A study of circulating neutrophils and exosomes associated with innate immune function in the periparturient grazing dairy cow

A thesis presented in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

in

Veterinary Science

at Massey University, Manawatū,

New Zealand.

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2017

Abstract

Dairy cows are at greatest risk of infectious and metabolic disease during the periparturient period. This period of three weeks either side of calving is also known as the transition period due to the transition into lactation. This thesis had several aims; one was to characterise innate immune function during the transition period in grazing dairy cows by investigating molecular changes in circulating neutrophils and to assess if common on-farm management strategies (pre-calving feeding level and body condition at calving) were able to influence these molecular changes. Next, metabolic stress on neutrophil function was assessed by establishing a model of cows divergent in metabolic health status. This model was further utilised with the aim to investigate nanoparticles (exosomes), which are regulators of innate immune function and indicators of disease state. To address these aims blood was collected from pasture-fed transition dairy cows. Cellular and molecular methods used included cell and exosome isolation, reverse transcriptase (RT)-quantitative PCR, RNA sequencing, cell culture, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results indicated that grazing dairy cows experience a change in innate immune function during the transition period, reflective of reduced functional capacity of the immune system to overcome infectious agents. This altered function was similar to that experienced by housed cows fed a total mixed ration, which adds evidence to support that the dysfunction is a natural part of the transition into lactation at calving. These results also indicated that the functional changes could be influenced by nutrition status, feeding level, and metabolic stress. Analysis of exosomes isolated from the blood of transition cows indicated that these particles carried cargo indicative of metabolic state during the transition period and that they had the ability to alter target cell processes (gene expression, protein expression, and cell proliferation). The conclusions from this thesis increase our understanding of transition cow immune function and how it is influenced by nutrition and cow metabolism. These data are particularly relevant for grazing dairy cows and the findings

will contribute to on-farm recommendations and the improvement in animal health and well-being.

Acknowledgements

First and foremost, I would like to acknowledge my supervisors, Drs John Roche, Alan Murray, Axel Heiser, and Rao Dukkipati. John, your endless support and encouragement kept me determined to persevere and your passion for science made moments of frustration wherein attempting to make sense of what seemed like erroneous results into exciting moments of discovery. Your infectious positivity made the tough times bearable and the fun times more merry, for that I am greatly appreciative. Alan, Axel, and Rao, thank you all for being so supportive of this work and providing valuable feedback (and in such a timely manner). Thank you for being a part of this work and making the process go so smoothly, it has been a pleasure working with you all.

Thank you to my colleagues and fellow students based at 3A Symonds St. You have provided answers to all my questions and are always there to lend a hand when required. Not to mention the numerous coffee runs, Friday wines, and most importantly, ear for my incessant chatter. You make day-to-day life not only bearable, but enjoyable. Special thanks to my fellow DairyNZ Auckland team members, Dr Caroline Walker and Dr Talia Grala, for teaching me the intricacies of molecular biology over the years (among other things).

I would also like to thank the brilliant minds of the DairyNZ Animal Science team, thank you for the useful comments and discussion. A very special thanks to Barbara Kuhn-Sherlock for being the most amazing biometrician I could ever wish for, I could not have done this without you. Also, thank you to the DairyNZ technical team, you are world-class and the incredible work you do is appreciated always.

Furthermore, I gratefully acknowledge MBIE and DairyNZ for providing the financial support for this work to be completed. Thank you to DairyNZ in particular, for supporting me and this project over its duration as well as the opportunities for conference travel and

collaboration. Also, thank you to the Ruakura ethics committee for approval to conduct the work undertaken in this study.

I would also like to acknowledge the overseas laboratory groups I had the pleasure of working with during my PhD tenure. These groups include Associate Professor Juan Loor's group in the Animal Sciences department at the University of Illinois, Assistant Professor Kasey Moyes' team in the department of Animal and Avian Sciences, University of Maryland, and Professor Murray Mitchell and his team at the University of Queensland's Centre for Clinical Research. I am grateful for the opportunity and privilege to visit and work with you all; it provided an invaluable learning experience.

