Genome-wide transcriptional response of primary alveolar macrophages following infection with porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory syndrome is a major cause of economic loss for the swine industry worldwide. Porcine reproductive and respiratory syndrome virus (PRRSV) triggers weak and atypical innate immune responses, but key genes and mechanisms by which the virus interferes with the host innate immunity have not yet been elucidated. In this study, genes that control the response of the main target of PRRSV, porcine alveolar macrophages (PAMs), were profiled in vitro with a time-course experiment spanning the first round of virus replication. PAMs were obtained from six piglets and challenged with the Lelystad PRRSV strain, and gene expression was investigated using Affymetrix microarrays and real-time PCR. Of the 1409 differentially expressed transcripts identified by analysis of variance, two, five, 25, 16 and 100 differed from controls by a minimum of 1.5-fold at 1, 3, 6, 9 and 12 h post-infection (p.i.), respectively. A PRRSV infection effect was detectable between 3 and 6 h p.i., and was characterized by a consistent downregulation of gene expression, followed by the start of the host innate immune response at 9 h p.i. The expression of beta interferon 1 (IFN-β), but not of IFN-α, was strongly upregulated, whilst few genes commonly expressed in response to viral infections and/or induced by interferons were found to be differentially expressed. A predominance of anti-apoptotic transcripts (e.g. interleukin-10), a shift towards a T-helper cell type 2 response and a weak upregulation of tumour necrosis factor-α expression were observed within 12 h p.i., reinforcing the hypotheses that PRRSV has developed sophisticated mechanisms to escape the host defence.

INTRODUCTION

Porcine reproductive and respiratory syndrome is a major cause of economic loss for the swine industry worldwide (Neumann et al., 2005) and causes high mortality of nursery piglets, reproductive failure in sows, respiratory distress in pigs of all ages and influenza-like symptoms in grow/finish swine (Mengeling & Lager, 2000; Nodelijk, 2002). The aetiological agent is porcine reproductive and respiratory syndrome virus (PRRSV), belonging to the family Arteriviridae with an enveloped, positive-stranded RNA genome of about 14.5 kb (Snijder & Meulenburg, 1998).

A typical hallmark of PRRSV is that it causes an acute viraemic phase (up to 14 days post-inoculation) during which the virus can be detected in serum and all susceptible organs (Beyer et al., 2000; Duan et al., 1997b). This acute phase is followed by virus elimination from serum and most organs, and by persistent replication in tonsils, lungs and some lymph nodes (Allende et al., 2000; Rowland et al., 2003; Wills et al., 2003). This prolonged replication does not represent a true persistent infection, as all animals clear the virus by 6 months after inoculation, thus indirectly showing that the immune system is capable of finally dealing with the virus, although not efficiently. Because of this persistent nature of PRRSV infections, numerous studies have analysed the immune responses that may control PRRSV infections or that may be altered by PRRSV (reviewed by Lopez & Osorio, 2004; Mateu & Diaz, 2007; Murthaugh et al., 2002).

The PRRSV-specific humoral immunity is generally characterized by a strong, non-neutralizing antibody
response, which is detected from 5–6 days post-infection (p.i.). In contrast, induction of neutralizing antibodies is severely delayed (starting at 3–4 weeks p.i.) and their levels remain low (Lopez & Osorio, 2004); antibodies were shown to be ineffective in eliminating PRRSV-infected macrophages in combination with complement (Costers et al., 2006). Cellular immune responses against PRRSV infection are characterized by a late onset of lymphocyte proliferative responses (4 weeks p.i.) and the late appearance of gamma interferon (IFN-γ)-secreting cells (Meier et al., 2003). Several studies have also shown weak and atypical innate immune responses, such as weak IFN-γ responses and high induction of interleukin (IL)-10. This inadequate recognition of virus infection by the innate defence mechanisms could be responsible for the initially crippled immune response (Albina et al., 1998; Buddaert et al., 1998; Murtaugh et al., 2002; Royaee et al., 2004; Suradhat et al., 2003; van Reeth et al., 1999; Xiao et al., 2004). The mechanism by which PRRSV interferes with innate immune responses has yet to be elucidated.

PRRSV has a highly specific tropism for cells of the monocyte/macrophage lineage, cells that are essential for immune function. In vivo, the virus mainly infects a subpopulation of differentiated macrophages that are present in tonsils, lungs and other lymphoid tissues (Beyer et al., 2000; Duan et al., 1997a, b). Besides macrophages, in vitro analysis of susceptible cells has identified cultivated monocytes and dendritic cells as potential targets, but their role during PRRSV infections in vivo remains to be established (Delputte et al., 2007; Duan et al., 1997a; Loving et al., 2007; Teifke et al., 2001; Voicu et al., 1994; Wang et al., 2007). Lung pathogenesis is another feature of PRRSV infections, and porcine alveolar macrophages (PAMs) are generally considered to be a major target for PRRSV.

The aim of this study was to gain insight into the putative mechanisms by which PRRSV can evade innate immunity, and consequently the adaptive response, using a genome-wide approach. A time-course gene expression profiling of PAMs infected in vitro with a reference strain (Lelystad) was conducted by utilizing an Affymetrix 24K Porcine Chip microarray. Collection of samples at different times during the infection cycle, from 1 h p.i. (virus entry) up to 12 h p.i. (virus release and cell death) allowed us to discriminate between changes in early and late gene expression during infection. Times later than 12 h p.i. were not analysed, as by that time PRRSV infection of macrophages has typically resulted in cell death.

**METHODS**

**Cells and treatments.** Six 3-week-old hybrid piglets from a PRRSV- and porcine circovirus 2-negative herd of the Rattlerow–Seghers genetic line (a cross-breed between English Landrace, Belgian Landrace, Large White and a synthetic company Landrace) were injected daily with 1 ml enrofloxacin (5 % solution) and 1 ml lincomycin/spectinomycin (5 or 10 % solution) for 3 days to eliminate eventual bacterial pathogens. Two weeks later, the piglets were sacrificed. PAMs were collected by bronchoalveolar lavage and frozen in liquid nitrogen as described by Wensvoort et al. (1991).

PAMs were thawed and cultured for 48 h before treatment as described previously by Delputte & Nauwynck (2004). One primary culture from each animal was split into two: one was infected at an m.o.i. of 10 with a 13th passage of PRRSV Lelystad virus (kindly provided by G. Wensvoort, Institute for Animal Science and Health, Lelystad, The Netherlands), which was semi-purified as described previously (Delputte & Nauwynck, 2004). The other culture was maintained as a control and was mock inoculated. The percentage of infected cells ranged between 60 and 70 % for all batches. Cells were collected at 1, 3, 6, 9 and 12 h p.i. in TRIzol (Invitrogen Life Technologies) for RNA extraction (Fig. 1).

