JOHNE'S DISEASE RESEARCH CONSORTIUM

GENE EXPRESSION IN DEER RESISTANT OR SUSCEPTIBLE TO JOHNE'S DISEASE

COLIN MACKINTOSH
AND
RUDIGER BRAUNING

AGRESEARCH INVERMAY

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HYPOTHESES

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- Resistant and susceptible animals respond differently to MAP challenge by "turning on" or "expressing" different genes involved in different immunological pathways
- These pathways change with time as a result of interactions between the host and MAP
- Key events happen early in the disease, which determine the pathways to mild or severe disease
- New methods of measuring the expression levels of genes in key tissues will assist in understanding this complex immune response

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TWO STUDIES: 2008/9 AND 2009/10

- 18/19 red weaners bred from unselected hinds
  - Resistant and susceptible sires
- Heavy oral challenge with MAP at 4 mo
- Mesenteric lymph node (LN) biopsied
  - 4 weeks pc
  - 12/13 weeks pc
  - at euthanasia if clinical Johne's disease
    or at slaughter 50 weeks pc
- LN samples
  - Snap frozen -80C in Liquid Nitrogen
  - Histopathology and culture
- Weighed & blood sampled over 50 weeks

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2008 STUDY DISEASE OUTCOME OVER 50 WEEKS

		Wk4	Wk4	Wk12	Wk12	Wk50	Wk50	
Tag	**Sire**	**LSS**	**JJLN dtp**	**LSS**	**JJLN dtp**	**LSS**	**JJLN dtp**	**Affected**
506	TBR	2	36	6	21	3	34	Least
511	TBR	0	36	4	21	4	34	Least
509	TBS	0	36	6	15	5	26	Least
Mean		**0.7**	**36**	**5**	**19**	**4**	**31**	**Least**
Mean			35	9	13	7	21	Rest
510	TBS	2	36	11	12	9	18	Most
508	TBR	0	32	11	15	9	18	Most
507	TBR	0	36	11	12	13	18	Most
Mean		**0.7**	**35**	**11**	**13**	**10**	**18**	**Most**

2009 STUDY DISEASE OUTCOME OVER 49 WEEKS

		Week 4	Week 4	Week 13	Week 13	Week 49 euthanasia*	Week 49	
Tag	**Sire**	**LSS**	**JJLN dtp**	**LSS**	**JJLN dtp**	**LSS**	**JJLN dtp**	**Affected**
92	R	0	29	6	22	0	>66	Nil
84	R	0	29	11	22	5	32	v mild
88	R	0	29	6	22	5	39	v mild
Mean		**0**	**29**	**8**	**22**	**3**	**46**	**mild**
Mean		0	33	11	16	9	26	moderate
82*	R	0	29	11	16	13	8	Clinical
85*	S	0	39	13	9	13	8	Clinical
93*	S	0	29	11	10	13	8	Clinical
Mean		**0**	**32**	**12**	**12**	**13**	**8**	**severe**

GENE EXPRESSION

When cells need to produce particular proteins, the genes are transcribed into “messenger RNA” (mRNA). mRNA is the template that is used to build the proteins etc. This process is called gene expression.

This study is aimed at measuring what genes are expressed in either very resistant (R) or very susceptible (S) deer. We are targeting the genes involved with the immune response in the gut so we biopsied the mesenteric lymph nodes

✓ mRNA was extracted from frozen node samples
✓ mRNA was stabilized by converting it into copy DNA (cDNA) by reverse transcriptase
✓one short sequence per cDNA was selected
✓a huge number of short sequences was sequenced by “new generation sequencing”

