaken. I wish to thank Dr A. Pernthaner and Dr I. Sutherland of AgResearch Ltd for performing the cytokine assays and Dr P. O'Toole for advice on molecular biology, Mr Richard Green of AgResearch Ltd, Wallaceville for assistance with the antibody assay and Dr D. Thomas for assistance with skin biopsies and histology. Antisera were kindly donated by Dr W. Hein, Dr J. Hansky and Dr. J. Walsh.

I am extremely thankful to my chief supervisor Assoc Prof Heather Simpson, whose patience I stretched on occasions to its absolute maximum. Always approachable and with the best for her students in mind she showed no tiredness to share her enthusiasm for research with me: "I had a thought while I was driving home last night..." became quickly the most dreaded phrase for me. Heather, many thanks for having me as a postgraduate student and for your good nature, support and encouragement throughout my studies. I also wish to thank Dr Ian Scott, who, first as postdoctoral fellow and later as one of my co supervisors, kept his calm and humour throughout all of my antics. His permission to use material as well as some of the data presented from the LI and AT experiments is gratefully acknowledged. Thank you very much for your support and encouragement during, and especially towards the end, of my studies. I am also indebted to Dr Gordon Reynolds for introducing me to sheep surgery and to Brett Guthrie, whose skills and calm were an irreplaceable asset during the days of surgery. The positive criticism and support from Dr Simon Brown is greatly appreciated and without his assistance I would be still a

headless chicken. Thank you very much for your kindness and willingness to help when it was needed most. Dr David Simcock, what shall I say? What had I done without his extraordinary sense of humour and perfect impersonations of Cartman during my years at Massey? Thank you so much for your invaluable help and kindness. Dr Kevin Pedley, the right man at the right place at the right time. Many thanks for your skilful help in the photograph section. The assistance of Mervin Birtles by taking some of my photographs is also greatly acknowledged. I want to thank Dr Mark Patchett and Dr Juliet Sutherland for their patience while introducing me to molecular biology techniques. Extra special thanks go to Juliet for sharing her experiences as a postdoc. Without the excellent technical assistance of Samera Khalaf, who spent countless hours especially hunting larvae and processing the histology, as well as Lois Taylor, who was a flexible allround help with fantastic catering skills, I could not have done all this work. The same has to be said about John Pedley, who came to my aid whenever technical equipment failed me as well as Barbara Addlington, who supplied me with countless parasites and books. Without sheep no thesis, so special thanks to the extremely helpful and friendly sheep crew: Debbie Chesterfield, Geoff Warren and Barry Parlane.
I wish to thank my fellow students and friends Stefanie Reinhardt, Lisa Walker, Heather Purnell, Alexandra Huber, Shu-Er Zeng, Zaid Mohamad, Alexander McLachlan and Janice Lloyd, without their friendship, moral support, resilience, humour and understanding lots of my days would have been a real drag! Keep in touch. To sum it up: The friendliness and kindness I have experienced was amazing. Thank you so much for it.

I am extremely grateful and utterly indebted to my parents without whose help my Ph.D. studies in New Zealand never could have taken place in the first instance. They not only supported me financially, but also believed in me when I did not. For my brothers Werner and Wolfgang a big hug for being so supportive and understanding. A big hug and special thanks go to my brother Gerhard, whose experience and understanding was an enormous reassurance. Perhaps I can help you one day? ;-) Silvia, favourite sister of mine, I can't thank you enough! Thanks Anci, for being such a source of funny craziness! Last but not least many thanks to my colleagues in Liverpool, especially to Mark for his

patience and understanding and to Alice and Kevin for bringing Caffrey's to my attention, while finishing off this beast.

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µg	Microgram
µM	Micromolar
µm	Micrometre
Ab	Antibody
ACh	Acetylcholine
ALN	Abomasal Lymph Node
ANOVA	Analysis of Variance
AP	Aminopyrine
AR	Amphiregulin
AT	Adult Transplant
ATV	Antibiotic-Trypsin-Versene solution
BSA	Bovine Serum Albumin
C	Control flock
cAMP	Cyclic Adenosine Monophosphate
CCK	Cholecystokinin
CCK-A/B	Cholecystokinin Receptor type A or B
CEM	Complete Essential Medium
CNS	Central Nervous System
CGRP	Calcitonin Gene Related Peptide
conA	Concavalin A
DAG	Diacyl Glycerol
e.p.g.	eggs per gram
EBSS	Earle's Balanced Salt Solution
EC	Enterochromaffin

ECL	Enterochromaffin-like
EDTA	di-sodium Ethylene Diamine Tetra-Acetate
EGF	Epidermal Growth Factor
ES	Excretory/Secretory products
FBS	Fetal Bovine Serum
FEC	Faecal Egg Count
g	Gram
g	Gravitational force
Gal	Galanin
GIP	Gastric Inhibitory Peptide
Gm-CSF	Granulocyte-macrophage Colony-Stimulating Factor
GRP	Gastrin Releasing Peptide
GRPR1	Gastrin Releasing Peptide Receptor type 1
h	Hour
H2	Histamine receptor type 2
H&E	Haematoxalin & Eosin
H. pylori	*Helicobacter pylori*
H.contortus	*Haemonchus contortus*
HB-EGF	Heparin Binding-Epidermal Growth Factor
HBSS	Hank's Balanced Salt Solution
HDC	Histidine Decarboxylase
HFW	High Fleece Weight flock
I.A.	Immature Adult
i.m.	intramuscular
i.v.	intravenous
IFN-γ	Interferon-gamma

