MUCIN CHANGES ASSOCIATED WITH ABOMASAL PARASITISM IN SHEEP

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ABSTRACT

Mucins play important roles in host-pathogen interactions, influencing host resistance, establishment of infection, as pathogen recognition sites and a source of nutrients. They are highly glycosylated molecules and changes in monosaccharide composition during parasitism have been reported. Effects of parasites on monosaccharide component of fundic and duodenal mucins of sheep were investigated in 3 age ranges (i) 4-4.5, (ii) 6 and (iii) 8-9 months old: (1) non-infected; (2) infected with 10,000 *Haemonchus contortus* and euthanased 21 days post infection (p.i.); (3) infected with 50,000 *Teladorsagia circumcincta* and euthanased 28 days p.i. Three days-old lambs and 9 weeks-old lambs: (a) milk-fed, (b) solid-fed and (c) solid-fed, infected with *T. circumcincta* were also included. The effects of *H. contortus* and *T. circumcincta* infection in mucin changes were significantly different in the fundus, however, both of them shared some similarities. Infected sheep showed lower proportion of fucose and sialic acids in fundic and duodenal mucins compared with non-infected animals, the level of sulphation varied depending on the age of infected sheep: decrease in young sheep but increase in older animals. *H. contortus* infection also caused increased proportions of GlcNAc and Gal in fundic mucins and duodenal mucins respectively at all ages, however, in *T. circumcincta* infection, it was shown that the alterations of mucins were age-dependent. *T. circumcincta* infected sheep showed the significant changes at young ages (4-6 months-old) while 8-9 months-old animals showed less change in fundic mucins compared with non-infected animals. Effects of *H. contortus* and *T. circumcincta* infection differed in the fundic mucins but were similar in the duodenum. The study showed that parasitism caused the modifications of monosaccharide composition in gastrointestinal mucins of sheep. These alterations may result from parasite species differences, causing different effects from the host's immune response. The changes in mucin profiles observed in the duodenum of sheep infected with abomasal nematodes suggested that the host may respond to parasitism. This would facilitate the use of mucins from accessible sources, without euthanasing the animals, to investigate the changes in mucin compositions which can be used to diagnose the susceptibility or resistance of sheep to parasites.

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LIST OF ABBREVIATIONS

AB	Alcian blue
AMPS	ammonium persulphate
µC	microcoulon
µg	microgram
µl	microlitre
10X	10 times
AAL	*Aleuria aurantia* lectin
AB/PAS	Alcian blue/ Periodic acid Schiff
ANOVA	Analysis of variance
Asp	Asparagine
BPL	*Bauhinia purpurea* lectin
BSA	Bovine serum albumin
BSM	Bovine submaxillary mucin
Ca^{2+}	Calcium ion
CD4+	Co-receptor
CF	Cystic fibrosis
ConA	Concanavalin A
CsCl	Cesium chloride
Da	Dalton
DAB	Diamino benzidine
DBA	*Dolichos biflorus* agglutinin
ddH_2O	Distilled water

DNA	Deoxyribonucleotide
DTT	Dithiothreitol
E. trivolvis	*Echinostoma trivolvis*
EDTA	Ethylene diamine tatra acetic acid
ELLA	Enzyme-linked lectin assay
ES	Excretory/ secretory products
F. hepatica	*Fasciola hepatica*
FEC	Faecal egg count
Fuc	Fucose
g	gram
Gal	Galactose
GalN	Galactosamine
GalNAc	N-acetyl galactosamine
GalNAcTs	N-acetyl galactosaminyl transferase
Glc	Glucose
GlcN	Glucosamine
GlcNAc	N-acetyl glucosamine
GSA-I	*Griffonia simplicifolia* lectin I
GSA-II	*Griffonia simplicifolia* lectin II
GuHCl	Guanidinium chloride
h	hour
H. contortus	*Haemonchus contortus*
H. pylori	*Helicobacter pylori*
H_2SO_4	Sulphuric acid

HCl	Hydrochloric acid
HID	High iron diamine
HPAEC	High performance anion exchange chromatography
HPLC	High performance liquid chromatography
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
kDa	kiloDalton
KOH	Potassium hydroxide
L_1	First stage larvae
L_2	Second stage larvae
L_3	Third stage larvae
L_4	Fourth stage larvae
LCA	*Lens culinaris* agglutinin
LPA	*Limulus polyphemus* agglutinin
LTA	*Lotus tetragonolobus* lectin
MAL	*Maackia amurensis* lectin
Man	Mannose
min	minute
ml	millilitre
mM	millimolar
MMC	Mucosal mast cells
MNC	Mucous neck cell
MPL	*Maclura pomifera* lectin

