Caspase-mediated cleavage of the feline calicivirus capsid protein

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Feline calicivirus (FCV) is responsible for an acute upper respiratory tract disease in cats. The FCV capsid protein is synthesized as a precursor (76 kDa) that is post-translationally processed into the mature 62 kDa capsid protein by removal of the N-terminal 124 amino acids. Our previous studies have also detected a 40 kDa protein, related to the FCV capsid protein, produced during infection. Here we demonstrate that cleavage of the FCV capsid protein, during infection of cells in culture, was prevented by caspase inhibitors. In addition, caspase-2, −3 and −7 were activated during FCV infection, as shown by pro-form processing, an increase in N-acetyl-Asp-Glu-Val-Asp-7-amido-4-trifluoromethylcoumarin cleavage activity and in situ poly(ADP-ribose) polymerase cleavage. Caspase activation coincided with the induction of apoptosis and capsid cleavage to the 40 kDa fragment. An in vitro cleavage assay, using recombinant human caspases and in vitro-derived FCV capsid protein, revealed that caspase-2, and to a lesser extent caspase-6, cleaved the capsid protein to generate a 40 kDa fragment. Taken together, these results suggest that FCV triggers apoptosis within infected cells and that caspase-induced capsid cleavage occurs concomitantly with apoptosis. The possible role of capsid cleavage in the pathogenesis of FCV infection is discussed.

INTRODUCTION

The family Caliciviridae includes several significant pathogens of man and animals. Feline calicivirus (FCV) is a major cause of upper respiratory tract disease in cats. The genome is a single-stranded positive sense RNA of about 7.5 kb (Carter et al., 1992a). The genome is polyadenylated and covalently linked to a 15 kDa viral protein, termed VPg, at the 5′ end and contains three open reading frames (ORF). ORF1 encodes the non-structural proteins, ORF2 encodes the capsid protein and ORF3 a small highly basic structural protein (reviewed in Clarke & Lambden, 1997). ORF2 and ORF3 are expressed from a single subgenomic mRNA (Herbert et al., 1996). Calicivirus particles contain a single capsid protein (58–76 kDa). The capsid of members of the vesivirus genus, such as FCV and San Miguel sea lion virus (SMSV), is formed initially as a larger precursor which is cleaved by the viral protease into the mature capsid protein (62 kDa in the case of FCV; Neill, 1992; Neill et al., 1991; Carter et al., 1992b; Sosnovstev et al., 1998). The mature FCV protein is created by removal of the N-terminal 124 amino acids from the precursor (Carter, 1989). The mature protein is incorporated into virions.

A smaller form of the capsid protein (approx. 40 kDa by SDS-PAGE; called p40) has been observed during FCV infection in cell culture at late times post-infection (Carter et al., 1989). Similar truncated forms of the capsid protein have been reported in the late stages of rabbit haemorrhagic disease virus (RHDV) infection (reviewed in Carter & Cubitt, 1998). In addition, cleaved soluble forms of the Norwalk virus (NLV) capsid protein have been associated with enteric infection in man (Hardy et al., 1995). This cleaved capsid protein was also present within infected stools. However, it is not known if these cleaved forms are generated by similar mechanisms or if they have any role in infection.