**RNA extraction, reverse transcription, RNA labelling and cRNA hybridization.** Total RNA extraction from PAMs was performed using TRIzol following standard instructions (Invitrogen) and a clean-up was carried out using RNeasy columns (Qiagen). RNA quality was assessed by microcapillary electrophoresis on an Agilent 2001 Bioanalyzer (Agilent Technologies) with RNA 6000 Nanochips. RNA was quantified by spectrophotometry (ND-1000; NanoDrop Technologies). Reverse transcription of 20 μg total RNA and synthesis of biotin-labelled cRNA with one round of amplification were carried out following the standard Affymetrix one-cycle protocol according to the manufacturer’s instructions.

Transcriptional profiles were assessed using Affymetrix 24K GeneChip Porcine Genome Arrays (http://www.affymetrix.com/products/arrays/specific/porcine.affx). Based on previous evidence that sample pooling does not significantly affect the results of Affymetrix chip analysis (see, for example, Han et al., 2004), three samples each from control and infected-cell cultures were pooled for

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Fig. 1. Schematic representation of the experimental design used in this study to challenge PAMs with PRRSV in vitro. PAMs were obtained from six piglets (a). Each PAM culture was split into two and infected with PRRSV or mock infected as a control (b). The total RNA from PAMs of each piglet was extracted at different time points (0, 1, 3, 6, 9 and 12 h p.i.). The RNA of three piglets was pooled (pools I and II) for the subsequent microarray and real-time analyses (c).
each time point (Fig. 1), resulting in two control (pools I" and II") and two infected pools (pools I" and II").

Hybridization and scanning of the arrays were carried out according to standard Affymetrix protocols (Shen et al., 2005) using a GeneChip Scanner 3000 7G.

**Microarray data analysis.** Signal intensities were evaluated using the GeneChip Operating Software algorithm (GOS version 1.4; Affymetrix). Raw data and statistical analyses were performed with GeneSpring version 7.3.1 software (Agilent). Normalization was performed per chip (normalized to 50th percentile) and per gene (normalized to the median).

A statistical analysis of variance (ANOVA) model was applied to the data and significance was declared accepting a false discovery rate (FDR) of 0.05. Fixed effects of time point and status (infected—non-infected cells) were included in the ANOVA model. A further cut-off threshold was applied based on a fold change of 1.5 between infected and control PAMs. Hierarchical clustering of the conditions was performed using Pearson’s correlation coefficient (r) as a measure of similarity and the average linkage method as the clustering algorithm.

In order to test for the presence of outliers in the two pools, the transcriptional profiles of infected animals were analysed separately at each time point (Fig. 1), resulting in two control (pools I and II) and two infected pools (pools I and II). Two micrograms of total RNA from pools I and II were reverse-transcribed using the Superscript II RT-PCR System (Invitrogen Life Technologies) and standard procedures. The real-time reaction mixture (total 20 μl) included 5 μl cDNA as template (diluted 1:50), 200 nM of each of the two primers (forward and reverse), 100 nM Roche probe and 1× master mix (Applied Biosystems). Real-time PCR was performed in 384-well optical plates using a Tecan Freedom EVO-150 liquid handling workstation (Tecan Trading) and an ABI 7900HT real-time PCR machine (Applied Biosystems) with the GeneAmp 7900HT sequence detection system software (PerkinElmer).

A control cDNA dilution series (1:50, 1:100, 1:500 and 1:5000) was created for each transcript to establish a standard curve for each plate; real-time reactions of the same pools described for the microarray analysis were performed in triplicate. Briefly, the log input amount of the standard curve was plotted against the output Ct values; all amplifications had a slope of between −3.48 and −2.99 and were accepted as quantitative. The log input amount of each sample was then calculated according to the formula (Ct−b)/m, where b is the y-intercept and m is the slope. The log input amount was converted to input amount according to the formula 10^[log input amount] and triplicate input amounts were averaged for each sample. The mean input amount of each gene was normalized to the mean input amount of HPRT1. A t-test (with thresholds for statistical significance set to 0.1 and 0.05) was applied to each gene to verify whether the difference between control and infected macrophages at each time point was significant.

Pearson’s correlation coefficient (r) was calculated for each gene on the normalized data to quantify the consistency between microarray experiments and real-time PCR.

**Microarray data.** The data of the microarray analysis were deposited in the ArrayExpress repository (http://www.ebi.ac.uk/arrayexpress) with ArrayExpress accession number MEXP-1350, following the guidelines of the rationale of minimum information about a microarray experiment (MIAME) (Brazma et al., 2001).

**RESULTS**

**Microarray analysis**

ANOVA analysis (FDR=0.05) showed that 1409 genes were differentially expressed in macrophages after PRRSV infection. After applying a further filter of 1.5-fold change in expression, two, five, 25, 16 and 100 transcripts were differentially expressed at 1, 3, 6, 9 and 12 h p.i., respectively, compared with the controls at the same time points. Overall, the effect of PRRSV on the host transcription machinery was one of downregulation (115/148 transcripts). The differentially expressed transcripts were annotated based on a previous work (Tsai et al., 2006) and are reported in Table 1. The distribution of signal intensities of the 100 differentially expressed transcripts at 12 h p.i. and the hierarchical clustering of controls and infected replicates for the five time conditions (plus the time 0) are shown in Fig. 2.

At early time points (1 and 3 h p.i.), the profiles of gene expression in the control and infected conditions were very similar and clustered together, i.e. only two (1 h p.i.) and five (3 h p.i.) transcripts were significantly altered. The expression profiles clearly changed between 3 and 6 h p.i., with greater differences detected at the later time points (9 and 12 h p.i.), when PRRSV has been shown to complete its replication (Halbur, 2001; Rossow et al., 1995).
Table 1. Transcripts differentially expressed in PAMs at 1, 3, 6, 9 and 12 h p.i. following PRRSV infection

A total of 148 transcripts showed differential expression and are listed from the highest to the lowest fold change at the different time points p.i. The Affymetrix probe set IDs are reported with fold changes, gene symbols and gene description (Tsai et al., 2006).