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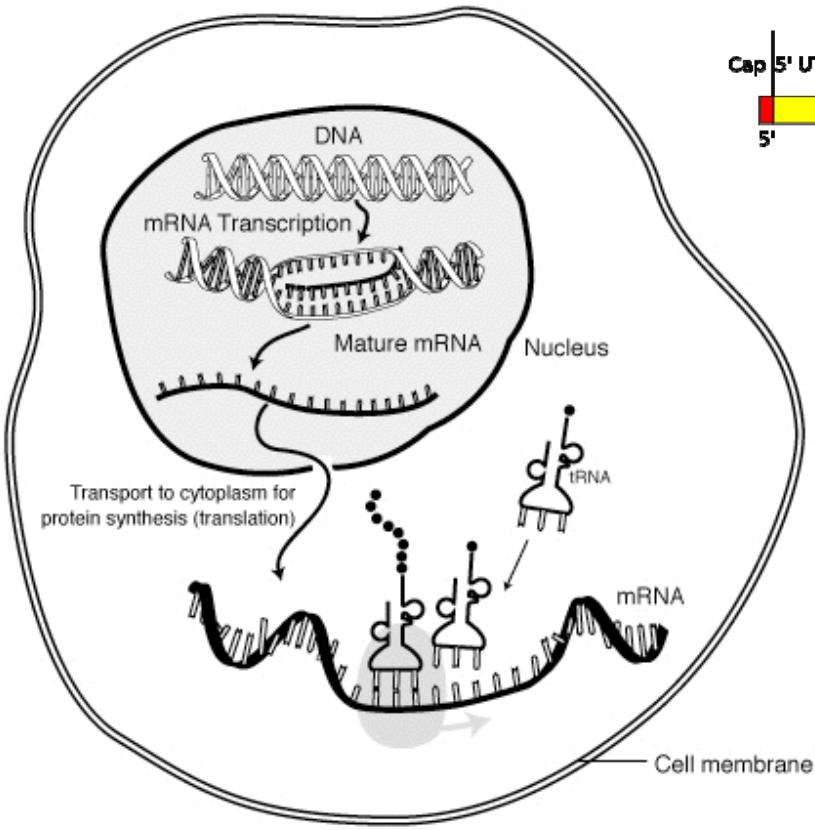
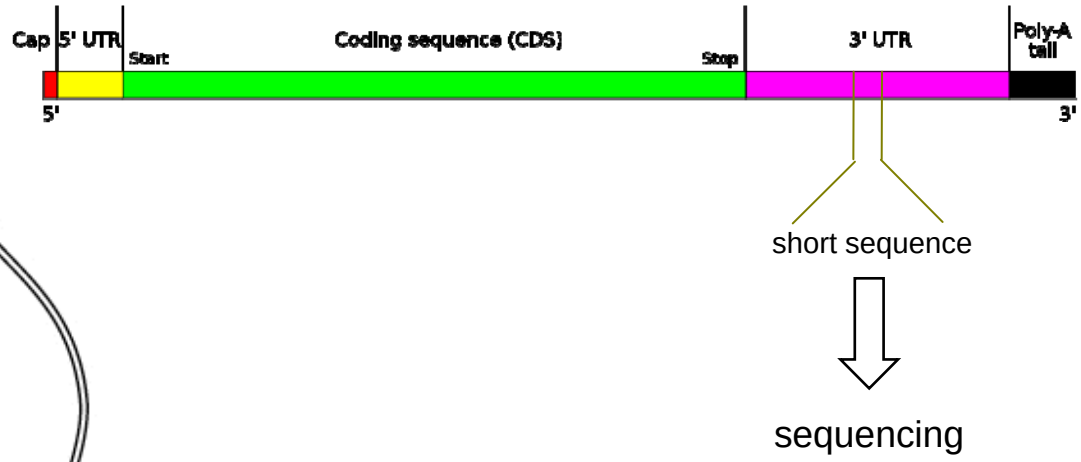
CARTOON OF GENE EXPRESSION





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NEW GENERATION SEQUENCING

24 samples sequenced
2008: 3 least affected (R) and 3 worst affected (S) at Weeks 4, 12 and 50
2009: 3 least affected (R) and 3 worst affected (S) at Weeks 4

Sequencing

- A total of 566 million reads (24 samples)
- 15-30 million reads were generated per sample

Quality control

- 6.7 million reads (1.2%) were discarded because of ambiguities.
- No bias for a specific sequence composition was detected.
- The remaining 560 million reads represent between 9.9 and 19.4 million different sequences per sample point with copy numbers ranging from 1 to 167,389.