Apoptosis is a process of cell death used by organisms to eliminate superfluous, cancerous or virus-infected cells (Arends & Wyllie, 1991; Kaufmann & Hengartner, 2001; Zimmerman et al., 2001). Viruses have been found to either inhibit or promote this process in host cells, or even to do both at different stages in their replicative cycles (reviewed in Everett & McFadden, 1999; Roulston et al., 1999; Alcamì, 2001). Many viruses block the apoptotic response to ensure efficient virus production but some induce apoptosis, resulting in virus dissemination and protection from an immune response. Apoptosis progresses through a series of morphological and biochemical changes: for example, chromatin condensation,
nuclear disruption, DNA fragmentation, plasma membrane blebbing and cell shrinkage. Most, if not all, of these changes are effected by members of a family of cysteine proteases called caspases. The caspase gene family contains 14 mammalian members, of which 11 human enzymes have been identified. All caspases are expressed as pro-enzymes that contain an N-terminal pro-domain, a large subunit and a C-terminal small subunit (Stennicke & Salvesen, 1998). Phylogenetic and functional analysis has shown that the caspase gene family can be divided into two subfamilies that are related to either interleukin-1β-converting enzyme and play a role in inflammation, or to the mammalian counterparts of Ced-3 and are involved in apoptosis. Some of the latter caspases (e.g. caspase-8, -9 and -10) are characterized by a long pro-domain and are called initiator caspases because their pro-domains contain interaction motifs that allow these caspases to form dynamic complexes with other proteins and transduce various apoptotic signals into protease activity. This in turn initiates a caspase cascade that culminates in the activation of downstream effector or executioner caspases that are characterized by short prodomains. The activated effector caspases-3, -6 and -7 cleave a number of target proteins, and this is responsible for the ultimate destruction of the cell (Budihardjo et al., 1999; Hengartner, 2000; Adrain & Martin, 2001; Bruton & Cohen, 2001).

To date there have been few reports on the molecular effects of calcicviruses on cells. Apoptosis has been observed in rabbit liver following infection with RHDV (Alonso et al., 1998) and in San Miguel sea lion virus infection (J. D. Neill, personal communication). We set out to study the mechanism of FCV capsid cleavage in tissue culture and were led to look at potential apoptotic changes in FCV-infected cells. We report here that, during FCV infection, the activation of caspases is responsible for proteolysis of the viral capsid protein as well as cellular apoptosis. In addition, caspase-2 and -6 were shown to cleave the FCV capsid protein to a p40 product in vitro.

**METHODS**

**Viruses and cells.** Crandell-Rees feline kidney (CRFK) cells were grown in modified Eagle’s medium (MEM; Gibco-BRL) supplemented with 10% foetal bovine serum, non-essential amino acids (1%), penicillin (100 U ml⁻¹) and streptomycin (100 μg ml⁻¹) at 37°C and 5% CO₂. FCV F9 strain was propagated in confluent monolayers.

**Morphological analysis of apoptosis.** Confluent monolayers of CRFK cells were grown on microscope slides and infected (or mock-infected) with FCV F9 at an m.o.i. of 100 p.f.u. per cell. At various times post-infection (p.i.), cells were fixed in PBS plus 4% (v/v) formaldehyde, washed and stained with Hoechst 33358 (Calbiochem) in PBS (3 μg ml⁻¹). Cells were visualized using a Zeiss Axiovert 135 fluorescence microscope under UV light.

**Western blot analysis of cellular and viral proteins.** For analysis of apoptotic proteins in FCV-infected cells, CRFK monolayers (in 35 mm dishes) were infected or mock-infected with FCV F9 as described above. At various times p.i., cells were harvested in 400 μl buffer C (120 mM NaCl, 50 mM Tris pH 8-0, 0-5% NP40). Supernatants were assayed for protein concentration (Bio-Rad DC protein assay) and equal amounts of protein (30 μg) subjected to SDS-PAGE (10% gels for caspase analysis and 7% for poly(ADP-ribose) polymerase (PARP) and FCV capsid protein analysis) and immunoblotting. Blots were probed with anti-caspase-2 (1:2000, SantaCruz, N19), anti-caspase-3 (1:5000; Calbiochem) and anti-caspase-7 (1:2000; gift from G. M. Cohen, MRC Toxicology Unit, Leicester, UK) antisera followed by peroxidase-labelled donkey anti-rabbit IgG (1:2000, Amersham), or with anti-PARP monoclonal antibody (clone C2-10; 1:10000; Alexis) or anti-FCV capsid antibody (1G9; 1:2000; Carter, 1989) followed by rabbit anti-mouse IgG (1:3000; Amersham). Detection onto X-ray film was achieved using chemiluminescence reagents (Pierce).

For the analysis of PARP cleavage, cell extracts were resuspended in reducing loading buffer (62.5 mM Tris pH 6.8, 6 M urea, 10% glycerol, 2% SDS, 0.003% bromophenol blue and 5% 2-mercaptoethanol) and sonicated on ice for 20 s prior to separation by SDS-PAGE in 7% gels (as described in Jones et al., 1999). The proteins were transferred to Immobilon membranes and detection was performed as described above.

