GENE EXPRESSION IN RED DEER RESISTANT OR SUSCEPTIBLE TO MAP

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Introduction

Paratuberculosis (Johne's disease) results in serious losses of farmed red deer (*Cervus elaphus*) in New Zealand. Young deer are particularly susceptible, especially to heavy challenge with the bovine strain of MAP, and clinical disease occurs in deer as young as 8 months. Field data suggests that some breed-lines of red deer display heritable resistance to paratuberculosis. This paper presents results of a study of gene expression in jejunal lymph nodes (JJLN) of red deer that were either resistant (R) or susceptible (S) to paratuberculosis after heavy oral challenge with MAP.

Method

Two challenge studies were carried out in 2008 and 2009. In 2008, 18 offspring were bred from two unrelated stags by AI across unselected red hinds. In 2009, semen from two stags designated R or S, based on the outcome of natural MAP field challenge of their offspring, were used across unselected red hinds to produce 9 offspring of each. In both years the offspring received heavy oral challenge with a bovine strain MAP (4 daily doses of 10^9 cfu) extracted from JJLNs of clinically affected deer. Samples of posterior JJLN were surgically biopsied at Weeks 4 and 12/13 post challenge (pc). JJLN samples were also collected at euthanasia of clinically affected or at trial end 49/50 weeks pc. These samples were snap-frozen in liquid nitrogen and then stored at -80C. The disease status for animals in both studies was scaled on clinical outcome, histopathology, culture and serology (Tables 1 and 2). The frozen JJLNs from the 3 least affected and 3 most affected at 3 time points (Weeks 4, 12, 50) in the 2008 study, and from Week 4 in the 2009 study, were processed and the RNA extracted and subjected to next generation Life Technologies SOLiD SAGE sequencing for gene expression.

Table 1: 2008 study results of JJLN culture and histopathology.

Week		4		12		50		
Tag	Sire	LSS	JJLN dtp	LSS	JJLN dtp	LSS	JJLN dtp	Affect
506	A	2	36	6	21	3	34	mild
511	A	0	36	4	21	4	34	mild
509	B	0	36	6	15	5	26	mild
Mean		0.7	36	5	19	4	31	mild
510	B	2	36	11	12	9	18	mod
508	A	0	32	11	15	9	18	mod
507	A	0	36	11	12	13	18	severe
Mean		0.7	35	11	13	10	18	mod

Table 2: 2009 study results of JJLN culture and histopathology.

Week		4		13		Euth or 49		
Tag	Sire	LSS	JJLN dtp	LSS	JJLN dtp	LSS	JJLN dtp	Affect
92	R	0	29	6	22	0	>66	nil
84	R	0	29	11	22	5	32	mild
88	R	0	29	6	22	5	39	mild
Mean		0	29	8	22	3	46	mild
82	R	0	29	11	16	13	8	severe
85	S	0	39	13	9	13	8	severe
93	S	0	29	11	10	13	8	severe
Mean		0	32	12	12	13	8	severe

Week: weeks post challenge with MAP; JJLN: jejunal lymph node; dtp: days to positive for Bactec culture of MAP; Euth: euthanasia of clinical cases
LSS: 0=nil, 1-3 nonspecific, 4-7 mild, 8-10 moderate, 11-13 severe.

Results

In 2008 no offspring were clinically affected and there was no sire effect on outcome. In 2009 there was a significant sire effect, with the three animals clinically affected and the offspring of the S sire were significantly worse affected than R offspring (Tables 1 and 2; also see Poster P045 "Heritable resistance / susceptibility in red deer to experimental MAP challenge").
Sequencing generated a total of 373 million "tags" with lengths between 26 and 28 bases. Genes were mapped against three datasets; the deer and elk transcriptome, the cattle transcriptome and the bovine genome. 31,500 genes were uniquely identified, and of these so far 18,000 genes have been annotated

and 17,500 were recognized by the Ingenuity Pathway Analysis (IPA) programme. The number of genes significantly (P<0.05) upregulated in either the 3 S animals or 3 R animals changed over time (Table 3).

Table 3: Number and fold range of significantly upregulated genes in R and S offspring at time points in two studies

	S offspring genes upregulated		R offspring genes upregulated	
	Number	Fold range (mean)	Number	Fold range (mean)
2008 Week 4	161	2.5 – 33.5 (5.0)	38	2.48 – 22.8 (6.5)
2008 Week 12	405	2.4 – 141.9 (6.9)	188	2.4 – 17 (5.0)
2008 Week 50	408	1.7 – 25.4 (3.1)	204	1.7 – 32.9 (3.4)
2009 Week 4	142	3.8 – 178 (16.2)	222	2.6 – 34.3 (5.5)

In the 2008 study the overall number of significantly upregulated genes in both R and S animals increased between Weeks 4 and 50, and the greatest fold changes were in the S animals at Week 12. The 2009 Week 4 R animals had significantly more upregulated genes than the 2008 Week 4 R animals (222 vs 38). While the number of S upregulated genes in 2009 remained similar, the range and mean of upregulation was higher, and the highest fold change in the study was recorded in S animals at 2009 study Week 4 (TCN2 - transcobalamin II; 178 fold). 2008 Week 12 and 2009 Week 4 animals had the greatest number of genes upregulated >10 fold (Table 4).

