HOST-PARASITE INTERACTIONS DURING ABOMASAL PARASITISM AND POTENTIAL ROLES FOR ES PRODUCTS

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

at

Massey University,
Palmerston North,
New Zealand

WIEBKE BÜRING

2009

Table of Contents

Abstract

Parasite excretory/secretory (ES) products are believed to play a role in the initiation of the host response to the abomasal parasites *Haemonchus contortus* and *Teladorsagia circumcincta*. Both parasites inhibit and cause loss of the acid-producing parietal cells. Three days after transplantation of adult *T. circumcincta* into parasite-naive sheep, a subpopulation of their parietal cells no longer expressed the proton pump β-subunit, but still stained for Transforming Growth Factor-α, suggesting loss of the proton pump preceded cell death. To investigate the ability of parasites to modify the function of mammalian cells *in vitro*, HeLa, AGS and CaCo-2 cells were exposed to ES products. ES products vacuolated all three cells, causing the development of large numbers of small vacuoles, which differed in appearance from those produced by *Helicobacter pylori* bacterial toxin VacA or ammonia. The vacuoles were unlike those which develop in parietal cells in the parasitised abomasum. Neither lipids nor prostaglandins appeared to play a role in vacuolation and the vacuolating factor *in vitro* is likely to be a protein because of its heat and acid lability. Vacuolation occurred within one hour and was partially reversible. ES products were also able to cause cytoskeletal rearrangement and detachment of HeLa cells, similar processes to those caused by bacterial pathogens, which also disrupt tight junctions in mammalian cells. *H. contortus* ES products also disrupted tight junctions of CaCo-2 cell monolayers, a model cell system used for these studies. The increased epithelial permeability was associated with structural rearrangements of the tight junction proteins occludin and ZO-1. This could explain protein loss and back-diffusion of pepsinogen into the blood, a marker of abomasal parasitism. Cell detachment and disruption of cell-cell adhesion in parasitised sheep may inhibit acid production by parietal cells, which cannot function when separated from adjacent cells. Increased permeability of the surface epithelium would allow parasite ES products to penetrate the mucosal barrier, causing further damage. This could also allow inhibition of parietal cells deeper in the abomasal glands and also allowing adult parasites living in the gastric lumen to modulate host immunity to enhance their survival.

Acknowledgements

Below is a list of persons/institutions (in alphabetical order), who crossed my path during my PhD and to whom I am very grateful, whether it be for inspiring and motivational discussions, administrative support, financial support, or teaching skills beyond the PhD:

Katharina Hillrichs, Van Hoang, Mike Hogan, Peter Jessop, Veronika Kristova, Arturo Luque, Meat and Wool New Zealand, Kevin Pedley, David Simcock, Heather Simpson, Lois Taylor and Lisa Walker.

Special thanks to:

- David Simcock and Katharina Hillrichs for the help with all sheep-related issues, e.g. infections
- Mike Hogan (IVABS, Pathobiology) for euthanasing the sheep
- Lisa Walker and Kevin Pedley for the help with the CaCo-2 cell experiments
- Michelle McGrath (IFNHH, Animal Nutrition and Physiology) for providing the plate reader for the cell proliferation assay
- Leiza Turnbull (IFNHH, Animal Nutrition and Physiology) for freeze-drying samples
- the histopathology lab, especially Pat Davey and Mary Gaddam, for processing tissue and cutting tissue samples

Ethical requirements applicable to this study have been approved as required by Massey University (ethical authorisations: MU 03/139 and 06/117).

Finally, I would like to thank Lars, my family and friends for their continuous support, understanding and love.

List of Figures

List of Tables

List of Abbreviations

#	Number
$[Ca^{2+}]_i$	Intracellular Ca^{2+} concentration
$[cAMP]_i$	Intracellular cAMP concentration
~	Approximately
µg	Microgram
µl	Microlitre
µm	Micrometre
µM	Micromolar
1D	1-dimensional
2D	2-dimensional
3T3	Fibroblasts isolated from mouse embryo
7H6	Tight junction associated antigen
A. caninum	*Ancylostoma caninum*
AA	Aminoacid
Ac	*A. caninum*
ACase	Adenylate cyclase
ACh	Acetylcholine
ADP	Adenosine diphosphate
AEBSF	4-(2-Aminoethyl)-benzenesulfonyl fluoride
AF-6	ALL-1 fusion partner from chromosome 6, also called afadin
AGS	Human gastric adenocarcinoma cell line derived from the stomach
Akt	Serine-threonine protein kinase
Amot	Angiomotin
AP	Adaptor protein
aPKC	Atypical protein kinase C
APR	Aspartic protease
AQ	Aquaporin