| Affymetrix probe set ID | Fold change | Gene symbol | Gene description |
|-------------------------|-------------|-------------|-----------------|
| **1 h p.i.**             |             |             |                 |
| Ssc.10997.1.S1_at        | 1.699       | GRP58       | Protein disulfide-isomerase A3 precursor |
| Ssc.1313.1.A1_at         | 0.661       | NP_077001   | XTP3-transactivated protein A (Homo sapiens) |
| **3 h p.i.**             |             |             |                 |
| Ssc.20199.2.S1_at        | 0.666       | HIVEP2      | Human immunodeficiency virus type I enhancer-binding protein 2 |
| Ssc.30752.1.S1_at        | 0.659       | IFT1        | IFN-induced protein with tetratricopeptide repeats 1 |
| Ssc.23248.1.S1_at        | 0.653       | PTPRC       | Leukocyte common antigen precursor |
| AFFX-Ss_IRP_3_at         | 0.648       | IRG6        | Sus scrofa inflammatory response protein 6 |
| Ssc.390.2.S1_at          | 0.550       | HIF1-α      | Hypoxia-inducible factor 1-α |
| **6 h p.i.**             |             |             |                 |
| Ssc.12512.1.A1_at        | 1.544       | DDX17       | Probable RNA-dependent helicase p72 (DEAD-box protein 17) |
| Ssc.20344.1.S1_at        | 0.667       | WBP2        | WW domain-binding protein 2 |
| Ssc.13400.2.S1_at        | 0.666       | C3orf4      | Protein C3orf4 (membrane protein GENX-3745) (HSPC174) |
| Ssc.8570.1.A1_at         | 0.665       | DLC1        | Rho-GTPase-activating protein 7 |
| Ssc.13657.1.A1_at        | 0.663       | ATF2        | Cyclic-AMP-dependent transcription factor ATF-2 |
| Ssc.16363.1.S1_at        | 0.662       | TMOD3       | Ubiquitous tropomodulin |
| Ssc.3420.1.S1_at         | 0.660       | C1orf111    | Protein C1orf111 (CGI-35) |
| Ssc.7164.1.A1_at         | 0.660       | NP_060530   | Mitochondrial isoleucine tRNA synthetase (Homo sapiens) |
| Ssc.17091.2.A1_at        | 0.637       | C3orf52     | Protein C3orf52 (membrane protein GENX-3745) (HSPC174) |
| Ssc.2354.1.S1_at         | 0.625       | PTPRC       | Leukocyte common antigen precursor |
| Ssc.1333.1.A1_at         | 0.611       | ZCCHC11     | Zinc finger, CCHC domain containing 11 isoform b |
| Ssc.26084.1.S1_at        | 0.607       | ATP2B1      | Plasma membrane calcium-transporting ATPase 1 |
| Ssc.22212.1.S1_at        | 0.600       | GKP3        | Glycerol kinase, testis-specific 1 |
| Ssc.13219.1.S1_at        | 0.599       | NP_689905   | Core 1 UDP-galactose : N-acetylgalactosamine-α-R β1,3-galactosyltransferase 2; core 1 β-galactosyltransferase-specific molecular chaperone (Homo sapiens) |
| Ssc.19975.1.S1_at        | 0.592       | TEBP        | Telomerase-binding protein p23 (Hsp90 co-chaperone) (progerin receptor complex p23) (Homo sapiens) |
| Ssc.4004.1.A1_at         | 0.577       | SYNE2       | Nesrin 2 (nuclear envelope spectrin repeat protein 2) (Syne-2) (nuclear envelope protein 2) (nucleus and actin connecting element protein) (NUANCE protein) |
| Ssc.10997.1.S1_at        | 0.535       | GRP58       | Protein disulfide-isomerase A3 precursor |
| Ssc.4472.1.A1_at         | 0.524       | NNT2        | NTF2-related export protein 2 (p15-2 protein) (DC9) (BM-025) |
| Ssc.390.2.S1_at          | 0.511       | HIF1-α      | Hypoxia-inducible factor 1-α |
| **9 h p.i.**             |             |             |                 |
| Ssc.30752.2.A1_at        | 3.195       | IFT1        | IFN-induced protein with tetratricopeptide repeats 1 |
| Ssc.30532.1.A1_at        | 2.546       | XRCC2       | DNA-repair protein XRCC2 (X-ray repair cross-complementing protein 2) |
| Ssc.286.1.S1_s_at        | 2.182       | cig5        | Viperin; similar to inflammatory response protein 6 (Homo sapiens) |
| AFFX-Ss_IRP_3_at         | 1.919       | IRG6        | Sus scrofa inflammatory response protein 6 |
| Ssc.15761.1.A1_at        | 1.881       | TCRA        | T-cell receptor α-chain C region (Homo sapiens) |
| Ssc.314.1.S1_at          | 1.745       | ADM         | ADM precursor [contains adrenomedullin (AM)] |
| Ssc.286.1.S1_at          | 1.639       | GBP1        | IFN-induced guanylate-binding protein 1 |
| Ssc.14474.1.S1_at        | 1.538       | LOC396897   | Sus scrofa apomucin |
| Ssc.336.1.S1_at          | 1.521       | USP18       | Ubl carboxyl-terminal hydrolase 18 |
Table 1. cont.

