Classical swine fever virus induces proinflammatory cytokines and tissue factor expression and inhibits apoptosis and interferon synthesis during the establishment of long-term infection of porcine vascular endothelial cells

Emmanuelle Bensaude, Jane L. E. Turner, Philip R. Wakeley, David A. Sweetman, Claire Pardieu, Trevor W. Drew, Thomas Wileman and Penelope P. Powell

Correspondence
Penelope P. Powell
penny.powell@bbsrc.ac.uk

1Department of Virology, Veterinary Laboratories Agency, Weybridge, Surrey KT15 3NB, UK
2Department of Immunology and Pathology, BBSRC Institute for Animal Health, Ash Road, Pirbright, Surrey GU24 0NF, UK

Infection with virulent strains of classical swine fever virus (CSFV) results in an acute haemorrhagic disease of pigs, characterized by disseminated intravascular coagulation, thrombocytopenia and immunosuppression, whereas for less virulent isolates infection can become chronic. In view of the haemorrhagic pathology of the disease, the effects of the virus on vascular endothelial cells was studied by using relative quantitative PCR and ELISA. Following infection, there was an initial and short-lived increase in the transcript levels of the proinflammatory cytokines interleukins 1, 6 and 8 at 3 h followed by a second more sustained increase 24 h post-infection. Transcription levels for the coagulation factor, tissue factor and vascular endothelial cell growth factor involved in endothelial cell permeability were also increased. Increases in these factors correlated with activation of the transcription factor NF-κB. Interestingly, the virus produced a chronic infection of endothelial cells and infected cells were unable to produce type I interferon. Infected cells were also protected from apoptosis induced by synthetic double-stranded RNA. These results demonstrate that, in common with the related pestivirus bovine viral diarrhoea virus, CSFV can actively block anti-viral and apoptotic responses and this may contribute to virus persistence. They also point to a central role for infection of vascular endothelial cells during the pathogenesis of the disease, where a proinflammatory and procoagulant endothelium induced by the virus may disrupt the haemostatic balance and lead to the coagulation and thrombosis seen in acute disease.

INTRODUCTION

The innate cellular response against virus infection includes production of inflammatory and antiviral cytokines, as well as induction of cell death through apoptosis. Many viruses have evolved mechanisms that inhibit inflammation and prevent apoptosis and, as a consequence, are able to establish chronic infections. Classical swine fever virus (CSFV) is a member of the Pestivirus genus in the Flaviviridae family and causes classical swine fever, an OIE List A disease of pigs. Strains of low to moderate virulence can persist in vivo, whereas strains of high virulence cause an acute disease with high mortality rates, characterized by haemorrhagic fever, thrombocytopenia and disseminated intravascular coagulation (Thiel et al., 1996; Moennig & Plagemann, 1992). Interestingly, CSFV replicates in macrophages and vascular endothelial cells in pigs (Trautwein, 1988). Vascular endothelial cells maintain the haemostatic balance by providing a quiescent, anti-thrombotic barrier. However, they are rapidly activated by pathogens to express a proinflammatory and procoagulant phenotype to eliminate infection (Bierhaus & Nawroth, 2003). If this is not controlled, factors produced by infected endothelial cells may disrupt the haemostatic balance and cause pathological damage. Indeed, endothelial cell involvement for several viral haemorrhagic diseases, such as African swine fever, Ebola and dengue virus infections, has been demonstrated (Vallee et al., 2001; Avirutnan et al., 1998; Yang et al., 1998). In each case, it includes the appearance of microthrombi, disseminated intravascular coagulation, lymphocytopenia and fibrinolysis (Summerfield et al., 2000; van Oirschot, 1988). The changes in haemostatic balance are thought to be caused by proinflammatory and antiviral factors, cell
adhesion molecules and blood coagulation factors induced in endothelial cells. A central regulator of the expression of these proinflammatory genes is the transcription factor NF-κB and many viruses manipulate the NF-κB pathway, resulting in suppression of antiviral responses or prevention of apoptosis (Hiscott et al., 2001; Tait et al., 2000).