MQ-water	MilliQ water
MUC	Mucin type
MW	Molecular weight
MWCO	Molecular weight cut-off
n	number
N. brasiliensis	*Nippostrongylus brasiliensis*
NaCl	Sodium chloride
NANA	N-acetyl neuraminic acid
NaOH	Sodium hydroxide
ND	Non-diseased
NEM	N-ethylmaleimide
NGNA	N-glycolyl neuraminic acid
nm	nanometre
O. dentatum	*Oesophagostomum dentatum*
O. ostertagi	*Ostertagia ostertagi*
p	probability
P. aeruginosa	*Pseudomonas aeruginosa*
PAD	Pulse Amperometric Detection
PAGE	Polyacrylamide gel electrophoresis
PAS	Periodic acid Schiff
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PES membrane	Polyethersulfone membrane

PHA	*Phaseolus vulgaris* agglutinin
p.i.	post infection
PMSF	phenylmethylsulphonyl fluoride
PNA	Peanut agglutinin
PSA	*Pisum sativum* agglutinin
PSM	Porcine submaxillary mucin
PTL	*Psophocarpus tetragonolobus* lectin
PVDF	Polyvinylidene fluoride
RCA	*Ricinus communis* agglutinin
RNA	Ribonucleic acid
RO water	Reverse osmosis water
rpm	Revolution per minute
S. venezuelensis	*Strongyloides venezuelensis*
SBA	Soybean agglutinin
SD	Standard deviation
SDS	sodium dodecyl sulphate
sec	second
Ser	Serine
SMC	Surface mucous cell
SNA	*Sambucus nigra* agglutinin
T. axei	*Trichostrongylus axei*
T. circumcincta	*Telardosagia circumcincta*
T. colubriformis	*Trichostrongylus colubriformis*
T. muris	*Trichuris muris*

T. spiralis	*Trichinella spiralis*
T. taeniaformis	*Taenia taeniaformis*
TBS	Tris-buffered saline
TEMED	N, N, N', N'- tetramethylethylenediamine
TFF	Trefoil peptide family
Th	T-helper
Thr	Threonine
TLC	Thin layer chromatography
TNF	Tumour Necrosis Factor
UEA	*Ulex europaeus* agglutinin
UV	ultraviolet
V	violet
V_0	Void volume
V_e	Elution volume
V_t	Total volume
WGA	Wheat germ agglutinin
Y. enterocolitica	*Yersinia enterocolitica*

INTRODUCTION

For many years, the agricultural industry has played a key role in the New Zealand economy. The products obtained from sheep and cattle contribute a large part to the total national income. With 40 million sheep in the country (stated on the 30 June 2005 by Statistics New Zealand), products from sheep not only supply the domestic population, but they are exported to many other countries. Since sheep are living on pasture, they can easily become infected by helminths. Once these parasites infect the sheep, they may cause significant economic losses due to reduced wool production and acute disease and death in susceptible young lambs.

Gastrointestinal nematodes cause serious loss to farm production. In sheep, parasitism by *H. contortus* and *T. circumcincta* cause anemia, anorexia, reduction in body weight and wool growth and death. The emergence of multiple-drug resistant nematodes has increased the need for improved and sustainable methods of controlling nematodes, as well as selection of resistant or resilient lines of sheep. To develop novel therapies or select for hosts resistant to parasites, more needs to be learned about the host-parasite interaction.

Mucus and mucins are at the site of interaction of host and parasite. Many pathogens targeting epithelial surfaces recognize their niche for invasion and interact with the tissues through specific carbohydrate residues on the cell membranes and mucins. Parasitic nematodes may also identify their site of infection in this way. Many parasites degrade mucins to invade host tissues and for nutrition. Gastrointestinal mucus is involved in expulsion of parasites, yet little is known about the changes in mucin glycosylation during parasitism. Answers to these questions may open new opportunities to interfere with parasite establishment.

The present project aims to identify the changes in the mucins of the fundus and duodenum of sheep while the parasites *H. contortus* and *T. circumcinta* are present in the abomasum. Little is known about the changes in carbohydrate content of mucins that occur during the infection with these nematodes. It is hoped that increased knowledge of these carbohydrate residues may lead to a better

understanding of the host-parasite interactions and the role of these moieties in host protection or parasite establishment.

The project involves both chemical analysis of mucins, histochemistry and lectin binding to tissues collected from the abomasum and duodenum. Chemical analysis can be used to identify changes in the proportions of hexoses and hexosamines making up the mucins and histochemistry can be used to study post-translational modifications, such as sialylation and sulphation. Lectin histochemistry provides an insight into the presence and distribution of specific carbohydrate residues in different cell types which secrete mucins. The overall objective of the project is to gain more knowledge about which alterations occur in mucins in the gastrointestinal tract with age and infection with abomasal parasites.