| Affymetrix probe set ID | Fold change | Gene symbol | Gene description |
|-------------------------|-------------|-------------|------------------|
| Ssc.11901.1.S1_at       | 0.652       | C10orf22    | Chromosome 10 open reading frame 22 |
| Ssc.4462.1.S1_at        | 0.630       | SDHC        | Succinate dehydrogenase cytochrome b560 subunit, mitochondrial precursor (integral membrane protein CII-3) |
| Ssc.17091.2.A1_s_at     | 0.621       | C3orf52     | TPA-induced transmembrane protein |
| Ssc.5333.1.S1_at        | 0.620       | ZDHHC3      | Zinc finger DHHC domain containing protein 3 (zinc finger protein 373) (DHHHC1 protein) |
| Ssc.15559.1.A1_s_at     | 0.552       | NP_060114   | DRE1 protein (Homo sapiens) |
| 12 h p.i.               |             |             |                  |
| Ssc.29006.1.S1_at       | 20.150      | IFN-β1      | IFN-β precursor |
| Ssc.30752.2.A1_at       | 4.917       | IFT1        | IFN-induced protein with tetra-tricopeptide repeats 1 |
| Ssc.3032.1.A1_at        | 4.079       | XRCC2       | DNA-repair protein XRCC2 (X-ray repair cross-complementing protein 2) |
| AFFX-Ss_IRP_3_at        | 3.827       | IRG6        | Sus scrofa inflammatory response protein 6 |
| Ssc.286.1.S1_s_at       | 3.778       | cig5        | Viperin; similar to inflammatory response protein 6 (Homo sapiens) |
| Ssc.5085.1.A1_at        | 2.912       | TNF-αIP3    | Tumour necrosis factor-α-induced protein 3 |
| Ssc.15761.1.A1_at       | 2.689       | TCR-α       | T-cell receptor α-chain C region (Homo sapiens) |
| Ssc.336.1.S1_at         | 2.034       | USP18       | Ubl carboxyl-terminal hydrolase 18 |
| Ssc.148.1.S1_at         | 1.934       | IL-10       | Interleukin-10 precursor |
| Ssc.12284.1.A1_at       | 1.923       | SGK         | Serine/threonine-protein kinase Sgk1 |
| Ssc.11048.1.S1_at       | 1.918       | PLAC8       | Placenta-specific gene 8 protein |
| Ssc.16288.1.S1_at       | 1.859       | IGHM        | Ig α-1 chain C region |
| Ssc.29054.3.S1_at       | 1.738       | GBP1        | IFN-induced guanylate-binding protein 1 |
| Ssc.314.1.S1_at         | 1.718       | ADM         | ADM precursor [contains adrenomedullin (AM)] |
| Ssc.18038.1.A1_at       | 1.642       | MAP3K8      | Mitogen-activated protein kinase kinase kinase 8 |
| Ssc.10754.1.A1_at       | 1.621       | PIK3R1      | Phosphatidylinositol 3-kinase regulatory α subunit (PI3-kinase p85-alpha subunit) |
| Ssc.26507.2.S1_at       | 1.585       | NP_073596   | Endo-β-N-acetylgalactosaminidase (Homo sapiens) |
| Ssc.25855.1.S1_at       | 1.531       | XP_846553   | PREDICTED: hypothetical protein |
| Ssc.1701.2.S1_at        | 1.529       | Q6PK96      | Cytochrome b, ascorbate-dependent 3 |
| Ssc.100.1.S1_at         | 1.513       | TNF-α       | Tumour necrosis factor precursor (TNF-α) |
| Ssc.13657.1.A1_at       | 1.665       | ATP2       | Cyclic-AMP-dependent transcription factor ATP2 (activating transcription factor 2) |
| Ssc.4498.1.S1_at        | 0.663       | IXL         | Intersex-like |
| Ssc.3420.1.S1_at        | 0.661       | C14orf111   | Protein C14orf111 (CGI-35) |
| Ssc.8311.1.A1_at        | 0.660       | MPI         | Mannose-6-phosphate isomerase |
| Ssc.8541.1.A1_at        | 0.659       | STAG2       | Cohesin subunit SA-2 (Stromal antigen 2) |
| Ssc.16422.2.A1_at       | 0.658       | PLAA        | Phospholipase A-2-activating protein |
| Ssc.21796.1.S1_at       | 0.657       | SORL1       | Sortilin-related receptor precursor (sorting protein-related receptor containing LDLR class A repeats) |
| Ssc.5404.1.S1_at        | 0.657       | MOSPD1      | Motile sperm domain-containing 1 (Homo sapiens) |
| Ssc.27060.1.A1_at       | 0.656       | SSSCA1      | Sjogren's syndrome/scleroderma autoantigen 1 (autoantigen p27) |
| Ssc.26533.1.S1_at       | 0.655       | AP1-γ1      | Adaptor-related protein complex 1 γ1 subunit (γ-adaptin) (adaptor protein complex AP-1 γ1 subunit) |
| Ssc.3656.1.S1_at        | 0.654       | KHLX        | Kelch-like protein X (Homo sapiens) |
| Ssc.24239.1.S1_at       | 0.653       | C14orf11    | Protein C14orf11 (CGI-35) |
| Ssc.9314.2.S1_at        | 0.653       | TP53RK      | TP53 regulating kinase |
| Ssc.17314.1.S1_at       | 0.653       | C3orf10     | Probable protein BRICK1 |
| Ssc.16057.2.S1_a_at     | 0.650       | GANC        | Calpain 3 (EC 3.4.22.-) (Calpain L3) |
| Ssc.2756.1.A1_at        | 0.650       | MRPL22      | Mitochondrial ribosomal protein L22 (Homo sapiens) |
| Ssc.26084.1.S1_at       | 0.649       | ATP2B1      | Plasma membrane calcium-transporting ATPase 1 |
| Ssc.8283.1.A1_at        | 0.649       | PTPN11      | Protein-tyrosine phosphatase, non-receptor type 11 |
| Ssc.26735.1.A1_at       | 0.649       | Q96BP3      | Peptidylprolyl isomerase domain and WD repeat containing 1 (Bos taurus) |
| Ssc.8430.1.A1_at        | 0.649       | Q86W74      | Ankyrin repeat domain 46 (ANKRD46) (Bos taurus) |
| Ssc.1441.1.S1_at        | 0.649       | DCTN3       | Dynactin 3 isoform 1; dynactin light chain (Homo sapiens) |
### Table 1. cont.