Ig	Immunoglobulin
IL	Interleukin
IP_3	Inositol 1,4,5-triphosphate
kg	Kilogram
l	Litre
L_3	Third-stage larva
L_4	Fourth-stage larva
L_5	Fifth stage larva
LI	Larval infection
LT	lymphotoxin
M	Molar
M3	Muscarinic receptor type 3
MEM	Minimal Essential Medium
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
MMC	Mucosal Mast Cell
mRNA	Messenger Ribonucleic Acid
MWCO	Molecular Weight Cut-Off
n	Number
N. brasiliensis	*Nippostrongylus brasiliensis*
NA	Noradrenalin
NHE2	Sodium/Hydrogen Exchanger 2
NK	Neurokinin
nm	Nanometers
NR	Neutral Red

O. circumcincta	*Ostertagia circumcincta*
O. leptospicularis	*Ostertagia. leptospicularis*
O. ostertagi	*Ostertagia ostertagi*
OD	Optical Density
P follicle	Primary follicle
p.i.	Post infection
PACAP	Pituitary Adenylate Cyclase-Activating Peptide
PAC-R1	Pituitary Adenylate Cyclase-Activating Peptide Receptor type 1
PAS	Periodic Acid Schiff
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PG	Prostaglandin
PHI	Peptide Histidine Isoleucine
PKA	Protein kinase A
PKC	Protein kinase C
PLC	PhospholipaseC
PP	Pancreatic Polypeptide
PSN	Penicillin-Streptomycin-Neomycin
reg	Regenerating gene
RO water	Distilled water
S follicle	Secondary follicle
s.c.	subcutaneous
SD	Standard Deviation
SEM	Standard Error of the Mean
SP	Substance P
SPSS	Statistic Package for Social Scientists

SST	Somatostatin
SST2	Somatostatin receptor subtype 1
T. axei	*Trichostronglus axei*
T. colubriformis	*Trichostronglus colubriformis*
TAE	Tris Acetate EDTA
TGF-α	Transforming Growth Factor-alpha
Th	T helper cell
TNF-α	Tumor Necrosis Factor-alpha
v/v	volume per volume
VIP	Vasoactive Intestinal Polypeptide
w/v	weight per volume

Introduction

The agricultural industry, especially pastoral farming, plays an important role in the New Zealand economy. Since livestock are almost exclusively kept on pasture in New Zealand, parasites, especially internal helminths, have a significant impact on the outcome of the sheep and beef and dairy industries.

The alarming number of reports of drench resistance, the increasing interest in organic farming and the recognition of the long-term impact of agricultural chemicals on the environment are all incentives to develop alternative strategies to the current chemical anthelmintics. High concentrations of anthelmintics are excreted in the faeces onto the pasture where free-living nematodes and other soil and pasture dwellers are also being affected by highly potent chemicals. Further, there is a significant incidence of anthelmintic resistance amongst the common nematodes infecting ruminants in New Zealand, as is the case elsewhere in the world. This alone has highlighted the need to supplement current drenches with other measures to minimise the use of these chemicals to slow the rate of development of resistance.

Alternative strategies to drenching include better management to minimise larval intake, exploiting the genetic variation in susceptibility of animals to parasites and to their impact on productivity, the use of plants containing natural anthelmintics as fodder, trapping the free-living stages of nematodes by fungi and the development of vaccines against the parasites. Another alternative would be biological control by interfering with parasite establishment, metabolism or reproduction. This approach will depend on a much more detailed understanding of the host-parasite interaction, parasite biology and the pathophysiological basis for resistance, resilience and susceptibility of hosts.

The objective of the present experiments was to study the responses of sheep of different genetic backgrounds to infection with abomasal parasites in order to learn more about the communication between the parasite and the host tissues and the relative importance of host inflammatory responses and the

excretory/secretory products of the worms in causing abomasal dysfunction. The effects of infection with *Ostertagia circumcincta* or *Haemonchus contortus* in outbred sheep are well described: inhibition of gastric acid secretion, hypersecretion of gastrin, increased levels of circulating pepsinogen, loss of functioning parietal cells, inflammation characterised by large numbers of eosinophils and mucous cell hyperplasia in the infected abomasum. What is still unknown is whether the parasites actively inhibit acid secretion to promote their survival, if so, how do they do it and could this be a target for biological control of abomasal nematodes.

Alternatively, it may be more practical to breed for resistant or resilient hosts to reduce the impact of the parasites with a minimum of chemical intervention. Whereas it has been established that resistance has an immunological basis, there seems to be no information on the physiological basis for resilience to parasitism. If resistance and resilience are not opposite extremes of the same phenomenon, then selection may provide a way for the incorporation of both traits into a breeding programme. The experiments described in this thesis are also aimed at establishing the underlying reasons for different host responses to abomasal parasites and from these studies also to gain a better understanding of the pathophysiological processes initiated by the parasites.