A SEARCH FOR GENETIC FACTORS INFLUENCING IMMUNE RESPONSES TO A KILLED *MYCOBACTERIUM AVIUM* SUBSPECIES *PARATUBERCULOSIS* VACCINE IN AUSTRALIAN FINE-WOOL MERINO SHEEP

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ABSTRACT

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A study was conducted to identify associations between genetic markers and immune responses in Australian fine-wool Merino sheep to a killed *Mycobacterium avium* subspecies *paratuberculosis* (*Map*) vaccine (Gudair^{TM}). Blood samples and immune response data (antibody and interferon gamma, IFN-γ results) were obtained from 934 sheep from a long-term *Map* vaccination trial undertaken on three independent properties in New South Wales, Australia. Blood samples were genotyped for eight microsatellite markers that included four (DYMS1, OLADRW, OLADRB and SMHCC1) from the *Ovar-Mhc* region, two each from the SLC11A1 (OVINRA1 and OVINRA2) and IFN- γ (o(IFN)γ and OarKP6) gene regions.

Vaccination with Gudair^{TM} induced strong antibody and IFN-γ responses as early as two weeks post-vaccination. Between-property differences in magnitude and trend of immune responses, concomitant with season of vaccination and magnitude of natural infection prevalent in individual flocks, were evident. Immune responses in controls on all the three properties remained consistently low, except for slightly elevated IFN-γ levels at a few time points in controls of properties 2 and 3, concomitant with exposure to natural infection.

There were only 2 alleles and 3 genotypes for marker o(IFN)γ but other loci exhibited extensive polymorphisms, the most occurring at OLADRW which had 42 alleles and 137 genotypes. Heterozygosities varied between 33% (OVINRA2) and 87% (SMHCC1), while polymorphic information contents ranged from 0.31 (o(IFN)γ) to 0.88 (OLADRW). Genotypes at loci DYMS1, OLADRB, SMHCC1, OVINRA1 and o(IFN)γ were in Hardy-Weinberg equilibrium (HWE), while those at OarKP6 were in HWE only when rare alleles (<1.0% frequency) were pooled with the closest size class. Departure from HWE, resulting from possible preferential amplification of alleles in heterozygotes, was evident at OLADRW and OVINRA2.

Associations between immune responses and genetic polymorphisms at the marker loci were examined by analysing both genotypic and allelic affects. The study revealed several genotypes/alleles at different marker loci to be significantly associated with antibody and IFN-γ responses to vaccination with Gudair™. However, the majority of those effects were inconsistent across the three properties. Based on significance and consistency in effects across the three properties, five genotypes (two at DYMS1 and one each at OLADRB, SMHCC1 and OVINRA1) and three alleles (one each at DYMS1, OLADRB and o(IFN)γ) were considered either 'probable' or 'most likely' to be associated with low IFN-γ responses, while a genotype at o(IFN)γ was considered 'most likely' to influence high IFN-γ responses. An allele at OarKP6 was considered 'probable' to be associated with low antibody responses to vaccination. Considering the significance of IFN-γ responses in protection against *Map*, it is likely that the identified genotype/alleles influencing IFN-γ responses to vaccination would also influence immune responses to natural *Map* infections. However, further studies need to be conducted to determine the role of these marker genotypes/alleles in protection against paratuberculosis under natural infection conditions.

Key words: paratuberculosis, OJD, Johne's disease, sheep, immune response, genetic markers, gene polymorphisms, MHC, SLC11A1, IFN-γ

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LIST OF ABBREVIATIONS

1C7	MHC classIII gene
AFO	acid-fast organism
AIC	Akaikes information criterion
AR 1	first-order auto-regressive model
Bf	B factor
BLV	bovine leukemia virus
Bota	*Bos taurus*
bp	base-pairs
C2, C4A and C4B	complement factors
CARD15	caspase recruitment domain-containing protein 15
CD	Crohn's disease
CD38	cluster of differentiation 38
CD4+	T-helper cells expressing cluster of differentiation 4
CD8+	T-helper cells expressing cluster of differentiation 8
cDNA	complementary DNA
CLIP	class II associated invariant chain peptide
CMI	cell-mediated immunity
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSRD226	microsatellite in MHC class I region
CTL	cytotoxic T lymphocytes
CYP21	MHC classIII gene
DMA and DMB	genes encoding α- and β-chains of MHC class II DM molecules
DNA	deoxyribo nucleic acid
dNTP	deoxyribo nucleotide phosphate
DP, DM, DN/DO, DQ and DR	MHC class II molecules
DQA and DQB	genes encoding α- and β-chains of MHC class II DQ molecules
DRA and DRB	genes encoding α- and β-chains of MHC class II DR molecules
DRB1	ovine functional class II DRB gene
DRB2, DRB3 and DRB3	ovine non-functional DRB genes
DTH	delayed-type skin hypersensitivity
DYA and DYB	genes encoding α- and β-chains of MHC class II DY molecules
DYMS1	microsatellite in ovine DYA gene
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EM	expectation-maximization
ER	endoplasmic reticulum
FEC	faecal egg count
FGMT	footrot gene-marker test
G15	MHC class III gene
HIV	human immuno-deficiency virus
HLA	human leukocyte antigen
HSP70	heat-shock protein 70
HWE	Hardy-Weinberg equilibrium
IFN-γ	interferon gamma
IL	interleukin
IMF	international mapping flock
Ipr1	intracellular pathogen resistance 1
kb	kilo base-pairs
kDa	kilo Daltons

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LMP	low-molecular-mass protein
LRR	leucine-rich repeat
LSM	least square mean
LST1	MHC classIII gene
LT	lymphocyte transformation
LTA and LTB	MHC class III genes
Map	*Mycobacterium avium* subspecies *paratuberculosis*
MAS	marker-assisted selection
MBL	mannan binding lectin
MHC	major histocompatibility complex
MSMD	Mendelian susceptibility to mycobacterial disease
NK	natural killer cells
NOD2	nucleotide oligomerization binding domain 2
NRAMP1	natural resistance-associated macrophage protein 1
o(IFN)-γ	microsatellite in ovine interferon gamma gene
OD	optical density
OLA	ovine leukocyte antigen
OLADRB	microsatellite in ovine DRB2 gene
OLADRW	microsatellite in ovine DRB1 gene
Ovar	*Ovis aries*
OVINRA1 and OVINRA2	microsatellites in the ovine SLC11A1 gene
PBR	peptide binding region
PCR	polymerase chain reaction
PPD	purified protein derivative
PSO	polymorphism-specific oligonucleotide
PTB	paratuberculosis
QTL	quantitative trait loci
RFLP	restriction fragment length polymorphism
RSCA	reference-strand-mediated conformation analysis
RT-PCR	reverse transcription polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate - polyacrylamide gel electrophoresis
SE	standard error
Sh-LA	sheep leukocyte antigen
SLC11A1	solute carrier family 11 member 1
SMHCC1	microsatellite in MHC class I region
SNP	single nucleotide polymorphisms
SSCP	single strand conformational polymorphism
sst1	susceptibility for tuberculosis 1
STR	simple tandem repeat
TAP	transporter-associated protein
TAPBP	transporter-associated protein binding protein
T-cells	thymus-derived lymphocytes
TCR	T-cell receptor
Th1	T-helper cells subset 1
Th2	T-helper cells subset 2
TLR	toll like receptor
TNF	tissue necrosis factor
VDR	vitamin D receptor