Online material

The moderately virulent CSFV strain induced mild and delayed modulations of PBMC gene transcription compared to the highly virulent CSFV strain

The PBMC collected before infection (D0) were used as reference samples for these one-class SAM analyses of microarray results. The expression of 650 genes was statistically modified over the 3 days post-infection by the highly virulent Eystrup strain, whereas the expression of only 250 genes was modulated by the moderately virulent Paderborn strain (Fig. S1, Tabs. SI and SII). Examination of the Eystrup results revealed that the expression of 151 genes was modified on D1 pi, most of which were up-regulated (114 genes up-regulated, 37 down-regulated). The maximum impact of infection on gene expression was apparent on D2 pi, with 398 genes showing altered expression but, in contrast to D1, most of the modulated genes were down-regulated (289 genes down-regulated and 109 genes up-regulated). On D3 pi, 160 of the 251 modulated genes were up-regulated and 91 were down-regulated. Examination of the Paderborn results revealed that only 46 genes were modulated on D1 (10 up-regulated and 36 down-regulated), whereas the number of variant genes increased on D2 to 119 (109 up-regulated genes versus 10 down-regulated genes). The highest response to Paderborn infection was obtained on D3 pi with 132 variant genes, most of which were down-regulated (92 down-regulated and 40 up-regulated) (Fig. S1).

The extent of host pathogen interactions, revealed by statistical analysis of the microarray results, appeared to differ considerably between these two strains. These discrepancies were reinforced when the gene expressions which were similarly altered in both strains was examined on a day to day basis. The results, (see Fig. S1) revealed few variant genes that were common to both the Paderborn and Eystrup data sets. No common genes were apparent in the host response to viral aggression on D1. On day 2, 10% of the genes modulated by Eystrup (37 up-regulated and only 3 down-regulated) were similarly modulated by Paderborn (these genes represented 34% of the Paderborn modulated genes on D2). On D3, the number of common genes increased to 22% of those modulated by Eystrup

(respectively 41% of Paderborn's) with 32 up-regulated and 22 down-regulated by both strains. One gene (CD69) behaved in the opposite way, being up-regulated by Eystrup but down-regulated by Paderborn. Examination, on a day to day basis, among all the genes which expression was altered, showed that only 70 variant genes were common to Eystrup and Paderborn infections, and 25 of these genes were similarly regulated on both D2 and D3 pi.

Whereas a decrease in the number of blood lymphocytes from pigs infected with Eystrup was observed as early as D1 pi, this lymphocyte depletion was apparent one day later (D2 pi) in pigs infected with Paderborn (Fig. 1A). These results and the apparent paucity of genes commonly affected by Eystrup and Paderborn infection led us to hypothesize that this low similarity of host response to the two CSFV strains might be due to unsynchronized responses to infection in Paderborn-infected pigs compared to Eystrup-infected pigs. We tested this hypothesis by looking for all genes regulated by both strains over the 3 days of infection and identified 47 additional genes, producing a total of 117 common genes. The expression of 80 of these genes was modulated earlier by Eystrup than by Paderborn (1 day earlier for 72 of them, 2 days earlier for 8 of them), 23 were regulated earlier by Paderborn and 14 were regulated at the same time. According to this microarray analysis, the host response towards these two closely related CSFV strains was firstly associated with a faster modification of host gene expression with the highly virulent strain, as illustrated in Fig 3, and secondly, with an apparently low similarity in host response toward the two infections. Only 117 (15%) of the total of 783 genes exhibiting altered expression were common to both CSFV strains and conversely 666 (85%) genes were different.