**Effect of caspase inhibitors on FCV capsid cleavage.** For investigation of the role of caspases in FCV capsid protein cleavage, infections were carried out in the absence or presence of the caspase inhibitors Z-Val-Ala-dt-Asp-fluoromethyl ketone (Z-VAD-FMK; Bachem; 100 μM) or Acetyl-Asp-Glu-Val-Asp-aldehyde (Ac-DEVD-CHO; Bachem; 100 μM) or the corresponding solvent (dimethyl sulfoxide). Lysates were subjected to SDS-PAGE (7%) and immunoblotting for FCV capsid protein as described above.

**Detection of caspase activity.** Mock-infected or FCV-infected CRFK cells were harvested by scraping, washed in buffer A (40 mM β-glycerophosphate, 50 mM NaCl, 2 mM MgCl₂, 5 mM EGTA, 10 mM HEPES, pH 7-0), freeze–thawed three times and centrifuged (13000 g for 30 min). The supernatants were assayed for DEVDase activity in the presence of 40 μM Ac-DEVD-AFC using a fluorescence microtitre plate reader as previously reported (Jones et al., 1999).

**In vitro FCV capsid cleavage assays.** The entire FCV F9 capsid gene has been cloned and expressed previously (Carter et al., 1992b). For this study, an initiation codon was inserted immediately adjacent to the cleavage site to permit the expression of the mature protein (62 kDa) without the requirement for proteolytic cleavage. This section was amplified by PCR using the primers For 5'-CGAATTCGAGTCATGGCGGAT and Rev 5'-TTATGAATCTAATTTAGGCC. EcoRI sites were added to enable insertion into EcoRI-digested pGEM-4Z (Promega) for use in vitro coupled transcription and translation (TNT-T7Promega) reactions. Plasmids were sequenced to check for correct orientation and to verify the capsid coding sequence. [³⁵S]Methionine-labelled FCV capsid protein was produced using the TNT T7 Quick kit (Promega) using 1 μg plasmid, as per the manufacturer’s instructions. Samples were cooled to stop the reaction and recombinant human capsase-1, -2, -3, -6, -7, -8, -9 and -10 (Calbiochem) were added to initiate FCV capsid proteolysis. The final reaction mixture volume was 15 μl and contained 12.5 μl of TNT sample and 12.5 μl of recombinant capsase in 10 mM DTT, 0-1% CHAPS and 5% sucrose. Following incubation for 1, 2-5 and 5 h at 37°C, 5 μl samples were removed from the reaction, mixed with SDS-PAGE loading buffer and were analysed by SDS-PAGE (10%) and autoradiography.
RESULTS

Caspase inhibitors prevent cleavage of the FCV capsid during infection of CRFK cells

In agreement with a previous report (Carter, 1989), the production of the mature capsid protein during FCV infection of CRFK cells was followed by the appearance of an approximately 40 kDa fragment (p40) by 6–8 h p.i., as detected by Western blot analysis. Modification of other viral capsid proteins has been reported, for example the influenza virus nucleocapsid protein is cleaved during apoptosis, as is the capsid protein of transmissible gastroenteritis virus (TGEV) (Zhirnov et al., 1999; Eleouet et al., 2000). As these capsids were cleaved by caspases, we studied the possible involvement of this family of proteases in the FCV capsid cleavage event. In the first approach, CRFK cells were treated with the pan-caspase inhibitor Z-VAD-FMK prior to, and during, infection with FCV. In the absence of caspase inhibitors, cleavage of the 62 kDa protein into a p40 product was observed at about 8 h p.i. (Fig. 1, panel a). However, addition of the caspase inhibitor Z-VAD-FMK completely prevented this cleavage. Likewise, addition of the caspase-3 specific inhibitor Ac-DEVD-CHO also prevented the capsid cleavage (Fig. 1, panel b). These results suggested that caspases were involved in the FCV capsid cleavage event.

