

EADGENE Cytokine response during experimentally induced mastitis

Quantitation of the immune alert

The RIBFA group in the EADGENE

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2nd EADGENE Mastitis Working Group Meeting, Roslin 1/2nd March, 2007 **RIBFA & EADGENE Mastitis** Leibniz Gemeinschaft

EADGENE Cytokine-Induction *in vivo in the udder: E. coli* induces, *S. aureus* ~not!

IL10: TH1 Antagonist
 TNF α : proinflammatory cytokine recruits neutrophils
 IL8: also measured: TGF- β , IL12

E. coli N = 4
S. aureus N = 4

Inadequate recognition of *S. aureus* pathogens?
 Cell models!

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EADGENE MEC as cell models: MEC are highly immune competent

Perviously:
 Murine model cell line HC-11, challenged, analysed on Affymetrix 430A
 Result:
 350 genes regulated, many of proinflammatory relevance

See examples:

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EADGENE Preparation of bovine primary Mammary Epithelial Cells

- Method: collagenase dissociation of udder tissue
- Removal of fibroblasts from MEC-culture by selective trypsination

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EADGENE MEC are highly immune competent

Bovine MEC
 Three replica, challengend o/n with heat killed *E. coli*, analysed on Affymetrix microarray, compared to pooled udder samples from live Infections (*S. uberis*, *E. coli*)
 Sorted by maximum stimulation, arbitrary list of top MEC-expressed genes

Top up-regulated genes				RefSeq	Gene Title
sig	S. Uber	E. coli	Log 2	Transcript ID	
ptMEC	Log 2	Log 2	fold		
mean					
Log					
2					
sig					
ratio					
9.364	1.687869	1.248217		NM_175827	chemokine (C-C motif) ligand 5
8.200	1.658894	1.121742		NM_203435	lingual antimicrobial peptide
8.109	1.632198	1.120222		XM_587897	haptoglobin
7.931	1.411181	1.175023		NM_181016	serum amyloid A 3
6.688	1.339138	1.471927		—	—
6.571	1.633868	1.168182		Transcribed locus, XP_547319.1 PREDICTED: 1	
6.499	1.609911	1.255222		NM_173041	myxovirus (influenza virus) resistance 2 (mouse)
6.370	1.638688	1.462013		Transcribed locus, C15 protein (BM-004) [Canis familiaris]	
6.314	1.489168	1.446224		Transcribed locus	
6.223	1.191612	1.170723		Transcribed locus	
6.067	1.118144	1.121012		NM_174283	chemokine (C-C motif) ligand 20
6.014	1.840162	1.176023		NM_173925	interleukin 8 [neutrophil activating peptide 1]
5.814	1.332021	1.117802		Transcribed locus	
5.697	1.664838	1.492225		XM_583696	similar to putative ISG12(a) protein
5.547	1.118177	1.164922		Transcribed locus, XP_245246 [Canis familiaris]	
5.539	1.431302	1.301222		XM_583817	similar to Interferon-induced protein 44 n1

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EADGENE MEC and Fibroblasts differ in immunocompetence

- TNF α is mainly expressed in MEC after *E. coli* induction
- Fibroblasts do not produce defensins

Important to purify MEC cultures from fibroblasts

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