| Affymetrix probe set ID | Fold change | Gene symbol | Gene description |
|-------------------------|-------------|-------------|------------------|
| Ssc.10542.1.S1_at       | 0.647       | EXOSC1      | 3′→5′ ExoRNase CSL4 homologue |
| Ssc.772.1.S1_at         | 0.643       | CARHSP1     | Calcium-regulated heat-stable protein 1 |
| Ssc.3281.1.S1_at        | 0.643       | C11orf10    | UPF0197 protein C11orf10 (HSPC005) |
| Ssc.16495.1.A1_at       | 0.643       | DFSL        | Gasdermin domain containing protein 1 (*Homo sapiens*) |
| Ssc.4306.1.A1_at        | 0.642       | ME3DC1      | Mesoderm development candidate 1 |
| Ssc.26318.1.S1_at       | 0.638       | DNCL1       | Dynein light chain 1, cytoplasmic |
| Ssc.24811.1.A1_at       | 0.638       | Q6NSH4      | Nuclear receptor-binding protein 2 (NRBP2) (*Bos taurus*) |
| Ssc.1153.1.A1_at        | 0.638       | C9orf28     | C9orf28 protein |
| Ssc.22634.1.S1_at       | 0.637       | RB1CC1      | Rbl-inducible coiled-coil protein 1 (*Homo sapiens*) |
| Ssc.3154.1.S1_at        | 0.627       | GRM5        | Metabotropic glutamate receptor 5 precursor (mGlur5) |
| Ssc.6833.1.S1_at        | 0.625       | BTG1        | B-cell translocation protein 1 (*Homo sapiens*) |
| Ssc.1160.1.S1_at        | 0.624       | PSMC3       | 26S protease regulatory subunit 6A (TAT-binding protein 1) (TBP-1) (proteasome subunit P50) |
| Ssc.12944.1.A1_at       | 0.623       | RPA3        | Replication protein A 14 kDa subunit |
| Ssc.10037.1.A1_at       | 0.622       | NLK         | Serine/threonine kinase NLK |
| Ssc.22120.1.S1_a_at     | 0.617       | RYR2        | PREDICTED: similar to RIKEN cDNA 311009E18 (*Homo sapiens*) |
| Ssc.18253.1.S1_at       | 0.616       | F8          | Coagulation factor VIII precursor |
| Ssc.24739.1.A1_at       | 0.616       | SLC16A7     | Monocarboxylate transporter 2 (MCT 2) |
| Ssc.16936.2.S1_a_at     | 0.615       | Q9P0T8      | Similar to hypothetical protein HSPC111 (*Bos taurus*) |
| Ssc.16691.1.S1_at       | 0.609       | H2AF-J      | H2A histone family, member J isoform 1 (*Homo sapiens*) |
| Ssc.30182.1.A1_at       | 0.607       | RER1        | RER1 protein (*Homo sapiens*) |
| Ssc.21559.1.S1_at       | 0.606       | ANKRDI0     | Ankyrin repeat domain protein 10 |
| Ssc.11369.1.S0          | 0.605       | NP_077271   | Derlin-1 (Der1-like protein 1) |
| Ssc.1029.1.S1_at        | 0.603       | PHF6        | PHD finger protein 6 (PHD-like zinc finger protein) |
| Ssc.13954.1.A1_at       | 0.602       | QSVV17      | PREDICTED: similar to hypothetical protein DKFZp761A052 |
| Ssc.11878.1.S1_s        | 0.601       | HMBS        | Porphobilinogen deaminase |
| Ssc.16677.1.S1_a_at     | 0.599       | C17orf37    | Uncharacterized protein C17orf37 (protein C35) (HBV X-transactivated gene 4 protein) |
| Ssc.24943.1.S1_at       | 0.597       | NDUFA11     | NADH-ubiquinone oxidoreductase subunit B14.7 |
| Ssc.3426.1.A1_at        | 0.595       | MAPK6       | Mitogen-activated protein kinase 6 |
| Ssc.21783.1.S1_at       | 0.595       | MRPL2       | Mitochondrial ribosomal protein L2 (*Homo sapiens*) |
| Ssc.13370.1.A1_at       | 0.595       | Q8NA66      | RIKEN cDNA 1810054D07 gene (1810054D07Rik) (*Mus musculus*) |
| Ssc.1206.1.A1_at        | 0.595       | ADAMTS19    | ADAMTS-19 precursor |
| Ssc.6979.1.S1_at        | 0.587       | TPP2        | Tripeptidyl-peptidase II |
| Ssc.13218.1.A1_at       | 0.587       | NP_660155   | Testis development protein NYD-SP29 (*Homo sapiens*) |
| Ssc.19975.1.S1_s        | 0.582       | TEBP        | Telomerase-binding protein p23 (Hsp90 co-chaperone) (*Homo sapiens*) |
| Ssc.16392.2.A1_a_at     | 0.574       | MKNK2       | MAP kinase-interacting serine/threonine kinase 2 |
| Ssc.6230.2.A1_at        | 0.573       | SDCCAG3     | Serologically defined colon cancer antigen 3 (*Homo sapiens*) |
| Ssc.21987.1.A1_at       | 0.57       | IFRD1       | IFN-related developmental regulator 1 |
| Ssc.5163.1.S1_at        | 0.569       | GCNT2       | N-Acetyllactosaminide β-1,6-N-acetylgalcosaminyl-transferase |
| Ssc.6189.1.A1_at        | 0.565       | SLC7A11     | Cystine/glutamate transporter (amino acid transport system x_{c^-}) |
| Ssc.1333.1.A1_at        | 0.565       | ZCHC11      | Zinc finger, CCHC domain containing 11 isoform b |
| Ssc.29047.1.S1_at       | 0.561       | HIG2        | Hypoxia-inducible protein 2 (*Homo sapiens*) |
| Ssc.22287.1.S1_at       | 0.561       | GABR-a3     | γ-Aminobutyric-acid receptor α-3 subunit precursor [GABA(A) receptor] |
| Ssc.2354.1.S1_at        | 0.554       | GPR160      | Probable G protein-coupled receptor 160 |
| Ssc.4004.1.A1_at        | 0.552       | SYNE2       | Nesrin 2 (nuclear envelope spectrin repeat protein 2) |
| Ssc.13400.2.S1_at       | 0.550       | C3orf4      | Protein C3orf4 (membrane protein GENX-3745) |
| Ssc.26309.1.A1_at       | 0.548       | CHES1       | Checkpoint suppressor 1 (Forkhead box protein N3) |
| Ssc.6513.1.S1_at        | 0.542       | LRRC28      | Leucine-rich repeat-containing 28 (*Homo sapiens*) |
| Ssc.3451.1.S1_at        | 0.540       | SLC11A2     | Natural resistance-associated macrophage protein 2 (NRAMP2) |

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genes (IFIT1, GBP1, USP18 and cig5) that encode accessory proteins related to the immune response, and in particular to the pro-inflammatory cytokine IFN-β, but also genes with a known anti-apoptotic function (ADM and TNF-αIP3).

At 12 h p.i., the downregulated transcripts were also largely predominant over the upregulated ones (80 vs 20, respectively). The latter confirmed the main pattern of anti-apoptotic and antiviral response already observed at 9 h p.i., with the addition of two new transcripts representing TNF-α and IL-10. The overall highest fold change (FC) was observed for IFN-β (FC=20.15 at 12 h p.i.), whilst the most downregulated transcript was NP_060114 (FC=0.306 at 12 h p.i.). NP_060114 corresponds to the human DRE1 protein, a member of the kelch-repeat family, which modulates host immune response to viral infection (Prag & Adams, 2003). KHLX belongs to the same family and also showed a consistent downregulation at 12 h (FC=0.654).

The GO analysis assigned the 100 differentially expressed transcripts at 12 h p.i. to 34 biological processes, five molecular functions and three cellular components.