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BIOINFORMATICS

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- The output was “filtered” to remove “rubbish”
- 373 million “tags” with lengths between 26 and 28 bases were generated
- Genes were mapped against three datasets
  - the deer and elk transcriptome (in-house)
  - the cattle transcriptome
  - the bovine genome
- Of the 373 million tags, 31,500 genes were uniquely mapped
- 18,000 genes annotated
- Repeated “tags” were counted to give an expression levels per gene and levels compared between R and S deer at the 3 time points
- Of 18,000 genes, 17,500 were recognized by the Ingenuity Pathway Analysis (IPA) programme

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APPROACHES TO ANALYSIS

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- Naïve approach
  - Investigate most upregulated genes in R and S groups to determine their function
- Interesting genes/candidates
  - Check out genes previously found upregulated in microarrays studies
- Focusing on immune candidates
  - TLRs, IFN, ILs, TNFs, chemokines, histocompatability, integrins, killing factors
- Pathway analysis
  - IPA analysis of innate and adaptive immune signalling cascades, inflammation, apoptosis etc
  - Patterns associated with particular diseases eg Tb

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SUMMARY OF GENES FILTERED OUT USING IPA

Group	S	S	S	R	R	R
	n	Total	Up-reg Fold Range	n	Total	Up-reg Fold Range
RS 0408	17		8-33.5	10		8-22.8
Week 4	58		4-7.9	20		4-7.9
	60		3-3.9	5		3-3.9
	34	169	2.5-2.9	5	40	2.5-2.9
RS 0608	110		8-142	26		8-16.9
Week 12	142		4-7.9	75		4-7.9
	86		3-3.9	54		3-3.9
	81	429	2.5-2.9	41	196	2.5-2.9
RS 0309	18		8-25.9	14		8-32.9
Week 50	55		4-7.9	22		4-7.9
	52		3-3.9	27		3-3.9
	63	188	2.5-2.9	21	84	2.5-2.9
RS 0409	89		8-178.3	43		8-34.3
2nd Week 4	67		4-7.9	73		4-7.9
	3		3-3.9	61		3-3.9
	0	159	2.5-2.9	53	230	2.5-2.9

FINDINGS TO DATE – INTERESTING GENES

	Symbol	Fold Change	p-value	Entrez Gene Name	Function
Week 4	TCN2	-7.523	0.000048	transcobalamin II	cobalamin transport, cell proliferation, mitochondrial dysfunction
Week 12	TCN2	-2.552	0.042	transcobalamin II	
2nd Wk 4	TCN2	-178.274	0.000000683	transcobalamin II	
Week 12	SLC11A1	-12.193	0.000000614	NRAMP	metal transporter, iron metabolism, host resistance
Week 50	SLC11A1	2.193	0.00389	NRAMP	
2nd Wk 4	CTSH	-81.654	0.0000016	cathepsin H	lysosomal cysteine proteinase, degradation of lysosomal proteins
2nd Wk 4	TOLLIP	-45.07	0.000316	TOLLIP	toll interacting protein
2nd Wk 4	MYD88	3.581	0.0107	MYD88	myeloid differentiation primary response gene 88, TLR signalling to NF-κB
2nd Wk 4	CAMP	18.916	0.00401	cathelicidin	antimicrobial peptide in macrophage lysosomes, assoc with Vit D

