Determination of Cytokine Gene Expression Profiles after Infection of Porcine Cells with *Classical swine fever virus*

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Aim of the project

- Overview of mechanisms of pathogenesis of CSF
- Determination of changes of cytokine gene expression profiles
- Gene expression profiles depend on virus strains and host (age, breed of pigs)?

- CSF = haemorrhagic fever
  - Characterised by leukopenia, vascular permeability, petechial bleedings and haemorrhages
- Neither leukopenia nor haemorrhages are a consequence of viral replication
- Hypothesis: networking of different cytokines
  - TNF-α, IL-1α, IL-1β, IL-6 and IL-8
  - Possible marker for haemorrhagic form?

Analysis of gene expression changes

by Serial Analysis of Gene Expression (SAGE)

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in infected cell culture with three CSFV strains of different virulence

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in *in vitro* infected peripheral blood mononuclear cells (PBMCs) infected with a highly virulent CSFV strain

SAGE

- Effective method
- Changes in gene expression profiles
  - Pathogen-host interactions
  - Cytokine gene expression
  - 'Tags' are sufficient for the identification of the mRNA transcript
- Quantitative determination of expression profiles

SAGE: Procedure

- Infection of porcine kidney cell line with highly virulent CSFV strain CSF0382
- Generation of four libraries
  - Consideration of early time points

	PK15(A) 24 h control	PK15(A) 2 hpi CSF0382	PK15(A) 6 hpi CSF0382	PK15(A) 24 hpi CSF0382
Total no of *tags*	42,474	40,648	36,200	37,809

SAGE: Results

Gene	Function	24 hrs control	2 hpi CSF0382	6 hpi CSF0382	24 hpi CSF0382
β-actin	Housekeeping gene	50	59	56	54
NADH-dehydrogenase	Electron transport chain	49	70	73	77
Tubulin-α	Formation of cytoskeleton	13	27	26	11
GAPDH	Glycolytic enzyme	30	46	48	27
Annexin I	Cytoskeletal protein	41	46	57	47
IL-8	**Chemokine**	**68**	**101**	**80**	**68**

Analysis of gene expression changes in cell culture

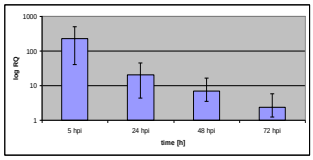
- Porcine kidney cell line infected with three CSFV strains of different virulence: highly virulent strain CSF0382, moderate virulent strain CSF0864 and non virulent C-strain
  - Analysis of gene expression changes by qRT-PCR after: 0 hpi, 5 hpi, 24 hpi, 48 hpi and 72 hpi
- Analysed cytokines: IL-1β, IL-6, IL-8 and TNF-α

→ **Only TNF-α showed a 50-fold increase 5 hpi**

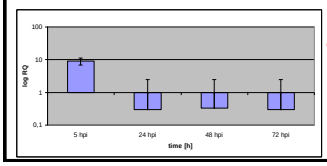
In vitro infection of PBMCs

- Isolation of PBMCs from pigs of different ages (12 weeks and 1.5 years old)
- Infection of PBMCs with highly virulent CSFV strain CSF0382
- Analysis of gene expression changes by qRT-PCR after: 0 hpi, 5 hpi, 24 hpi, 48 hpi and 72 hpi

Analysis of *in vitro* infected PBMCs: IL-1β

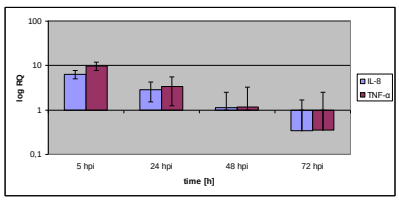


PBMCs from 12 weeks old pigs: 230-fold increase 5 hpi



PBMCs from 1.5 years old pigs: 9-fold increase 5 hpi

Analysis of *in vitro* infected PBMCs: IL-8 and TNF-α



PBMCs from 12 weeks old pigs: increase of IL-8 and TNF-α gene expression 5 hpi

No changes in gene expression in PBMCs from 1.5 years old pigs

Conclusion

- SAGE: first overview of up- and down-regulated genes after CSFV infection
  - Only IL-8 was detected
- Cell culture: only TNF-α showed increase in the beginning of the infection
  - Only highly virulent strain showed changes
- *In vitro* infected PBMCs from pigs of different ages
  - Young animals: increase of cytokines in the beginning of infection
  - Old animals: slight or no changes in gene expression
- Correlation between severe disease and high levels of IL-8 was shown for Dengue fever

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