Pathophysiology and immunomodulation associated with *Haemonchus contortus* infection

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In the Name of Allah the Most Gracious, the Most Merciful.

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ABSTRACT

The aim of this project was to investigate host-parasite interactions, which might lead to alternate strategies to control the sheep abomasal nematode *Haemonchus contortus*. The project focused on two aspects of host parasite interactions: the initiation of host pathology and suppression of host immune responses associated with the onset of infection.

Adult *H. contortus* ES products increased the permeability of Caco-2 cell monolayers and this increase could be blocked by single chain antibodies against ES products displayed on phage. Recombinant *H. contortus* enolase may be one of the active components of ES as it mimicked the action of ES products on Caco-2 cells.

This is the first study of immunomodulation by adult *H. contortus* ES products of the phenotypic and functional properties of human monocyte-derived dendritic cells (mdDCs). Incubation with ES products resulted in semi-maturation of mdDCs, with weak up-regulation of the co-stimulatory molecules CD40 and CD80 and increased surface expression of the tolerogenic markers CD32, CD305 and galectin-1. The highly variable responses of mdDCs of individual donors biased the group data, particularly in response to co-stimulation with ES products and LPS. This highlights genetic diversity in the immune system and possible difficulties in developing worm-based therapies.

The blastogenic activities of cells from lymph nodes collected from two groups of infected and vaccinated sheep were measured by ^{3}H-thymidine uptake after exposure to ConA or ES products. The Stimulation Index (SI) with ConA was 10-fold higher in cells from the older animals. Cells only from younger infected sheep had a reduced

response to ConA and vaccinated groups with reduced parasite burdens had the highest SI. There was little response to ES products in older sheep, but in younger animals there was a trend for lymphocyte SI to be greater with 10% ES in sheep with the fewest parasites.

These experiments show that *H. contortus* ES products may facilitate the initiation of host pathology and the potential to modulate responses of dendritic and lymph node cells during parasitism. Further identification of the specific ES components responsible may allow disruption of their actions, resulting in resilient and immune sheep.

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AAMs	alternatively activated macrophages
Ab	antibody
ADCC	antibody dependent cellular cytotoxicity
ADJ	adjuvant
ADP	adenosine diphosphate
AEC	3-Amino-9-ethylcarbazole
AJ	adherens junctions
AK	arginine kinase
A. lumbricoides	*Ascaris lumbricoides*
AMcase	acidic mammalian chitinase
Amot	angiomotin
ANOVA	analysis of variance
APC	antigen presenting cell
aPKC	atypical protein kinase C
ASIP	aPKC isotype-specific interacting protein
ASP	*Ancylostoma* secreted proteins
B cells	B lymphocytes
BES	*Brugia malayi* ES
B. malayi	*Brugia malayi*
bp	base pair
BRSV	bovine respiratory syncytial virus
BSA	bovine serum albumin
CAR	coxsackievirus and adenovirus receptor
CarLA	Carbohydrate larval antigen
CCR7	chemokine (C-C motif) receptor 7
cDC	conventional dendritic cells
Cdc42	cell devision control protein 42 homolog
cDNA	complementary deoxyribonucleic acid
CDP	common DC progenitor

CHaFFs	chitinase and FIZZ family members CMP common myeloid progenitor
CIP4	Cdc42- interacting protein 4
CLA	cutaneous lymphocyte-associated antigen
CLR	C-type lectin receptors
ConA	concancavilin A
CPI	cysteine proteinase inhibitor (cystatin)
CTGF	connective tissue growth factor
Cy	cyanine
DC	dendritic cells
DC-SIGN	dendritic cell specific intercellular adhesion molecule-3-grabbing non-integrin
Der p 1	*Dermatophagoides pteronyssinus* allergen
DNA	deoxyribonucleic acid
DS	desmosome
EDN	eosinophil derived neurotoxin
ECL	enterochromaffin-like
E. coli	*Escherichia coli*
EGF	epidermal growth factor
E. granulosus	*Echinococcus granulosus*
ES	excretory secretory
ESGPs	eosinophil secondary granule proteins
ETP	early thymic progenitor
EU	endotoxin unit
FBS	foetal bovine serum
FEC	faecal egg count
F. hepatica	*Fasciola hepatica*
FITC	fluorescein isothiocyanate
FIZZ	found in inflammatory zone
FMOs	fluorescence minus one
g	gram
g	gravitational force
GAP	GTPase-activating protein

GAPDH	glyceraldehyde-3-phosphate
G. duodenalis	*Giardia duodenalis*
GM-CSF	granulocyte-macrophage colony-stimulating factor
GPCR	G protein-coupled receptor
GTP	guanosine triphosphate
h	hour
*Hc*ES	*Haemonchus contortus* excretory secretory products
H. contortus	*Haemonchus contortus*
HES	*Heligmosomoides polygyrus* ES
HLA-DR	human leukocyte antigen-DR
H. polygyrus	*Heligmosomoides polygyrus*
H. pylori	*Helicobacter pylori*
HRP	horse radish peroxidase
HSC	hematopoietic stem cell
ICAM-1	intracellular adhesion molecule-1
iDC	inflammatory dendritic cell
IFN	interferon
Ig	immunoglobulin
IL	interleukin
INF	infected
IP-10	IFN-γ-inducible protein-10
IPSE	IL-4-inducing principle pf schistosome eggs
JAM	junctional adhesion molecules
JEAP	angiomotin-like-protein 1
kDa	kilodalton
KGF	keratinocyte growth factor
L	litre
L-NES	*N. brasiliensis* larval ES
LAIR-1	leucocyte-associated Ig-like receptor-1
LAP	latency-associated protein
LC	Langerhans cell
LDH	lysine dehydrogenase