INTERFERON FAMILY

	Symbol	Fold chg	p-value	Entrez Gene Name
Week 4	IRF3	-2.823	0.0307	interferon regulatory factor 3
	IFNG	-2.676	0.0461	interferon, gamma
	Ifi47	2.483	0.0496	interferon gamma inducible protein 47
	ISG20	2.499	0.0479	interferon stimulated exonuclease gene 20kDa
	IFI6	2.753	0.0276	interferon, alpha-inducible protein 6
	IFI44	2.872	0.0249	interferon-induced protein 44
	MX1	5.078	0.0011	myxovirus resistance 1, interferon-inducible protein p78
	IFIT3	5.41	0.0005	interferon-induced protein, tetratricopeptide repeats 3
	IFIT2	16.535	1E-05	interferon-induced protein, tetratricopeptide repeats 2
	ISG15	22.766	2E-09	ISG15 ubiquitin-like modifier
Week 12	IL6	-5.584	0.048	interleukin 6 (interferon, beta 2)
	ISG20	-3.139	0.0129	interferon stimulated exonuclease gene 20kDa
	IFNG	-2.909	0.0203	interferon, gamma
	Ifi47	-2.449	0.0498	interferon gamma inducible protein 47
Week 12	IFNA5	-2.492	0.0217	interferon, alpha 5
	Ifi47	-2.344	0.002	interferon gamma inducible protein 47
2nd Wk 4	CTSH	-81.654	2E-06	cathepsin H
	PRKRIR	-4.893	0.0464	protein-kinase, interferon-inducible inhibitor, P58 repressor
	DAP	-5.337	0.0191	death-associated protein

INFLAMMATION

	Symbol	Fold Change	p-value	Entrez Gene Name
Week 4	CXCR6	-2.839	0.0249	chemokine (C-X-C motif) receptor 6
Week 12	CXCL14	-31.951	1.56E-10	chemokine (C-X-C motif) ligand 14
	CXCL2	-30.567	1.39E-09	chemokine (C-X-C motif) ligand 2
	CXCL5	-22.261	4.55E-09	chemokine (C-X-C motif) ligand 5
	CXCR2	-4.584	0.0124	chemokine (C-X-C motif) receptor 2
	CXCL2	-3.812	0.00485	chemokine (C-X-C motif) ligand 2
	CXCR5	2.803	0.0415	chemokine (C-X-C motif) receptor 5
Week 12	CXCR7	-2.288	0.0237	chemokine (C-X-C motif) receptor 7
	CXCR6	-2.131	0.00831	chemokine (C-X-C motif) receptor 6
	CXCL2	3.04	0.022	chemokine (C-X-C motif) ligand 2
	CXCL14	3.497	0.000539	chemokine (C-X-C motif) ligand 14
	CXCL5	8.442	0.000031	chemokine (C-X-C motif) ligand 5
2nd Wk 4	*			

CANDIDATES

	Symbol	Fold Change	p-value	Entrez Gene Name
Neibergs et al				
Week 4	PIK3R3	-4.805	0.00972	phosphoinositide-3-kinase, regulatory subunit 3 (gamma)
Week 12	TGFB1I1	-3.225	0.0127	transforming growth factor beta 1 induced transcript 1
2nd Wk 4	EDN1	2.749	0.0445	endothelin 1
*	TDGF1	*	*	
Pant et al				
Week 12	TNFAIP6	-8.778	0.0000091	tumor necrosis factor, alpha-induced protein 6
2nd Wk 4	SLC39A2	12.611	0.0205	solute carrier family 39 (zinc transporter), member 2