Finally, I would like to thank my family. Dad and Mum, you have always encouraged my higher education (and this is as high as I'll get!). Your never-ending support has helped me succeed. Thank you for always believing in me, I appreciate everything you have done for me more than you will ever know. Thank you to my partner Anton, for always bringing a smile to my face. Your enthusiasm for life keeps me going, thank you for being so patient with me on this journey.

Preface

I have undertaken this thesis in the form of publishable experimental chapters using a format of thesis by publication. The current status and publication outlet are described in the following list. Published papers do not appear in chronological order.

Chapter 1: General Introduction

Chapter 1A: Biological literature review

Chapter 1B: Technical literature review

Chapter 2: Evaluation of endogenous control gene expression in bovine neutrophils by reverse transcriptase-quantitative PCR using microfluidics gene expression arrays. M. A. Crookenden, C. G. Walker, B. Kuhn-Sherlock, A. Murray, V. S. R. Dukkipati, A. Heiser, and J. R. Roche.

Accepted: Journal of Dairy Science, 2017, 100(8):6763-6771.

All cellular and molecular work and manuscript write-up and results interpretation was completed by M. A. Crookenden. Experimental plan was completed under the guidance of C. G. Walker and J. R. Roche. Statistical analysis was completed with the assistance of B. Kuhn-Sherlock.

Chapter 3: Parturition in dairy cows temporarily alters the expression of genes in circulating neutrophils. M.A. Crookenden, A. Heiser, A. Murray, V.S.R. Dukkipati, J.K. Kay, J.J. Loor, S. Meier, M.D. Mitchell, K.M. Moyes, C.G. Walker and J.R. Roche.

Published: Journal of Dairy Science, 2016, 99(8):6470-6483

Animal handling and management was completed under the guidance of J. K. Kay, S. Meier, and J. R. Roche. All cellular and molecular work and manuscript write-up and results interpretation was completed by M. A. Crookenden.

Chapter 4: Effects of pre-calving body condition and prepartum feeding level on gene expression in circulating neutrophils during the transition period.

M.A. Crookenden, C.G. Walker, A. Heiser, A. Murray, V.S.R. Dukkipati, J.K. Kay, S. Meier, K.M. Moyes, M.D. Mitchell, J.J. Loor, and J.R. Roche.

Published: Journal of Dairy Science, 2017, 100(3):2310-2322.

Animal handling and management was completed under the guidance of J. K. Kay, S. Meier, and J. R. Roche. All cellular and molecular work and manuscript write-up and results interpretation was completed by M. A. Crookenden.

Chapter 5: Transcriptomic analysis of circulating neutrophils in metabolically-stressed peripartal grazing dairy cows.

M.A. Crookenden, C.G. Walker, K.M. Moyes, B. Kuhn-Sherlock, K. Lehnert, A. Heiser, A. Murray, V.S.R. Dukkipati, J.J. Loor, and J.R. Roche.

Submitted: BMC Genomics. May 2017.

Cellular and molecular work, manuscript write-up, and results interpretation was completed by M. A. Crookenden. Selection of cows for RNA sequencing analysis was completed by M.A. Crookenden, K. M. Moyes, J. J. Loor, J. R. Roche and B. Kuhn-Sherlock. Bioinformatics and statistical manipulation was completed by K. Lehnert and B. Kuhn-Sherlock. Animal handling and management was completed under the guidance of J. K. Kay, S. Meier, and J. R. Roche.

Chapter 6: Evidence that circulating exosomal proteins represent metabolic state in transition dairy cows.

M. A. Crookenden, C. G. Walker, H. Peiris, Y. Koh, A. Heiser, J. J. Loor, K. M. Moyes, A. Murray, V. S. R. Dukkipati, J. K. Kay, S. Meier, J. R. Roche, and M. D. Mitchell.

Published: Journal of Dairy Science, 2016, 99(9):7661-7668

Manuscript write-up, and results interpretation was completed by M. A. Crookenden. Exosome isolation was completed by H. Peiris and Y. Koh. Animal handling and management was completed under the guidance of J. K. Kay, S. Meier, and J. R. Roche.

Chapter 7: Effect of circulating exosomes from transition cows on Madin-Darby bovine kidney cell function.