This study has shown that CSFV can replicate in endothelial cells in vitro, where it activates NF-κB and increases expression of proinflammatory and procoagulant factors. If this were to occur in vivo, these changes in gene expression could contribute to the haemorrhagic pathogenesis of the disease. Interestingly, endothelial cells infected with CSFV did not die and a long-term virus infection was maintained in tissue culture. In addition, CSFV infection in cell culture did not result in type I interferon (IFN) production and suppressed both IFN-α production and apoptosis induced by double-stranded RNA (dsRNA). These findings suggest that CSFV persistence in vitro involves active inhibition of cellular responses to the viral replicative intermediate, dsRNA.

METHODS

Reagents. Virus antigen was stained using monoclonal antibody (mAb) WH303 against glycoprotein E2 (Edwards et al., 1988). Interleukin (IL)-6 was quantified in a Quantikine P porcine IL-6 ELISA (P6000; R&D Systems). IL-8 was quantified by ELISA using capture antibody anti-porcine IL-8 (MAB 535) and biotinylated detection antibody (BAF 535) from R&D Systems. IFN-α was quantified using capture antibody anti-porcine IFN-α K9 mAb (27100-1) and biotinylated detection antibody F17 mAb (27105-1) from R&D Systems. Polyinosinic-polyribocydyl acid sodium salt (pIpC) was from Sigma.

Virus and cells. The CSFV strain Alfort 187 (Ruggli et al., 1996) was kindly provided by the CSFV Community Reference Laboratory (Hanover, Germany). The virus was propagated in the pig kidney cell line PK15 (Paton et al., 1995). Porcine aortic endothelial cells (PAECs) were isolated from aorta (Vallee et al., 2001) and cultured in RPMI with 2 mM glutamine, 1 mM sodium pyruvate, 50 IU penicillin ml⁻¹ and 50 μg streptomycin ml⁻¹ plus 10 % foetal calf serum (FCS) demonstrated to be bovine viral diarrhoea virus (BVDV)- and antibody-free. The cells were maintained on gelatin-coated flasks.

Virus infection and titration. Before infection, PAECs were washed twice with PBS. At time 0, the viral inoculum (Alfort 187 at an m.o.i. of 2 TCID₅₀ per cell) or an equal amount of uninfected virus and cells. For control experiments, cells were treated either with PBS or with an inoculum from uninfected PK15 cells, prepared by an identical procedure to virus-infected cell inoculum (mock infection). At 1, 2, 3, 5, 8, 12, 18, 24, 30, 48 and 72 h p.i., cells were lysed in 1 ml TRizol (Invitrogen). Total RNA was extracted, resuspended in water and reverse transcribed using Moloney murine leukaemia virus reverse transcriptase (Promega) and random hexamer primers at 42 °C for 1 h, followed by 10 min at 94 °C. For amplification of cytokine DNA by PCR, the primers in Table 1 were used with Taq polymerase (Promega) and the products were separated by agarose gel electrophoresis and visualized with ethidium bromide staining. Relative quantitative PCR was carried out using Sybr Green reagent (Applied Biosystems). For all amplifications, the cycle conditions were 50 °C for 2 min and 95 °C for 10 min, followed by 30 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 1 min. For each sample, cDNA corresponding to the β-actin gene, as well as the gene of interest, was amplified. PCR reactions were set up in triplicate. The Ct values were used to compare each sample with time 0 from that experiment. Ct (cycle threshold) represents the PCR cycle at which an increase in reporter fluorescence above a baseline signal can first be detected. cDNA was quantified relative to β-actin as the endogenous control; the relative concentration of target gene mRNA was equal to 2⁻ΔΔCt. The primers listed in Table 1 were designed from porcine sequences in GenBank.