Table 4: Number and fold range of genes upregulated >10 fold in R and S offspring at time points in two studies

	S offspring genes upregulated		R offspring genes upregulated	
	Number	Fold range (mean)	Number	Fold range (mean)
2008 Week 4	12	10.8 – 33.5 (14.4)	6	10.5 – 22.8 (15.3)
2008 Week 12	59	10.5 – 141.9 (21.8)	13	10.1 – 17 (12.5)
2008 Week 50	7	10.5 – 25.4 (13.8)	10	10.2 – 32.9 (16.8)
2009 Week 4	70	10.2 – 178 (26.4)	24	10.2 – 34.3 (15.3)

A number of genes showed high levels of upregulation at different time points. At least 105 inflammation related genes, including chemokines (C-C and C-X-C), interferon family, tumour necrosis factor family, S100 calcium binding, FAU, NOS and ADIPOQ genes, were upregulated at these 4 time points, including 13 genes upregulated on two occasions and 9 genes on 3 occasions. These genes were especially upregulated in S animals at Weeks 4 and 12 in the 2008 study. ADIPOQ was upregulated on all 4 occasions and is an important adipokine involved in the control of fat metabolism and insulin sensitivity, with systemic anti-inflammatory activities associated with antagonizing TNF-alpha and inhibiting endothelial NF-kappa-B signalling. By contrast, apoptosis and autophagy genes tended to be upregulated more in R animals, especially at Week 4 in the 2009 study. For example genes upregulated in R animals 2009 Week 4 include MAP3K11 (mitogen-activated protein kinase kinase kinase 11) 33.1 fold, RASGRP4 (RAS guanyl releasing protein 4) 8.6 fold, THAP3 (THAP domain containing, apoptosis associated protein 3) 8.2 fold, RNF41 (ring finger protein 41) 8.2, CEBPE (CCAAT/enhancer binding protein (C/EBP), epsilon) 7.9, AMBRA1 (autophagy/beclin-1 regulator 1) 6.6 fold, RASL11A (RAS-like, family 11, member A) 3.8 fold, S100A9 (S100 calcium binding protein A9) 3.7 fold, NFKBID (NFKB nuclear factor of kappa light polypeptide gene enhancer in B-cells) 3.1 fold, TNIK (TRAF2 and NCK interacting kinase) 2.9 fold. At Week 4 in both 2008 and 2009 studies, 8 genes were significantly upregulated in S animals. One gene (ORMDL3) was significantly upregulated in R animals at 8.1, 3.4

and 32.9 fold for 2008 Weeks 4, 12 and 50, respectively, and 3.3 for 2009 Week 4. ORMDL3 is present in the cytoplasm in most tissues, it may indirectly regulate endoplasmic reticulum-mediated Ca(2+) signalling, and its expression is associated with chronic inflammation. It is a member of a gene family that encodes transmembrane proteins anchored in the endoplasmic reticulum and genetic variants regulating ORMDL3 expression appear to be determinants of susceptibility to childhood asthma. The above are a few examples of relative gene expression and further results will be revealed in due course.

Discussion

Access to biopsy samples of JJLN from 19 animals at these 3 time points in the 2008 study has given a powerful insight into the parallel changes in histopathology, immunology, culture and gene expression over the 12 month period that, in red deer, typically determines the outcome of paratuberculosis in terms of the animal recovering, becoming latently infected or succumbing to clinical disease. Unfortunately only the Week 4 samples from the 2009 study could be sequenced due to lack of resources, although this has enabled interesting comparison with the 2008 Week 4 samples. These 2009 animals showed much greater differences between R and S than the 2008 animals, with R animals minimally affected and S animals developing clinical disease. Work is currently underway to sequence this full set.

Innate immunity did not appear to have any significant influence on disease state in the first 4 weeks of either study, with similar numbers of MAP present in JJLN and no histopathological lesions or measurable antibody. Nevertheless, there appear to be significant differences in gene expression at Week 4, which is likely to reflect differences in the pathway the animals' immune systems were taking at that time and subsequently diverged at Weeks 12 and 50. There was quite a marked difference between R and S groups between Week 4 and 12, with S animals showing a more marked increase in number of MAP and severity of lesions. This is accompanied by a much greater number and degree of genes upregulated in the 3 S animals, compared with R animals, especially genes associated with inflammation. Unfortunately it is not possible to be sure whether these differences in gene expression are due to cause or effect The task of analysing these results is ongoing because of the number of genes involved, the complexity of the immune response and the fact that our knowledge of many of the genes is nil or incomplete.

Conclusion

Gene expression in JJLN of deer R or S to MAP challenge is at a very informative phase. The results to date appear to be meaningful and will contribute to understanding resistance to MAP in ruminants.