### Table 1. cont.

| Affymetrix probe set ID | Fold change | Gene symbol | Gene description |
|-------------------------|-------------|-------------|------------------|
| Ssc.4462.1.S1_at        | 0.536       | SDHC        | Succinate dehydrogenase cytochrome b560 subunit, mitochondrial precursor (integral membrane protein CII-3) |
| Ssc.14114.1.A1_at       | 0.525       | ABCD3       | ATP-binding cassette, subfamily D, member 3 (70 kDa peroxisomal membrane protein) (PMP70) |
| Ssc.16563.1.S1_at       | 0.513       | NP_067050   | DC2 protein (Homo sapiens) |
| Ssc.1527.1.A1_at        | 0.503       | SLC20A1     | Solute carrier family 20 (phosphate transporter), member 1; Glvr-1; gibbon ape leukemia virus receptor 1 (Homo sapiens) |
| Ssc.16475.1.S1_at       | 0.475       | COL4-α3     | Collagen α3(IV) chain precursor (Goodpasture antigen) |
| Ssc.17091.2.A1_s_at     | 0.463       | C3orf52     | TPA-induced transmembrane protein |
| Ssc.4472.1.A1_at        | 0.459       | NXT2        | NTF2-related export protein 2 (p15-2 protein) (DC9) (BM-025) |
| Ssc.15559.1.A1_s_at     | 0.306       | NP_060114   | DRE1 protein (Homo sapiens) |

**Fig. 2.** (a) Distribution of signal intensities of the 100 transcripts differentially expressed at 12 h p.i. over the period of infection. Left, control PAMs; right, infected PAMs. Each line represents a transcript. (b) Hierarchical clustering of the different time point conditions, based on the transcripts differentially expressed at 12 h p.i. in control (C) and infected (I) PAMs. Coloration in both figures refers to the condition of infected cells at 12 h p.i. and is directly proportional to the expression, ranging from red (high expression) to green (low expression).
et al. This confirms and reinforces previous knowledge that PRRSV does not induce a generalized suppression of host gene transcription. Real-time PCR confirmed that IFN-β was the most upregulated gene, whilst IFN-α was not differentially expressed between control and infected cells at 9 and 12 h p.i. Moreover, real-time PCR analysis of PAMs in an independent challenge experiment, with a different viral strain and lower m.o.i., confirmed that, at 24 h p.i., IFN-β was strongly induced whilst IFN-α was only slightly upregulated (data not shown). The downregulation of four genes encoding mitochondrial proteins (NP_060530, SDHC, MRPL2 and MRPL22) at different time points (6, 9 and/or 12 h p.i.) might add up to the emerging role of mitochondria in antiviral immunity. The mitochondrial antiviral signalling protein MAVS is critical for the IFN-β signalling pathway in response to dsRNA, and is required for both TLR3-mediated and TLR3-independent signalling pathways, such as that triggered by the RNA helicase RIGI (Moore et al., 2008; Xu et al., 2005; Yoneyama et al., 2004). RIGI is the product of DDX58, a member of the DEAD box family of RNA helicases that mediate nucleoside triphosphate-dependent unwinding of dsRNA and are involved in many diverse cellular functions (Lamm et al., 1996). Intriguingly, the only upregulated gene found by microarrays at 6 h p.i. in PAMs (DDX17) belongs to the same family.

The atypical pattern of expression of innate immunity genes indicates that PRRSV has probably developed sophisticated mechanisms to control the antiviral response. Indeed, only a subset (IFI174, GBP1, USP18 and TNF-xIP3) of genes commonly modulated by pathogens in response to dsRNA and/or stimulated by IFN (Jenner & Young, 2005) were found to be upregulated by PRRSV at 9 and/or 12 h p.i. When the 1.5-fold change threshold was not applied after ANOVA analysis, this subset also included CD44, PML, PRKRA, CCL4, CCL8 and MT2A. Upregulation of USP18 has been observed previously in PAMs following PRRSV infection (Zhang et al., 1999). The same study reported the upregulation of the antiviral gene MX1, but neither MX1 nor MX2 was found to be differentially expressed in the present investigation. Downregulation of NRAMP2 at 12 h p.i. was consistent with the effects observed previously in humans after human immunodeficiency virus infection (reviewed by Jenner & Young, 2005). Production of IFN-α and IFN-β is a well-known reaction of virus-infected cells; however, only the IFN-β gene was strongly upregulated by PRRSV in PAMs. The induction of IFN-β mRNA, but not IFN-α mRNA, has also been observed previously in human immunodeficiency virus infection (reviewed by Jenner & Young, 2005).
Table 2. GO analysis and ranking of the 100 transcripts differentially expressed at 12 h p.i.

Ranking and assignment is given for the differentially expressed transcripts at 12 h p.i. to the three GO categories: biological process, molecular function and cellular component. The number of transcripts for each process is shown, with the corresponding e-values.

| GO category                                      | Ranking | Number of transcripts | e-value       |
|--------------------------------------------------|---------|-----------------------|---------------|
| **Biological process**                           |         |                       |               |
| Response to stimulus                             | 1       | 22                    | $6.50 \times 10^{-4}$ |
| Response to stress                               | 2       | 14                    | $6.10 \times 10^{-3}$ |
| Immune response                                  | 3       | 11                    | $6.60 \times 10^{-3}$ |
| Physiological process                            | 4       | 68                    | $8.30 \times 10^{-3}$ |
| Defence response                                 | 5       | 11                    | $1.20 \times 10^{-2}$ |
| Response to biotic stimulus                      | 6       | 11                    | $1.60 \times 10^{-2}$ |
| Anion transport                                  | 7       | 5                     | $2.00 \times 10^{-2}$ |
| Regulation of apoptosis                          | 8       | 7                     | $2.30 \times 10^{-2}$ |
| Regulation of programmed cell death              | 9       | 7                     | $2.40 \times 10^{-2}$ |
| Meiosis                                           | 10      | 3                     | $3.00 \times 10^{-2}$ |
| M phase of meiotic cell cycle                    | 11      | 3                     | $3.00 \times 10^{-2}$ |
| Macromolecule metabolism                         | 12      | 34                    | $3.10 \times 10^{-2}$ |
| Meiotic cell cycle                               | 13      | 3                     | $3.10 \times 10^{-2}$ |
| Organismal physiological process                 | 14      | 16                    | $3.60 \times 10^{-2}$ |
| Anti-apoptosis                                   | 15      | 4                     | $4.30 \times 10^{-2}$ |
| M phase                                          | 16      | 5                     | $4.40 \times 10^{-2}$ |
| Inorganic anion transport                        | 17      | 4                     | $5.30 \times 10^{-2}$ |
| Response to virus                                | 18      | 3                     | $5.40 \times 10^{-2}$ |
| Negative regulation of apoptosis                 | 19      | 4                     | $6.00 \times 10^{-2}$ |
| Negative regulation of programmed cell death     | 20      | 4                     | $6.10 \times 10^{-2}$ |
| Apoptosis                                        | 21      | 8                     | $6.20 \times 10^{-2}$ |
| Programmed cell death                            | 22      | 8                     | $6.30 \times 10^{-2}$ |
| Activation of NF-κβ transcription factor         | 23      | 2                     | $6.40 \times 10^{-2}$ |
| Response to wounding                             | 24      | 6                     | $7.00 \times 10^{-2}$ |
| Cell death                                       | 25      | 8                     | $7.30 \times 10^{-2}$ |
| Death                                            | 26      | 8                     | $7.50 \times 10^{-2}$ |
| Protein metabolism                               | 27      | 25                    | $7.60 \times 10^{-2}$ |
| Negative regulation of cell proliferation        | 28      | 4                     | $7.80 \times 10^{-2}$ |
| Positive regulation of transcription factor activity | 29    | 2                     | $7.90 \times 10^{-2}$ |
| Leukocyte adhesion                               | 30      | 2                     | $7.90 \times 10^{-2}$ |
| B-cell proliferation                             | 31      | 2                     | $7.90 \times 10^{-2}$ |
| Negative regulation of cellular process          | 32      | 9                     | $8.30 \times 10^{-2}$ |
| Interaction between organisms                    | 33      | 3                     | $8.60 \times 10^{-2}$ |
| Ion transport                                    | 34      | 8                     | $9.20 \times 10^{-2}$ |
| **Molecular function**                           |         |                       |               |
| Antigen binding                                  | 1       | 3                     | $1.50 \times 10^{-2}$ |
| Receptor binding                                 | 2       | 8                     | $6.00 \times 10^{-2}$ |
| Phosphatase binding                              | 3       | 2                     | $8.70 \times 10^{-2}$ |
| Tumour necrosis factor receptor binding          | 4       | 2                     | $8.70 \times 10^{-2}$ |
| Cytokine activity                                | 5       | 4                     | $8.80 \times 10^{-2}$ |
| **Cellular component**                           |         |                       |               |
| Integral to membrane                             | 1       | 26                    | $6.90 \times 10^{-2}$ |
| Intrinsic to membrane                            | 2       | 26                    | $7.10 \times 10^{-2}$ |
| Extracellular space                              | 3       | 6                     | $8.80 \times 10^{-2}$ |
Table 3. Real-time PCR results of genes differentially expressed following PRRSV infection of PAMs