Quantification of cytokines by ELISA. PAECs were infected with Alfort 187 (m.o.i. of 2 TCID₅₀ per cell) as described above and overlaid with 2 ml culture medium with 10 % FCS. At time points 0, 30 and 72 h p.i., cell culture supernatants were harvested and stored at −70 °C. For IL-6 detection, the Quantikine kit was used according to the manufacturer’s instructions. For IL-8 and IFN-α detection, microtitre plates were coated overnight with capture antibodies diluted in PBS, then blocked for 1 h with 5 % BSA. Samples were incubated for 2 h, prior to detection with a biotinylated secondary antibody for 1 h, followed by peroxidase-conjugated Extravidin (Sigma) for 1 h. Peroxidase activity was measured using the substrate tetramethylbenzidine at A₄₅₀.

Quantification of dsRNA-induced apoptosis and IFN-α production. For apoptosis quantification, following 30 h of treatment with pIpC, cells were lysed and tested using a nucleosome ELISA kit

| Table 1. RQ PCR primer sequences |

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5'→3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-sel</td>
<td>ATGAGTGCCTCCATCGATTTTCTCT</td>
</tr>
<tr>
<td></td>
<td>TCAGATGTACAGTGTACATCTACAG</td>
</tr>
<tr>
<td>IFN-α</td>
<td>ATGGGCCCAACCTCACGCTCCT</td>
</tr>
<tr>
<td></td>
<td>TCACCTCTTCTTCTCCAGTCT</td>
</tr>
<tr>
<td>IFN-β</td>
<td>ATGGCTAACAGTGACATCCTCCAA</td>
</tr>
<tr>
<td></td>
<td>TCAGTTCGGAGTATCTGTAAG</td>
</tr>
<tr>
<td>IL-8</td>
<td>AGCCCCTGTCAACTCAGCTGTC</td>
</tr>
<tr>
<td></td>
<td>GAATTGTGGTGCTACCTTACTGAG</td>
</tr>
<tr>
<td>IL-β</td>
<td>ATTCCTCCTGGGGACGGACCTGTAAG</td>
</tr>
<tr>
<td></td>
<td>GCATCATCAGGATGCTAGCTGGT</td>
</tr>
<tr>
<td>IL-6</td>
<td>GCTGCTTGGTGTATGCGCTACGAG</td>
</tr>
<tr>
<td></td>
<td>TGAAACTCCACAAGGGACCGCTGTTGA</td>
</tr>
<tr>
<td>TF</td>
<td>TCCACTGAC(A/C)ATT(T/C)TGAGTGTTGG</td>
</tr>
<tr>
<td></td>
<td>AGGCGGAGTGTGTAAC</td>
</tr>
<tr>
<td>VEGF</td>
<td>ATGACACTTCTCGTCTGCTGGT</td>
</tr>
<tr>
<td></td>
<td>TCAGCCGCTTGCTGTGCTACATCT</td>
</tr>
<tr>
<td>β-Actin</td>
<td>GAGAAGCTGCTGACTCGGC</td>
</tr>
<tr>
<td></td>
<td>CCAGACAGACGACTGTGGGT</td>
</tr>
</tbody>
</table>
This technique allows the quantification of apoptotic cells \textit{in vitro} by nucleosome-free DNA affinity-mediated capture, followed by an anti-histone detection step. The test was performed according to the manufacturer’s instructions. Apoptotic indices were calculated as ratios of the $A_{450-595}$ for treated and untreated cells. For IFN-$
abla$ quantification, PK15 cells were incubated with complete medium containing GeneJuice transfection reagent (Novagen) and pIpC. After 8 h, cell culture supernatants were stored at $-70^\circ C$ before IFN-$
abla$ concentration was measured by ELISA.