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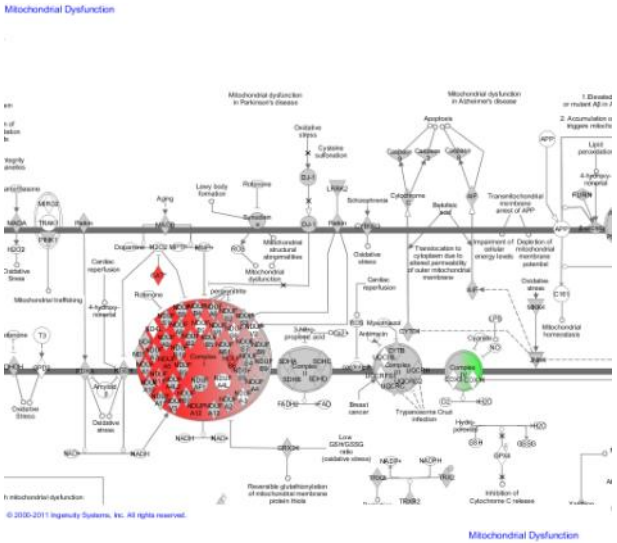
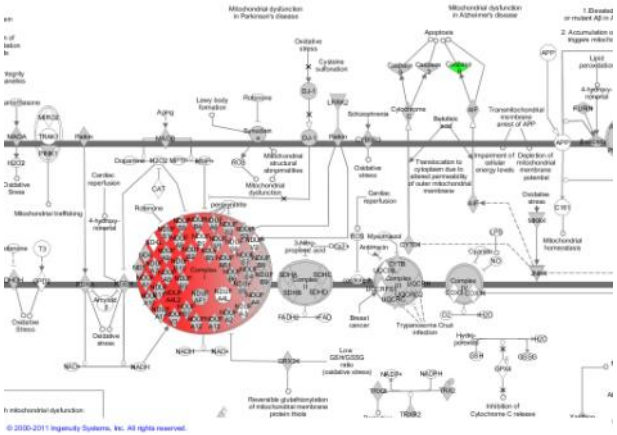
Mitochondrial dysfunction