Real-time PCR results of ten selected genes in two pools of PAMs infected with PRRSV (I) compared with control PAMs (C). The reported values are the means ± SD of technical triplicates and were calculated as described in Methods; values that significantly differ between infected and control PAMs are indicated in bold (*, \(P<0.10\); **, \(P<0.05\)). The last column gives the Pearson's correlation coefficient (\(r\)) between real-time and microarray data.

| Gene symbol | Status | Pool I | Pool II | \(r\) |
|-------------|--------|--------|---------|------|
|             | 1 h    | 3 h    | 6 h     | 9 h  | 12 h |
| IFN-\(\beta\) |        |        |         |      |      |
| I           | 0.01 ± 0.005 | 0.04 ± 0.016* | 0.01 ± 0.006 | 0.13 ± 0.032** | 2.03 ± 0.402** |
| C           | 0.02 ± 0.008 | 0.01 ± 0.005* | 0.004 ± 0.002 | 0.01 ± 0.002** | 0.01 ± 0.006** |
| TNF-\(\alpha\) |        |        |         |      |      |
| I           | 4.02 ± 1.614 | 1.37 ± 0.467 | 0.3 ± 0.106 | 0.25 ± 0.072 | 1.16 ± 0.425 |
| C           | 7.52 ± 2.786 | 0.99 ± 0.28  | 0.32 ± 0.088 | 0.16 ± 0.05  | 2.09 ± 0.598 |
| TNF-\(\alpha\)IP3 |  |    |         |      |      |
| I           | 2.1 ± 0.854 | 0.59 ± 0.2   | 0.32 ± 0.039 | 0.75 ± 0.203** | 1.31 ± 0.4*  |
| C           | 1.76 ± 0.484 | 0.33 ± 0.061 | 0.22 ± 0.035 | 0.2 ± 0.026** | 0.42 ± 0.12*  |
| USP18       |        |        |         |      |      |
| I           | 0.25 ± 0.085 | 0.41 ± 0.125 | 0.78 ± 0.12  | 0.92 ± 0.166* | 1.61 ± 0.393** |
| C           | 0.27 ± 0.079 | 0.3 ± 0.036  | 0.74 ± 0.121 | 0.54 ± 0.038* | 0.53 ± 0.106** |
| cig5        |        |        |         |      |      |
| I           | 0.13 ± 0.019 | 0.48 ± 0.165 | 0.97 ± 0.183 | 0.91 ± 0.083* | 1.72 ± 0.102** |
| C           | 0.14 ± 0.019 | 0.50 ± 0.102 | 0.79 ± 0.151 | 0.58 ± 0.123* | 0.86 ± 0.258** |
| IL-10       |        |        |         |      |      |
| I           | 0.62 ± 0.228 | 0.52 ± 0.199 | 0.19 ± 0.094 | 0.49 ± 0.151 | 1.6 ± 0.415*  |
| C           | 0.57 ± 0.107 | 0.18 ± 0.087 | 0.18 ± 0.047 | 0.23 ± 0.04  | 0.47 ± 0.06*  |
| GRP58       |        |        |         |      |      |
| I           | 1.92 ± 0.103** | 0.99 ± 0.175* | 0.66 ± 0.144** | 1.82 ± 0.248 | 2.22 ± 0.307** |
| C           | 1.00 ± 0.046** | 1.61 ± 0.179* | 1.49 ± 0.268** | 1.19 ± 0.161 | 0.66 ± 0.043** |
| IFN-\(\gamma\) |        |        |         |      |      |
| I           | 0.07 ± 0.013 | 0.18 ± 0.028** | 0.03 ± 0.002 | 0.09 ± 0.012 | 0.13 ± 0.020 |
| C           | 0.08 ± 0.015 | 0.03 ± 0.002** | 0.03 ± 0.002 | 0.06 ± 0.013 | 0.08 ± 0.013 |
| IFN-\(\gamma\)R1 |        |        |         |      |      |
| I           | 0.93 ± 0.274 | 0.96 ± 0.239 | 0.91 ± 0.165 | 0.85 ± 0.187 | 0.87 ± 0.25  |
| C           | 1.11 ± 0.450 | 1.096 ± 0.265 | 1.12 ± 0.220 | 0.84 ± 0.202 | 1.02 ± 0.192 |
| Sialoadhesin |        |        |         |      |      |
| I           | 0.25 ± 0.016** | 0.17 ± 0.019 | 0.14 ± 0.009 | 0.19 ± 0.026 | 0.22 ± 0.034 |
| C           | 0.17 ± 0.033** | 0.16 ± 0.018 | 0.15 ± 0.005 | 0.23 ± 0.051 | 0.15 ± 0.024 |