AR	Amphiregulin
ASH1	Absent, small, or homeotic-like (Drosophila), transcription factor
ASIP	Atypical PKC isotype specific interacting protein
ATP	Adenosin triphosphate
ATPase	Enzyme that can bind and hydrolyse ATP to ADP and P_i
B. malayi	*Brugia malayi*
Bcl	B cell lymphoma
biotin-XX	Biotinylated
BN-PAGE	Blue native-polyacrylamide gel electrophoresis
BSA	Bovine serum albumin
BSL	*Bandieraea simplicifolia* lectin
C1q	Complement component 1, subcomponent q
Ca^{2+}	Calcium ion
$CaCl_2$	Calcium chloride
CaCo-2	Human epithelial colonic adenocarcinoma cell line
CagA	*H. pylori* virulence factor encoded by cytotoxin-associated gene A
CaM	Calmodulin
CAM	Cell adhesion molecule
CaMK	Calmodulin kinase
cAMP	Adenosine 3', 5'-cyclic monophosphate
CAR	Coxsackie virus and adenovirus receptor
CaSR	Calcium-sensing receptor
CBL	Cathepsin B-like cystein protease
CCK	Cholecystokinin
CCK_B	Cholecystokinin B/gastrin receptor
CD95	Cluster of differentiation, added numbers are standing for distinct Antigens, death receptor, also called Fas, TNF receptor superfamily, member 6
Cdc42	Cell division cycle 42, small GTPase, belonging to the subfamiliy of Rho GTPases, superfamily Ras GTPases
cDNA	Complementary DNA
CEM	Complete essential medium

CGRP	Calcitonin gene-related peptide
CHO	Chinese hamster ovary epithelial cell line
Cl^-	Chloride ion
cm	Centimetre
cm^2	Square centimetre
CMC	Critical micelle concentration
Conc.	Concentration
CO_2	Carbon dioxide
CP	Cysteine protease
CRP	C-reactive protein
Ct	Control
d	Day
D. pteronyssinus	*Dermatophagoides pteronyssinus*
D10	DMEM medium with 10% FBS
DAG	Diacylglycerol
DBA	*Dolichos biflorus* agglutinin (horse gram)
Der p 1	Allergen 1 from *D. pteronyssinus*
DLG	Discs-large protein in *Drosophila*
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Desoxyribonucleinacid
E. caproni	*Echinostoma caproni*
E. coli	*Escherichia coli*
E. histolytica	*Entamoeba histolytica*
e.p.g.	Eggs per gram
EC	Enterochromaffin
E-cadherin	Cadherin in epithelial cells
ECL	Enterochromaffin-like
ECL1	Extracellular loop 1 of claudin
EDTA	Ethylenediaminetetraacetic acid
EEA1	Early endosome antigen 1
EGF	Epidermal growth factor

ERK	Extracellular signal-regulated protein kinase
ES	Excretory/Secretory (products)
ESAM	Endothelial cell selective adhesion molecule
EVOM	Epithelial volt-ohm meter
F. hepatica	*Fasciola hepatica*
F12-Hams	Originally developed to support growth of several clones of Chinese hamster ovary (CHO) cells
F-actin	Filamentous actin, filaments can be dissociated in their globular subunits (G-Actin)
FAK	Focal adhesion kinase
Fas	Death receptor, also called CD95, TNF receptor superfamily, member 6
FBS	Fetal bovine serum
FEC	Faecal egg count
g	Gram
g	Gravitational force
G protein	Guanine nucleotide binding protein
GAP	GTPase activating proteins
Gd^{3+}	Gadolinium ion
GDH	Glutamate dehydrogenase
GEF	Guanine nucleotide exchange factor
GEF-H1	Guanine nucleotide exchange factor H1
Gi	Guanine nucleotide binding protein, inhibitory
GIP	Gastric inhibitory peptide
Gq	Guanine nucleotide binding protein, q polypeptide, regulating PLC
GRP	Gastrin-releasing peptide
Gs	Guanine nucleotide binding protein, stimulatory
GTP	Guanosine triphosphate
GTPase	Enzyme that can bind and hydrolyse GTP to GDP (guanosine diphosphate) and P_i (inorganic phosphate)
h	Hour
H&E	Hematoxylin and eosin
H. contortus	*Haemonchus contortus*