\textbf{Quantification of NF-\kappa B p65 DNA-binding activity.} Following infection with Alfort 187 (m.o.i. of 2) or treatment with TNF-$
abla$, the level of p65 activity in PAECs was measured using the NF-\kappa B TransAM kit (Active Motif) according to the manufacturer’s instructions. Briefly, cells were lysed at 4 $^\circ C$ and protein concentrations were measured using the Bradford assay (Bio-Rad). Lysates (20 µg total proteins) were incubated in ELISA wells coated with the oligonucleotide motif recognized by active p65. p65 was then detected using a specific antibody, followed by a secondary antibody conjugated to peroxidase. The colorimetric reaction was measured at $A_{450}$, with 620 nm acting as the reference wavelength. The experiment was repeated three times and a representative figure is shown in the results.

\section*{RESULTS}

\subsection*{CSFV infection of PAECs \textit{in vitro}}

Since \textit{in vivo} studies have shown that vascular endothelial cells are host cells for CSFV (Trautwein, 1988), this study used primary vascular endothelial cell cultures to study phenotypic changes following infection. To characterize the nature of the infection, PAECs were infected with CSFV Alfort 187, fixed after 24 h and immunostained with an antibody against viral glycoprotein E2. E2 was localized in the cytoplasm and on the cell surface of over 90% of the cells (Fig. 1A and FACs analysis data not shown). As in continuous cell cultures (e.g. PK15 cells), CSFV Alfort 187 produced no cytopathic effect in these primary cells. CSFV-infected PAECs were maintained in culture for 4 weeks and the cells were passaged on the days indicated in Fig. 1(B). Cell-culture supernatants were sampled throughout the time course and cumulative totals of virus secreted into the medium between passages were determined. Virus production fell slightly over the first 10 days p.i. and then maintained a steady titre over the next 3 weeks. Virus secretion increased after passage, suggesting that there was an increased rate of virus replication in proliferating cells. Cell number was compared between infected and uninfected cells. Cell number did not alter between the two over a 2 week period, indicating that infection did not alter the kinetics of cell proliferation. Infection with an m.o.i. of 2 led to 100% of the cells being infected, as detected by E2 staining at 24 h p.i. The percentage of cells infected remained at 100% over the time course of the experiment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{CSFV infection of PAECs. (A) PAECs were infected with CSFV Alfort 187 and fixed at 24 h p.i. Virus was stained with an anti-E2 mAb (WH303) and visualized by immunohistochemistry. (B) Infected PAECs were maintained in culture for 4 weeks and passaged on days 3, 6, 10, 16 and 23 p.i. (dashed lines). Aliquots of cell culture supernatant were harvested throughout the experiment and infectious virus was titrated.}
\end{figure}
Activation of vascular endothelial cells following infection with CSFV

Expression of mRNA for the proinflammatory cytokines IL-1α, IL-6 and IL-8 was studied by isolation of total cellular RNA, reverse transcription and relative quantitative PCR (Fig. 2A). The fold increase in proinflammatory mRNA was relative to the zero time point and normalized against β-actin mRNA levels. Mock-infected PAECs, treated with either uninfected PK15 cell lysates or with PBS, showed no increase in cytokine gene expression throughout the 72 h time course. Cells infected with CSFV showed a biphasic increase in mRNA for IL-1α, IL-6 and IL-8. An initial rapid increase was detected between 1 and 3 h p.i., with a maximum at 2 h p.i. (when the virus inoculum was removed). This increase was short lived, with mRNA levels returning to normal by 4 h p.i. The decline suggested that there was a rapid removal or inhibition of the signal causing the increase in cytokine transcription and also that the cytokine transcripts were degraded very rapidly after synthesis. In addition, uninfected cell lysates did not cause the same increase in cytokine synthesis as infected cell lysates, indicating that endothelial activation was due to the virus in the preparation. The second peak in cytokine transcription occurred between 48 and 72 h p.i. (Fig. 2A). For IL-6, the second peak showed a 72-fold increase, for IL-8 a 282-fold increase and for IL-1α a 265-fold increase. Cytokines secreted from infected cells into the media were measured by ELISA (Fig. 2B). Both IL-6 and IL-8 increased in the culture supernatant, accumulating over 72 h p.i. to a concentration of 300 pg ml⁻¹ and 30 ng ml⁻¹, respectively. Protein synthesis and secretion of these factors, therefore, mirrored the increase in mRNA levels induced by the virus. A capture ELISA to measure porcine IL-1α secreted from cells was unable to detect any increase in this cytokine in the supernatants of infected endothelial cells (data not shown). IL-1α has no signal sequence and is not normally secreted from cells except after death (Dinarello, 1998). However, it can act as a potent intracellular autocrine factor, either by directly binding to the nucleus or by binding to an intracellular pool of IL-1 receptors. The increased IL-1α mRNA in infected cells may correlate with increased intracellular protein. The expression of several endothelial cell-specific genes that might play a role in the