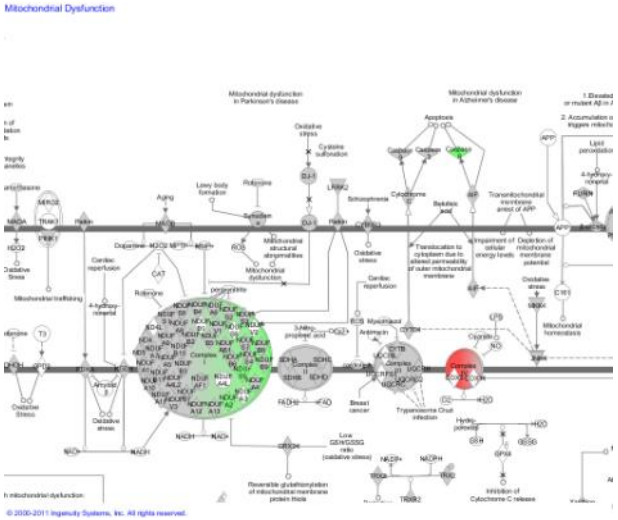


RS_0408 Week 4

Red: S group relatively upregulated
Green: R group relatively upregulated



RS_0608 Week 12



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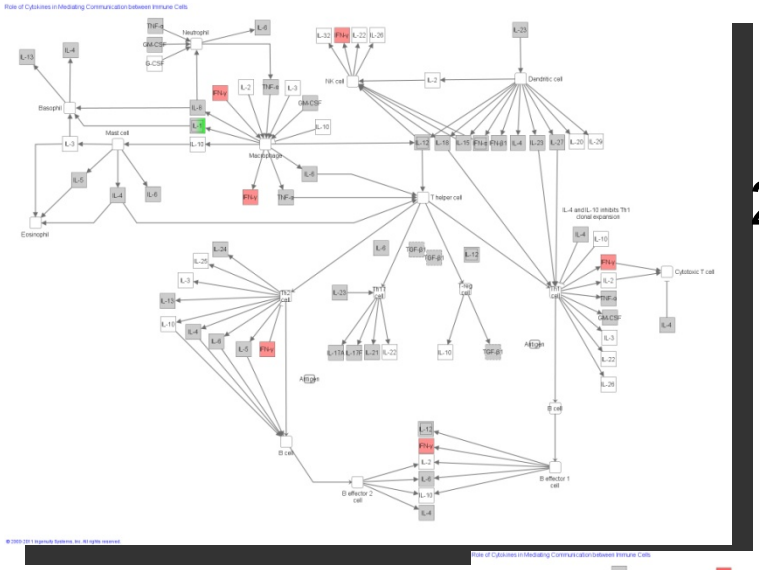
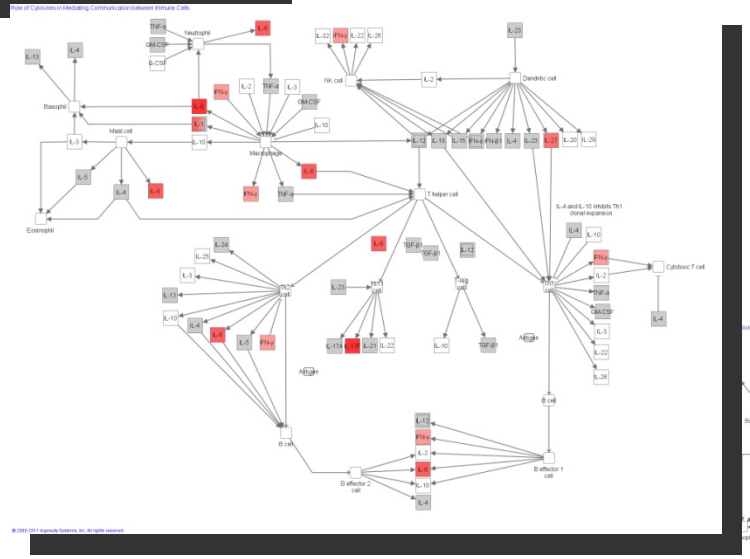
RS_0309 Week 50