H. pylori	*Helicobacter pylori*
H.c.	*Haemonchus contortus* infected
H^+	Hydrogen ion/proton
H^+/K^+-ATPase	Hydrogen/Potassium-ATPase, proton pump
H11	Hidden antigen of *H. contortus*
H_2/H_3	Histamine receptor
H_2CO_3	Carbonic acid
H_2O	Water
Hb	Hemoglobin
HB-EGF	Heparin binding-epidermal growth factor-like growth factor
HBSS	Hank's balanced salt solution
HCl	Hydrochloric acid
HCO_3^-	Hydrogen carbonate/bicarbonate ion
HDC	Histidine decarboxylase
HeLa cells	Derived from Henrietta Lacks, cell line of her cervical cancer
Hep2 cells	Originally thought to be derived from human epidermoid larynx carcinoma but actually derived via HeLa cell contamination
HES5	Hairy and enhancer-of-split homologue, basic helix-loop-helix protein
H-gal-GP	*Haemonchus* galactose-containing glycoprotein complex
HGF	Hepatocyte growth factor
HAT29-D4	Human colon adenocarcinoma cell line HAT 29, D4 is a clonal cell line of these
IEC-6	Epithelilal cell line from newborn rat intestine
IFN-γ	Interferon-γ
Ig	Immunglobuline
IgA1 protease	Secreted protease from *Neisseria gonorrhoeae* which inactivates human IgA1
IGF	Insulin-like growth factor
IgG_1	Immunglobulin G subclass 1
IL	Interleukin
ILK	Integrin linked kinase
INT407	Intestinal epithelial cell line from human embryo

IP_3	Inositol 1,4,5-triphosphate
IPEC-1	Intestinal epithelial cell line from neonatal piglet
IRD-98	Epithelial cell line from rat fetus intestine
I$\varkappa$B	Inhibitor of NF$\varkappa$B
JAM	Junctional adhesion molecule
JEAP	Junction enriched and associated protein, Angiomotin-like protein 1
K^+	Potassium ion
KCL	Potassium chloride
kDa	Kilodalton
kg	Kilogram
KGF	Keratinocyte growth factor
KH_2PO_4	Potassium dihydrogen phosphate
l	Litre
$L/L_1/L_3/L_4$	Larva/first stage larva/third stage larva/fourth stage larva
LAMP1	Lysosome-associated membrane protein 1
Lasp-1	Includes a LIM domain (*Lin*11, *Isl*-1, and *Mec*-3) in the NH_2-terminal region followed by two actin-binding repeats (R1, R2), and an Src homology 3 (SH3) domain in the COOH-terminal region
LEA	*Lycopersicon esculentum* agglutinin
LIM	Domain including *Lin*11, *Isl*-1, and *Mec*-3
M	Molar
M_3	Muscarinic acetylcholine receptor
MAGI	Membrane-associated guanyl kinase inverted proteins
MAPK	Mitogen-activated protein kinase
MASCOT	MAGI-associated coiled-coil tight junction protein, Angiomotin-like protein 2
max.	Maximum
MDCK	Madin-Darby canine kidney epithelial cell line
MEM	Minimum essential medium
MEP	Metalloprotease
mg	Milligram
Mg^{2+}	Magnesium ion

MIDAS	Metal ion dependent adhesion site
min	Minute
ml	Millilitre
MLC	Myosin light chain
MLCK	Myosin light chain kinase
mM	Millimolar
mm	Millimetre
mm^2	Square millimetre
MMP	Matrix metalloprotease
Mn^{2+}	Manganese ion
m-Numb	Mammalian Numb (notch inhibitor)
mOsM	Milliosmol
mRNA	Messenger ribonucleic acid
MUPP-1	Multi PDZ domain protein 1
MW	Molecular weight
n	Number/quantity
N. americanus	*Necator americanus*
N. brasiliensis	*Nippostrongylus brasiliensis*
Na^+	Sodium ion
Na^+/K^+-ATPase	Sodium/Potassium pump
Na_2HPO_4	Disodium hydrogen orthophosphate
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NaOCl	Sodium hypochlorite
NaOH	Sodiumhydroxide
neg.	Negative
NF$\varkappa$B	Nuclear factor $\varkappa$B
ng	Nanogram
NH_4Cl	Ammonium chloride
nm	Nanometre
No.	Number