![Fig. 2. CSFV infection increases IL-1α, IL-8 and IL-6 mRNA levels and IL-6 and IL-8 protein secretion. PAECs were infected with CSFV Alfort 187 (open circles) or mock-infected (filled circles). (A) Relative quantitative PCR was used to quantitate mRNA levels over 72 h p.i. Error bars show the maximal and minimal fold increase in mRNA (experiments carried out in triplicate). (B) IL-6 and IL-8 secretion into the medium of PAECs over 72 h p.i. was measured by ELISA.](image-url)
pathogenesis of the disease was investigated. Tissue factor (TF) is an initiator of the blood coagulation cascade and vascular endothelial cell growth factor (VEGF) controls vascular permeability, while E-selectin is a cell-surface adhesion molecule that recruits leukocytes to the site of infection. Expression of mRNA for these genes was also studied by relative quantitative PCR (Fig. 3). In control uninfected cells, the levels of all three mRNAs were low. In contrast, after CSFV infection, the mRNA levels of all three genes showed a similar biphasic increase to that seen for IL synthesis. An initial rapid and short-lived increase was followed by a more sustained increase, beginning 24 h p.i. The increase for each gene was lower than for the pro-inflammatory cytokines, possibly reflecting their function as membrane-bound regulatory molecules rather than signalling molecules. E-selectin mRNA, for example, had increased only 10-fold, VEGF mRNA 12-fold and TF mRNA 40-fold by 48 h p.i.

The rapid activation of endothelial cells, characterized by the production of proinflammatory cytokines and cellular adhesion molecules, is controlled by the transcription factor NF-κB (Ghosh et al., 1998). To investigate whether these changes in gene expression following CSFV infection were associated with activation of NF-κB, an assay was carried out to measure the activity of the 65 kDa subunit of NF-κB. Total cell lysates of infected cells were added to ELISA plates coated with an oligonucleotide sequence recognized by NF-κB p65. After washing, bound p65 protein was detected using an anti-p65 antibody (Fig. 4). NF-κB activity increased early in infection, maximally 1 h after addition of virus, and had fallen by 5 h p.i. A second increase in NF-κB activity was seen at 18 h p.i., which was sustained for up to 72 h p.i. These times of increase in NF-κB activity coincided with the times post-infection when IL secretion and E-selectin, TF and VEGF mRNA levels were greatest.

**PAECs infected with CSFV do not produce IFN**

PAECs were either mock-infected or infected with CSFV at an m.o.i. of 2. Expression of type I IFN genes (IFN-α and -β) was studied by relative quantitative PCR. There was no significant difference between the levels of mRNA in infected and mock-infected cells (Fig. 5A). This finding was supported by ELISA data showing no detectable IFN-α in culture supernatants over 72 h p.i. (Fig. 5B). A positive control, indicating that endothelial cells produce abundant IFN-α and -β mRNA when stimulated with IFN-α, is shown in Fig. 5(C). RNA samples were treated with RNase-free DNase to ensure that samples were not contaminated with genomic DNA. In a further experiment, PK15 cells were treated with the synthetic dsRNA pIpC, a potent inducer of IFN-α. Secretion of IFN-α into the supernatant of these cells was measured by ELISA (Fig. 5D). Addition of pIpC to cells in the presence of the transfection reagent GeneJuice further stimulated the secretion of IFN-α to over 1000 ng ml⁻¹. Interestingly, cells chronically infected with CSFV did not produce detectable amounts of IFN-α after pIpC treatment, even in the presence of transfection reagent (Fig. 5D).