M. A. Crookenden, C. G. Walker, H. Peiris, Y. Koh, F. Almughlliq, K. Vaswani, S. Reed, A. Heiser, J. J. Loor, J. K. Kay, S. Meier, S. S. Donkin, A. Murray, V. S. R. Dukkipati, J. R. Roche, and M. D. Mitchell.

Published: Journal of Dairy Science, 2017, 100(7):5687-5700.

Cellular and molecular work was completed by M. A. Crookenden with technical assistance from H. Peiris, Y. Koh, F. Almughlliq, K. Vaswani, and S. Reed. Manuscript write-up, and results interpretation was completed by M. A. Crookenden. Animal handling and management was completed under the guidance of J. K. Kay, S. Meier, and J. R. Roche.

Chapter 8: General Discussion

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Common Abbreviations

ANOVA	Analysis of variance
AST	Aspartate transaminase
BCS	Body condition score
BHBA	Beta-hydroxybutyrate
$BW^{0.75}$	Metabolisable liveweight
BW	Body weight
CD14	LPS/lipoteichoic acid receptor or cluster of differentiation factor 14
CD18	Cluster of differentiation factor molecule 18
CD62L	L-selectin
CDK	Cyclin-dependent kinase
cDNA	Complementary deoxyribonucleic acid
dsDNA	Double stranded DNA
COG	Cluster of orthologous groups of proteins
COX	Cycloxygenase
Cp	Crossing point
CXCR2	Chemokine (C-X-C motif) receptor 2 or CD182 or IL-8 receptor
CXCR4	Chemokine (C-X-C motif) receptor 4 or fusin or CD184
d	Day(s)
DC	Dendritic cell
DM	Dry matter
DMI	Dry matter intake
DMEM	Dulbecco's modified eagle medium
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
ECV	Extracellular vesicles
EDTA	Ethylenediaminetetraacetic acid
FA	Fatty acid
FBS	Foetal bovine serum
Fc-IgG2	Fragment, crystallisable region of IgG
FL	Feeding level
G-CSF	Granulocyte colony stimulating factor
GDP	Gross domestic product
HPC	Haematopoietic progenitor cell
HSC	Haematopoietic stem cell
IFC	Integrated fluidic circuit

IFITM	Interferon inducible transmembrane proteins
IFN	Interferon
IL-1	Interleukin 1
IL-17A	Interleukin 17A
IL-23	Interleukin 23
IL-8	Interleukin 8
IPA	Ingenuity pathway analysis
ITGAM/CD11b	Integrin alpha M or cluster of differentiation molecule 11B
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LPS	Lipopolysaccharide
LSM	Least squares mean
Mac-1	Macrophage activating factor 1 (CD11b/CD18 complex)
MDBK	Madin-Darby bovine kidney cells
min	Minute(s)
ME	Metabolisable energy
miRNA	micro RNA
MMP-8	Matrix metalloproteinase 8 or PMNL collagenase
MMP-9	Matrix metalloproteinase 9 or 92 kDa type IV collagenase or 92 kDa gelatinase or gelatinase B
MMP-23	Matrix metalloproteinase 23
MPO	Myeloperoxidase
mRNA	Messenger RNA
MS	Mass spectrometry
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NEV	Number of exosomal vesicles
NGS	Next generation sequencing
NK	Natural killer cell
NSAID	Non-steroidal anti-inflammatory drug
PBS	Phosphate buffered saline
PCNA	Proliferating cell nuclear antigen
PCR	Polymerase chain reaction
PMN	Polymorphonuclear cell (neutrophils)
RBC	Red blood cells
RNA	Ribonucleic acid
RNAseq	RNA sequencing
rRNA	Ribosomal RNA

ROS	Reactive oxygen species
RT-qPCR	Reverse transcriptase-quantitative polymerase chain reaction
SD	Standard deviation
SED	Standard error of the difference
SEM	Standard error of the mean
TAC	Total antioxidant capacity
TAG	Triacylglycerol
Th	T helper lymphocyte
TMR	Total mixed ration
TNF	Tumour necrosis factor alpha
WBC	White blood cells
Wk	Week(s)
UPL	Universal Probe Library