Notch	Transmembrane receptor, notch gene discovered in *Drosophila melanogaster* with notches in their wings
NR	Neutral Red
O. leptospicularis	*Ostertagia leptospicularis*
O. ostertagi	*Ostertagia ostertagi*
O. sinensis	*Ovomermis sinensis*
O. volvulus	*Onchocerca volvulus*
OH^-	Hydroxide ion
OMV	Outer membrane vesicles
p	Passage
p	Probability
p.i.	Past infection
p120CAS	p120 catenin isoform
p130CAS	v-Crk (oncogene product of avian sarcoma virus CT10) associated tyrosine kinase substrate
p120ctn	Catenin in adherens junctions
PACAP	Pituitary adenylyl cyclase-activating peptide
PALS-1	Protein associated with Lin-7
PAR	Partitioning defective protein (interacting partner aPKC)
PAR	Protease activated receptor
PATJ	PALS-1 associated tight junction
PBS	Phosphate buffered saline
PC	Parietal cell
PDZ	Binding domain first described in the proteins PSD-95, DLG and ZO-1
PEPI	Parasite pepsinogen-like aspartic protease
PG	Prostaglandin
P_i	Inorganic phosphate
PI3	Phosphatidylinositol 3
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PNA	Peanut agglutinin

pos.	Positive
PP	Pancreatic polypeptide
PP2A	Protein phosphatase 2A
Proteinase K	Broad specifity serine protease from *Engyodontium album*, it was called Proteinase K because it is able to digest native keratin
PSD-95	Post-synaptic density protein 95
PSN	Penicillin-Streptomycin-Neomycin
PTEN	Phosphatase and tensin homologue
PYK2	Protein tyrosin kinase 2, FAK homologue
PYY	Peptide YY
Rab	Rab family of small G proteins, role in vesicular transport
Rac	Ras-related C3 botulinum toxin substrate, small GTPase, belonging to the subfamiliy of Rho GTPases, superfamily Ras GTPases
Rap	Small GTPase, belonging to the superfamily of Ras GTP
Ras	Small GTPases, from rat sarcoma
RCA	*Ricinus communis* agglutinin
Reg	Regenerating gene product
Rf	Retention factor
Rho	Ras homologue gene family
RhoA	Ras homologue gene family, member A, small GTPase, belonging to the subfamiliy of Rho GTPases, superfamily Ras GTPases
RIC	Epithelial cell line from adult rabbit intestine
Rich-1	Cdc42 GAP
RNA	Ribonucleic acid
rpm	Rounds per minute
RPTPβ	Receptor protein tyrosine phosphatase beta
RT	Room temperature
s	Second
S. mansoni	*Schistosoma mansoni*
Sat	Secreted auototransporter toxin
SBA	Soybean agglutinin
SDS	Sodium dodecylsulfate

SDS-PAGE	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
Serpin	Serine protease inhibitor
SH	Sheath
SH3	Src homology region 3
SMC	Surface mucous cell
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein
SOD	Superoxide dismutase
SPATE	Serine protease autotransporters of *Enterobacteriaceae*
spp.	Species pluralis
SS	Somatostatin
SS2	Somatostatin receptor
STA	*Solanum tuberosum* agglutinin
T. brucei	*Trypanosoma brucei*
T. circumcincta	*Teladorsagia circumcincta*
T. colubriformis	*Trichostrongylus colubriformis*
T. cruzi	*Trypanosoma cruzi*
T. muris	*Trichuris muris*
T. spiralis	*Trichinella spiralis*
T. suis	*Trichuris suis*
T. vitrinus	*Trichostrongylus vitrinus*
T75	Tissue/cell culture flask 75cm^2
TEER	Transepithelial resistance
TEMED	N,N,N',N'-Tetramethylethylenediamine
TFF	Trefoil factor
TGF	Transforming growth factor
Tiam-1	T-lymphoma invasion and metastasis, Rac specific GEF
TJ	Tight junction
TLC	Thin-layer chromatography
TNF	Tumor necrosis factor
tPA	Tissue type plasminogen activator
tSNARE	SNARE protein on the target membrane
Tuba	Cdc42 specific GEF