**CSFV inhibits dsRNA-induced apoptosis of infected PAECs**

As shown above, endothelial cells became chronically infected with CSFV after 24–72 h p.i. There was an activation of the inflammatory pathway and an inhibition of IFN mRNA and protein synthesis after treatment with dsRNA. Therefore, experiments were performed to investigate whether CSFV could also block dsRNA-induced apoptosis. Cell death was induced in control uninfected PAECs using the synthetic dsRNA pIpC (Fig. 6A). The apoptotic index increased to 9·6 with increasing concentrations of pIpC ranging from 25 to 500 μg ml⁻¹. In contrast, when pIpC was added to CSFV-infected cells, cell death was inhibited, even at the highest concentration of pIpC tested (500 μg ml⁻¹).
In addition, viral proteins were shown to be necessary for the protection against apoptosis (Fig. 6B). PAECs were infected with CSFV for various times from 0 to 24 h and then treated with pIpC. CSFV did not protect the cells from apoptosis when added at the same time as pIpC, but the apoptotic indices decreased with increasing times post-infection, demonstrating a relationship between virus replication and protection from pIpC-induced apoptosis.

**DISCUSSION**

In this study, we have shown that CSFV activates endothelial cell proinflammatory responses but at the same time suppresses IFN production and apoptotic pathways. Eventually, CSFV establishes a long-term infection of endothelial cells with virus replication sustained over 4 weeks. Activation of endothelial cells was indicated by increased IL-1α, IL-6 and IL-8 mRNA levels. IL-1 showed the highest initial mRNA peak and may have played a role in the secondary induction of the other endothelial cell cytokines, IL-6, IL-8 and VEGF. We were unable to show the secretion of IL-1α protein from infected endothelial cells by ELISA. This cytokine has no signal sequence and in many cases remains cell-associated until cells are lysed after death. Increased intracellular IL-1 is a potent autocrine activator of several endothelial cell genes involved in vascular function. In contrast to our finding that there is no IL-1 secreted from infected endothelial cells, another study has shown that IL-1 is released from CSFV-infected macrophages (Knoetig et al., 1999). This led to secretion of prostaglandins, also important mediators of vascular tone and function, and suggests that there may be a significant paracrine activation of vascular cells by infected macrophages in vivo. IL secretion is also necessary to stimulate immune cell responses. IL-8 is, for example, an important chemoattractant for immune cells, while IL-1 and IL-6 prime B- and T-cell responses against infected cells. The immediate activation of the proinflammatory response seen at 2 h p.i. was transient and probably due to virus binding, but both were virus-specific, since the initial increase and the secondary increase after 24 h p.i. were not seen with uninfected cell lysates. We showed increased binding of NF-κB, indicating transcriptional activation of these genes. Cytokine mRNAs have a short half-life after synthesis and the rapid reduction in mRNA levels a few hours after addition of virus suggests rapid intracellular cytokine mRNA degradation. Virus replication and protein synthesis has been shown to commence 12–18 h p.i. (Mittelholzer et al., 2000), suggesting that the second increase in cytokine mRNA levels after 24 h p.i. was due to the presence of replicating virus. The second increase in cytokine synthesis at 18–72 h p.i. was more sustained and occurred together with an increase in the synthesis of TF, VEGF and E-selectin mRNA, molecules involved in vascular permeability and coagulation. Both initial and secondary peaks correlated with an activation of the 65 kDa/RelA subunit of NF-κB. However, p65 activation was highest following initial binding at 2 h p.i., while cytokine synthesis shows a more sustained increase 24 h p.i. NF-κB is an important modulator of the apoptotic pathway, controlling the synthesis of several anti-apoptotic genes such as bcl2 and bcl-xl (Karin & Lin, 2002) and these proteins may play a protective role against apoptosis following CSFV infection.
The early phenotypic changes described here suggest that endothelial cells may have a direct involvement in the pathogenesis of classical swine fever in vivo. Secretion of proinflammatory cytokines, growth factors and reactive oxygen species from infected endothelial cells and other factors, such as platelet activating factor from infected macrophages, disrupts the haemostatic balance, leading to thrombocytopenia and platelet aggregation followed by haemorrhagic fever (Knoetig et al., 1999; van Oirschot, 1988). In addition, CSFV infection causes immunosuppression and lymphocyte depletion, due to bystander apoptosis observed in non-infected cells, particularly B and T lymphocytes (Summerfield et al., 1998). The production of inflammatory cytokines by infected endothelial cells could play a role in this immunosuppression, as well as facilitating virus dissemination by attracting monocytic cells. This study was carried out using the virulent Alfort 187 strain and it will be important in future studies to compare these
changes with those induced by an attenuated virus, such as the C strain, which does not cause haemorrhagic fever.