LL	laminated layer
LPS	lipopolysaccharides
LSD	least significant difference
L1	first stage larva
L2	second stage larva
L3	third stage larva
L4	fourth stage larva
L5	fifth stage larva
M	molar
mAb	monoclonal antibody
MCP-1	monocyte chemo-attractant protein-1
mdDC	monocyte derived dendritic cell
MDP	macrophage DC progenitor
MFI	median fluorescence intensity
mg	milligram
MHC	major histocompatibility complex
MIF	macrophage migration inhibitory factor
min	minute
MIP	macrophage inflammatory protein
ml	millilitre
mM	millimolar
M. marshalli	*Marshallagia marshalli*
MMPs	matrix metalloproteinase
MNC	mucous neck cells
mRNA	messenger ribonucleic acid
MUC5AC	mucin 5AC
MUC6	mucin 6
MUPP	multi-PDZ domain protein
Mv	microvilli
n	number
N. americanus	*Necator americanus*
N. brasiliensis	*Nippostrongylus brasiliensis*
NAD^+	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide

NI	non-infected
Ng	nanogram
Ni-NTA	Nickel-nitrilotriacetic acid
NLR	NOD-like receptors
NOD-like	nucleotide-binding oligomerisation domain-like
NS	non-stimulated
O. ostertagi	*Ostertagia ostertagi*
O. volvulus	*Onchocerca volvulus*
OVA	ovalbumin
PAGE	polyacrelamide gel electrophrosis
PAMPs	pathogen-associated molecular patterns
PAR	partitioning defective protein
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffer saline
PCR	polymerase chain reaction
PD-L	programme death ligand
PE	phycoerythrin
PerCP	peridinin chlorophyll
PFU	plaque forming units
pg	picogram
PHA	phytohaemagglutinin
p.i.	post-infection
PK	pyruvate kinase
pmol	picomole
PRRs	pathogen recognition receptors
Rac1	Ras-related C3 botulinum toxin substrate 1
RELM	resistin-like molecule
RGM1	rat gastric mucosal first
Rich1	Rho GAP interacting with CIP4 homologues
r*Hc*AK	recombinant *Haemonchus contortus* arginine kinase
r*Hc*ENO	recombinant *Haemonchus contortus* enolase

RhoA	Ras homolog gene family, member A
RIG-1	retinoic acid-inducible gene-1
RNA	ribonucleic acid
RO	reverse osmosis
scFvs	single chain antibody fragments
SDS	sodium duodecyl sulphate
S.E.M	standard error mean
SI	stimulation index
Sm	*Schistosoma mansoni*
S. mansoni	*Schistosoma mansoni*
S. venezuelensis	*Strongyloides venezuelensis*
SMC	surface mucous cells
SPN	serine proteinase inhibitor (serpin)
TAE	tris-acetate-EDTA
TBS	tris-buffered saline
T cells	T lymphocytes
T. circumcincta	*Teladorsagia circumcincta*
TCR	T cell receptors
T. crassiceps	*Taenia crassiceps*
T. cruzi	*Trypanosoma cruzi*
TEER	transepithelial electrical resistance
TES	*T. canis* ES
TGF	transforming growth factor
T_H0	naïve T cell
T_H1	type 1 immune response
T_H2	type 2 immune response
Tiam-1	T-lymphoma invasion and metastasis-inducing protein-1
TJ	tight junction
TLR	Toll-like receptors
TNF	tumour necrosis factor
T_{regs}	regulatory T cells
TSLP	thymic stromal lymphopoietin
T. spiralis	*Trichinella spiralis*

ZO	zonula occluding
ZAK	ZO-1 associated kinase
µCi	microcurie
µg	microgram
µl	microliter

INTRODUCTION

In pasture-based grazing systems, like those in New Zealand, gastrointestinal nematodes are major contributors to serious health and welfare issues and fiscal losses, because of low productivity and high treatment and control costs (Leathwick et al., 2001). Haemonchosis, caused by the blood-feeding abomasal nematode *Haemonchus contortus,* is one of the major constraints on small ruminant health and production in warmer areas worldwide and in some parts of New Zealand, because of its blood feeding (Rowe et al., 1988; Le Jambre, 1995). At present, control of gastrointestinal nematodes depends heavily on the use of chemical anthelmintics (Wolstenholme et al., 2004) and, where feasible, pasture management. Under intensive grazing conditions, however, clean pastures are not readily available and extensive use of anthelmintics has resulted in increasing resistance to these chemicals (Jackson, 1993; Waller et al., 1995; Borgsteede et al., 1997; Van Wyk et al., 1997). Moreover, there are concerns about drug residues in meat and the environment (Madsen et al., 1990; Lumaret, 1993).

Problems associated with chemical treatment of livestock can be addressed by developing immunologically-based methods for gastrointestinal nematode control, ideally vaccination (Newton and Munn, 1999). Vaccines would provide protection during the susceptible period between weaning and development of natural immunity and would most likely use antigens responsible for causing host pathophysiology or necessary for parasite survival. An additional target may be the parasite immunomodulators which suppress the host immune response (Maizels et al., 2004).

This project investigated two aspects of the host-parasite interaction which are hoped to provide targets for new therapies. The

first was how the parasite initiates host pathology, which appears to be chemically mediated by their excretory-secretory (ES) products. Worm products were tested on a Caco-2 cell model to investigate the possibility that *H. contortus* can modulate epithelial cell permeability to facilitate entry of worm chemicals into host tissues. The second objective was to investigate the ability of abomasal parasites to suppress host immunity, first by an *in vitro* study of the effects of *H. contortus* ES components on dendritic cells and secondly by examining the ability of lymphocytes collected from infected and vaccinated sheep to proliferate *in vitro* in response to mitogens.