Tyr	Tyrosine
UEA	*Ulex europaeus* agglutinin (gorse)
uPA	Urokinase plasminogen activator
UV	Ultraviolet
V	Volt
v/v	Volume per volume
VacA	Vacuolating cytotoxin from *H. pylori*
VAMP	Vesicle-associated membrane protein
VASP	Vasodilator stimulated phosphoprotein
Vero cells	Kidney epithelial cells from African green monkey, Vero derived from "Verda Reno", meaning "green" and "kidney" in esperanto
VIP	Vasoactive intestinal peptide
VMAT-2	Vesicular monoamine transporter of subtype 2
vSNARE	SNARE protein on the vesicle membrane
V-type ATPase	Vacuolar-type ATPase
w/v	Weight per volume
Yes	Tyrosine kinase
ZAK	Sterile alpha motif and leucine zipper containing kinase AZK, which is also known as ZAK
ZO	Zonula occludens
ZONAB	ZO-1 associated nucleic acid binding protein
Ω	Ohm, SI unit for electric resistance

Introduction

Infections with gastrointestinal nematodes, particularly trichostrongylids, in domestic ruminants are an important economic burden worldwide (Holmes, 1985; Fox, 1997; Miller and Horohov, 2006). A major factor in the pathogenesis of abomasal parasitism of sheep (McLeay *et al.*, 1973; Anderson *et al.*, 1976) and cattle (Fox *et al.*, 1989a; b) is a depression in voluntary food intake (anorexia) that leads to decreased weight gain (Holmes, 1985; 1993; Parkins and Holmes, 1989) as well as diarrhoea, malabsorption (Koski and Scott, 2001) and increased protein loss due to a more permeable epithelium, which all in turn increases susceptibility to infection (van Houtert and Sykes, 1996; Scrimshaw and SanGiovanni, 1997). In particular, infections with the blood-feeding nematode *Haemonchus contortus* are concerning, as they additionally cause considerable anaemia in infected animals (Parkins and Holmes, 1989). Adding to the production loss are costs for anthelmintics, veterinary care and death of infected animals. Around 29% of the animal health market are anti-parasitic drugs, worth US$5.2 billion in 2007 (International Federation for Animal Health, 2007).

Parasite control relies on anthelmintic treatment, although alternative strategies also exist to cope with increasing anthelmintic resistance, including grazing management, breeding management, nutrition supplementation, use of plants containing natural antheltmintics and vaccine development (Coop and Kyriazakis, 1999; Newton and Munn, 1999; Koski and Scott, 2001; 2003; Bakker *et al.*, 2004; Miller and Horohov, 2006; Pomroy and Adlington, 2006). Anthelmintic resistance is widespread, even multiple drug resistant parasites have been reported worldwide (Kaplan, 2004; Wolstenholme *et al.*, 2004; von Samson-Himmelstjerna, 2006; Kaminski *et al.*, 2009). Although a new class of anthelmintics has been recently discovered and monepantel as the first component was launched earlier this year (Kaminski *et al.*, 2008; 2009), there is still need for the improvement of already existing alternative strategies and the development of new ones.

An alternative control method would be an antiparasitic vaccine. Attention has focused on using antigens present in the excretory/secretory (ES) products, as these are often recognised by immune animals (Schallig and Leeuwen, 1997; Schallig *et al.*, 1997; Bakker *et al.*, 2004) and may also be a source of ongoing antigenic stimulation to maintain immunity. ES products contain numerous proteins and glycoproteins with functions including depression of host immunity and probably also in initiating the host immune response and pathology.

The role of parasite ES products in abomasal pathophysiology and histopathology has been previously examined (Rhoads and Fetterer, 1996; Scott and McKellar, 1998; Lawton *et al.*, 2002; Merkelbach *et al.*, 2002; Haag *et al.*, 2005; Huber *et al.*, 2005; Przemeck *et al.*, 2005), but it is still unclear whether ES products act at least in part directly on abomasal cells or also indirectly through the inflammatory response. The aim of this study was to further investigate the host-parasite interactions with the main focus on the role of ES products. Particular interest was on the effects on parietal cells leading to impaired acid secretion. Tissue samples of infected and control animals were used as well as different cell lines as model systems.