In common with the related pestivirus BVDV (Schweizer & Peterhans, 2001), throughout CSFV infection of cells in vitro there was no up-regulation of IFN synthesis in response to virus or to synthetic dsRNA. Cells were also protected from dsRNA-induced apoptosis and this protection appeared to be dependent on viral protein synthesis. An interesting recent study (Ruggli et al., 2003) has shown that the resistance of CSFV-infected cells to dsRNA-induced apoptosis and IFN production can be abrogated by the deletion of the N-terminal protease, Npro. This indicates a novel function for this protease in inhibition of the cellular innate immune system, but its role remains to be established. Recent work has implicated IFNs as important regulators of viral-induced apoptosis (Tanaka et al., 1998). IFN synthesis is controlled through the combined action of the transcription factor NF-κB and IFN regulatory factors (IRFs), which together form part of the enhancer complex on the IFN-β promoter. Interestingly, and pertinent to this study, BVDV has been shown to inhibit IFN-β transcription by preventing IRF3 binding to DNA (Baigent et al., 2002). IRF3 is constitutively present in the cytoplasm of all cells and activated by phosphorylation to translocate to the nucleus and mediate gene expression (Schafer et al., 1998). IRF3 has been shown to be involved in regulating cellular apoptosis for several virus infections, such as Sendai virus (Heylbroeck et al., 2000). The importance of IRF3 in innate immune defences is illustrated by the number of virally encoded proteins that have been found specifically to target this transcription factor. These include the influenza virus NS1 protein (Talon et al., 2000) and the vaccinia virus protein E3L (Smith et al., 2001). Significantly, there is also a viral IRF3 encoded by human herpesvirus 8 (Lubyova & Pitha, 2000). Because of its similarity to BVDV, it is likely that CSFV also targets IRF3.

Commitment to apoptosis is thought to depend on IFN production (Tanaka et al., 1998) and viruses must evade this response before establishing persistence. However, it is often difficult to link this to virus pathogenicity. For BVDV, it has been shown that, whereas non-cytopathic biotypes fail to produce IFN in vitro (Schweizer & Peterhans, 2001), strong IFN responses were induced in calves in vivo (Charleston et al., 2002). However in the foetus, IFN was not produced by non-cytopathic BVDV and the virus persisted (Charleston et al., 2001). Transmission to the foetus is a very important step in viral evasion for both BVDV and CSFV, leading to persistently infected neonatal animals (Thiel et al., 1996). Elucidation of the viral mechanisms of activation and inhibition of signal transduction pathways in vitro will provide strategies to control the persistence of these diseases in vivo.

ACKNOWLEDGEMENTS

The work was funded by UK Defra grant SE0764. Our thanks go to M. Bailey for discussions and to G. Ibata and C. Watson, VLA Weybridge, for cell culture and virus production and J. Seekings and S. Eagle for technical assistance.

REFERENCES