In PBMC, genes modulated by the Eystrup and the Paderborn strains were similarly distributed between the same biological functions

Having identified differences between the modulations of gene expression induced by the highly virulent and moderately virulent CSFV strains, we then investigated the biological function of these genes in the two closely related infectious processes by organizing the genes according to their ontology, using the Ingenuity data mining software (www.ingenuity.com). Although the one-class SAM analysis showed that the host responded very differently to the two infections, the variant genes from the Eystrup and Paderborn data were similarly distributed between the same biological functions. Only one function ("Protein Synthesis") modulated by Eystrup was not found modulated by Paderborn (Figs. S3A and S3B). The three main biological functions were "Cell Death", "Immune Response" and "Cellular Growth and Proliferation" which grouped 12%, 9% and 14% respectively of the genes in Eystrup-infected samples and 12%, 11% and 8% of the genes in Paderborn-infected samples (Figs. S3A and S3B). However, a few slight differences between CSFV strains were noted in the relative weights (expressed as % of annotated genes) of these biological functions. For example, the relative weight of "Cellular Growth and Proliferation" was almost two times greater for Eystrup than for Paderborn (14% against 8%). Similarly, the modulation of "Cell Cycle" was greater with Eystrup than with Paderborn (5% against 1%) (Figs. S3A and S3B). Also, although the same biological functions were highlighted by the distribution of the variant genes in the Eystrup and Paderborn samples (with very similar relative weights), the number of genes involved in these biological functions was very different. For example, 124 genes for “Cell death” were regulated by Eystrup compared with 63 for Paderborn, 89 genes for “Immune response” compared with 55 and 140 genes for “Cellular growth and proliferation” compared with 43 for Paderborn.

Figure S1. Venn diagrams showing the distribution of modulated genes during 3 days post-infection with either Eystrup or Paderborn strains. A one-class SAM analysis of microarray data was performed with 8 microarrays for each infection day and each CSFV strain, in relation to a non infected gene expression reference. Red areas indicate the up-regulated genes and green areas the down-regulated genes.

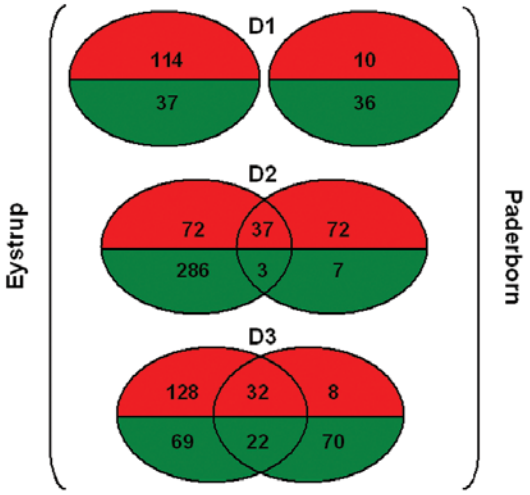

Figure S2. Clustering of the most variant genes in PBMC over the 3 days post-CSFV infection. A one-class SAM analysis of microarray data was performed with 8 microarrays for each infection day and each CSFV strain, in relation to a non infected gene expression reference. The SAM significant genes were then clustered using Cluster/TreeView programs. Up-regulated genes are illustrated in red and down-regulated genes in green. For each column the gene expression variations are the mean values of the data obtained for 8 pigs from either the Eystrup or Paderborn groups. Whereas numerous up- and down-regulated genes are clearly apparent as early as day 1 for the Eystrup samples (E) the D1 Paderborn sample (P) displays very few up- or down-regulated genes.

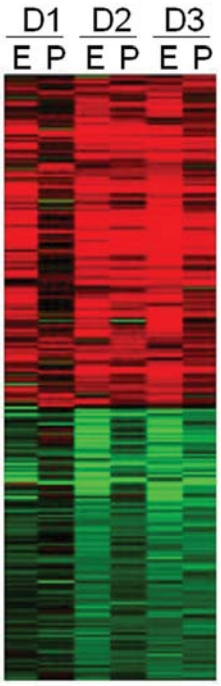

Figure S3. Distribution of the variant genes in biological function during CSFV infection. Genes obtained by the one-class SAM analysis over the 3 days post-infection with either Eystrup or Paderborn strains were organized according to their biological processes annotation with the Ingenuity Pathway Analysis software. A) modulated genes distribution during Eystrup CSFV infection, B) modulated genes distribution during Paderborn CSFV infection. The most important biological functions are shown. Percentages refer to the number of genes included in one function related to the remaining others.