*Correlation coefficient (\(r\)) between real-time and microarray data.
observed in monocyte-derived dendritic cells infected by PRRSV at 12 h p.i. (Loving et al., 2007). Previous studies, both in vitro and in vivo, have also shown that PRRSV is a poor inducer or even a suppressor of IFN-α compared with other respiratory viruses (Albina et al., 1998; Buddaert et al., 1998; Miller et al., 2004; van Reeth et al., 1999). Blocking IFN-α production clearly is beneficial for PRRSV replication, as IFN-α can efficiently block replication when present during infection (Delputte et al., 2007; Loving et al., 2007). IFN-β can also protect macrophages against PRRSV infection (Overend et al., 2007), but it has been suggested that IFN-β alone may be not sufficient to trigger the adaptive immune response (Loving et al., 2007). A recent report has shown that in vitro stimulation of monocytes and macrophages with IFN-α induces expression of sialoadhesin, the main PRRSV receptor in PAMs, and that treatment with IFN-α before inoculation strongly increases PRRSV infection of monocytes (Delputte et al., 2007). In agreement with this, in this study neither the gene encoding sialoadhesin nor that encoding IFN-αR1 (IFN receptor 1) showed consistent differential expression in infected cells.

Despite previous evidence that IFN-β expression by infected cells mediates and potentiates apoptosis (Tanaka et al., 1998), the present study showed a predominance of transcripts leading to prolonged cell survival within 12 h of infection (both upregulation of anti-apoptotic transcripts and downregulation of pro-apoptotic genes). Upregulation was observed for IL-10, ADM and TNF-zIP3. IL-10 has been demonstrated to protect cells against apoptosis (Sieg et al., 1996; Zhou et al., 2001). ADM has been shown (Kubo et al., 1998) to be overproduced by macrophages after inflammation and to modulate cytokine production (specifically TNF-α); several different independent studies support the fact that ADM is an anti-apoptotic peptide on different cell types (Bi et al., 2007; Uzan et al., 2006; Yin et al., 2004). TNF-zIP3 is a cytoplasmic zinc finger protein that inhibits NF-κB activity and TNF-mediated programmed cell death (Li et al., 2006; Qin et al., 2006). Downregulated genes included those encoding NLK, a stimulator of apoptosis (Yasuda et al., 2003), HIF1-α, which has been suggested to favour apoptosis in the absence of oxygen (Bruick, 2000), and GRM5, known to protect neurons from apoptotic death (Maiese et al., 2000).

Taken together, these findings suggest that PRRSV actively induces an anti-apoptotic state in order to complete its virus replication cycle. This is discordant with previous results showing that PRRSV induces infected cells, as well as uninfected bystander cells, to undergo apoptosis (for examples, see Chang et al., 2005; Sirinarumitr et al., 1998), but it should be noted that those data were obtained with in vitro infection treatments much longer than 12 h. On the other hand, the absence of apoptotic induction by PRRSV has been observed in MARC-145 cells (Miller & Fox, 2004) and HeLa cells (Lee et al., 2004). Interestingly, Kim et al. (2002) reported an atypical form of apoptosis that culminates in increased cell membrane permeability and late apoptosis after completion of virus replication.

The upregulation of IL-10 gene expression (FC=1.9) indicates that the IL-10-mediated downregulation of the T-helper cell type 1 (Th1) response may be an important mechanism operated by PRRSV, as well as by other viruses (for reviews, see Fickenscher et al., 2002; Redpath et al., 2001). Upregulation of IL-10 expression was found previously in PRRSV-infected porcine monocytes, macrophages and dendritic cells (Flores-Mendoza et al., 2008; Suradhat et al., 2003) and in vivo in PRRSV-infected pigs (Suradhat & Thanawongnuwech, 2003; Sutherland et al., 2007; Thanawongnuwech & Thacker, 2003; Thanawongnuwech et al., 2004). IL-10 in PRRSV-infected cells seems to be increased concurrent with the onset of viraemia and the development of clinical signs (Diaz et al., 2005). Also, PIK3R1 (upregulated in this study; FC=1.6), is known to positively regulate the production of IL-10 (Saegusa et al., 2007). These findings add to previous studies (Murtaugh et al., 2002; Wang et al., 2007), suggesting that PRRSV causes an imbalanced immune response characterized by an abundance of humoral immunity (Th2-mediated), which is less effective against viral pathogens.

The TNF-α gene was only slightly upregulated at 12 h p.i. (FC=1.5). The role of TNF-α in PRRSV infection is controversial: it has been reported that PRRSV is a potent inducer of TNF-α in PAMs at 18, 36, 54, 72, 90 and 108 h p.i. (Chang et al., 2005) and at 6 and 15 h p.i. (Thanawongnuwech et al., 2004). However, Charrentantakul et al. (2006) showed that, in porcine monocytes infected by PRRSV, IL-10 gene expression increased, and this response contributed to a reduction in TNF-α production. In fact, crucial anti-inflammatory activities of IL-10 may be due to its inhibitory effects on TNF-α production (Moore et al., 2001). Overall, this suggests that IL-10 may also participate in fine-tuning the production and effects of TNF-α.
Other differentially expressed genes (see Table 1) confirmed that a complex pattern of TNF-α regulation takes place upon PRRSV infection. TNF-αIP3, known to be induced by TNF-α (Dixit et al., 1990; Lee et al., 2000) and suggested to protect against the inflammatory response to influenza virus infection (Onose et al., 2006), was upregulated. STAG2, an enhancer of TNF production (Lara-Pezzi et al., 2004), and the member of the MAPK pathway, ATF2, a transcription activator of both IFN-β and TNF-α in response to virus infection (Biron & Sen, 2001; Tsai et al., 1996), were downregulated. The MAPK pathway was the most highly represented gene network identified in this study, with five differentially expressed genes at 12 h p.i. (ATF2, TNF-α, MAP3K8, MKNK2 and NLK). The MAPK pathway is one of the most important pathways for immune response to infection (Bruder & Kovesdi, 1997; Yang et al., 2007) and has been found to be modulated in PAMs after an antibody-mediated cross-linking treatment of sialoadhesin, the main PRRSV internalization receptor (Genini et al., 2008), although in this case different genes of the pathway were involved.

In conclusion, this work has provided a genome-wide gene expression catalogue of PRRSV pathogenesis and has allowed us to picture how different genes and gene pathways are co-modulated in the physiological context allowed us to picture how different genes and gene expression catalogue of PRRSV pathogenesis and has allowed us to picture how different genes and gene pathways were involved.

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