INTERFERON FAMILY

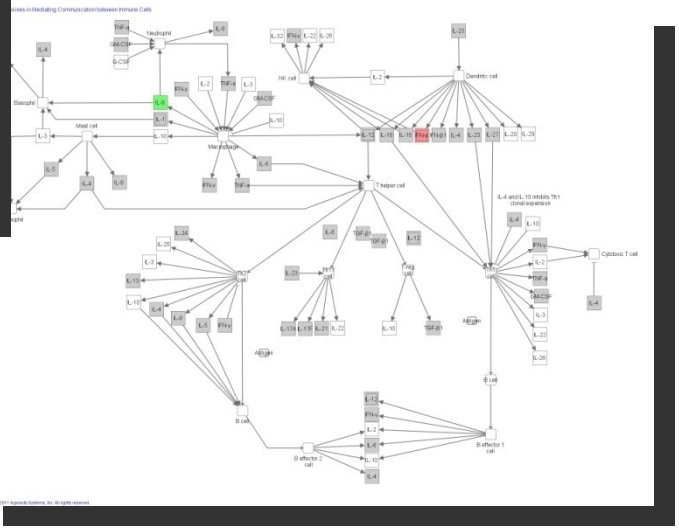


2008 Week 4

Red: S group relatively upregulated
Green: R group relatively upregulated



2008 Week 12



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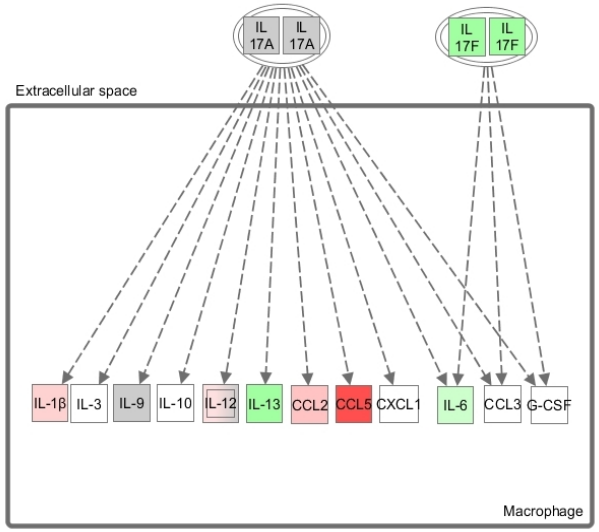
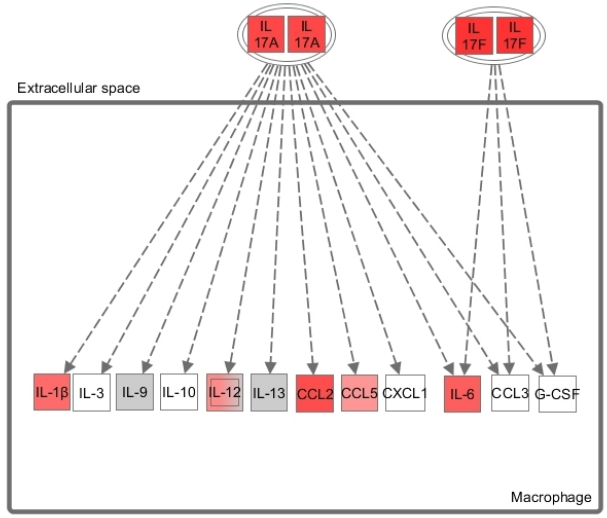
REGULATION OF CYTOKINE PRODUCTION IN MACROPHAGE AND T-HELPER CELLS BY IL17A AND IL17 F

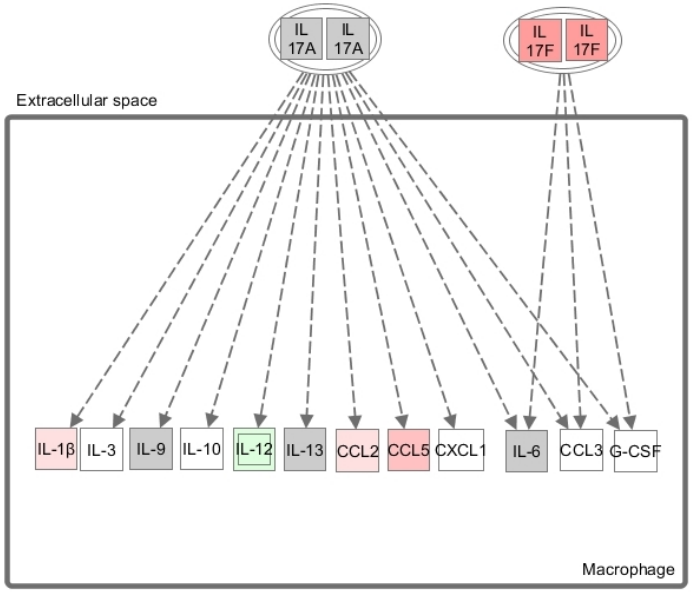


2008 Week 4

Red: S group relatively upregulated
Green: R group relatively upregulated



2008 Week 12



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WHAT COULD BE GENERATED FROM RESEARCH

- Key genes and pathways associated with R/S to Johne's disease will be identified
- Better understanding of the immunopathology of MAP infection and disease
- Underpin SNP panels
- Better strategies for the prevention, control and management of Johne's disease in deer and other domestic livestock, and better tools including marker-assisted selection, diagnostic tests and vaccines
- Development of gene expression panels that can be used to select high value breeding stock

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IMPACT ON FARM

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- JD control difficult
  - Intracellular niche of MAP
  - Complex host-parasite interactions
  - MAP subverts many host immune responses
- No current strategies or control measures completely effective

- Selection for increased resistance is a useful long term strategy, but will not eliminate the problem

- Better cost-effective tests, vaccines and management are needed, and these results will assist in their development

- Future prospect of treatment, such as somatic gene therapy, once the key immune pathways have been elucidated

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ACKNOWLEDGEMENTS

JDRC for funding
AgResearch Invermay staff
Disease Research Lab staff
Otago University staff