Caspase activation in FCV-infected cells

The involvement of caspases in FCV capsid cleavage implicates an activation of caspases during FCV infection. Therefore, we examined the activation of a number of caspases by Western blot analysis. Activation of caspase-3 in FCV-infected cells was observed from about 4 h p.i., as shown by the appearance of the p20 fragment, and by 8 h p.i. of p17, corresponding to the large subunit of the pro-caspase (Fig. 2). In parallel, a decrease in the levels of the pro-form (p32) was observed (Fig. 2). We also observed activation of caspase-7, with the pro-form starting to disappear between 4 and 6 h p.i., although the fragment (p19) representing the processed large subunit was never detected using the available antisera (Fig. 2). In the case of caspase-2, the pro-form was processed from a 48 kDa protein into a 33 kDa fragment that represents an intermediate cleavage product, most likely consisting of the large subunit plus pro-domain (Li et al., 1997; Fig. 2). The activation of caspase-3/-7 was further confirmed by an increase in caspase-3-like activity (DEVDase activity) that became detectable by 6 h p.i. (Fig. 3) and paralleled the pro-caspase processing described above.

The activation of caspases during FCV infection was

Fig. 1. Caspase inhibitors prevent cleavage of the FCV capsid protein. FCV infections were performed in the absence or presence of the caspase inhibitor Z-VAD-FMK (100 μM; panel a) or Ac-DEVD-CHO (100 μM; panel b). Cytoplasmic extracts were made at the times indicated p.i. and subjected to SDS-PAGE (7%) and Western blotting. Blots were probed with monoclonal antibody 1G9 as described in methods. Detection onto X-ray film was achieved using chemiluminescence. The capsid cleavage product (p40) is indicated by the arrow.

Fig. 2. Caspase activation in FCV-infected CRFK cells. CRFK cells were infected with FCV for the times indicated or treated with 1 μM staurosporine (St) for 20 h or mock-infected (C). Cell extracts were subjected to SDS-PAGE (10%) and Western blotting as described in methods. Caspase-3: the 32 kDa pro-form (p32) was processed to yield p20/p17 fragments that correspond to the (active) large subunit. Caspase-7: the 36 kDa pro-form and the inactive p29 intermediate are shown to be processed during FCV infection. The feline caspase-7 large fragment (p19) was not detected by the antiserum as shown by its absence following induction of apoptosis with staurosporine (data not shown). Caspase-2: the 48 kDa pro-form was processed to a p33 intermediate form that is indicative of activation.
furthermore confirmed by investigating the in situ cleavage of PARP, a protein normally involved in DNA repair (Lindahl et al., 1995). During apoptosis PARP is cleaved from a 116 kDa protein into an inactive 85 kDa form (Kaufmann, 1989; Lazebnik et al., 1994). Cleavage of PARP was detected in FCV-infected cells from about 4 h p.i. (Fig. 4) as shown by the appearance of a band corresponding to the 85 kDa form.

Another established target for caspases is ICAD/DFF45 (Liu et al., 1997; Enari et al., 1998) which becomes inactivated through proteolytic cleavage, enabling the DNase CAD/DFF40 to cleave the cellular DNA into oligonucleosomal length fragments. Cleavage of the cellular DNA during FCV infection of CRFK cells was detected between 4–8 h p.i. (data not shown) and this correlated well with the onset of chromatin condensation observed by microscopy (see below). However, the DNA fragments could not be resolved to give the appearance of a sharp ladder in apoptotic CRFK cells. This was observed even in CRFK cells induced to undergo apoptosis with a well characterized agent such as staurosporine (1 μM). We therefore assume that this is an effect of the cell type used in this study.

In vitro cleavage of the FCV capsid protein by recombinant human caspases

In an attempt to identify the caspase(s) responsible for FCV capsid cleavage, we studied the ability of recombinant

**Fig. 3.** Increase in caspase-3-like activity during FCV infection. CRFK cells were infected with FCV F9 and at the indicated time-points the cells were harvested. Cellular extracts were prepared and assayed for Ac-DEVD-AFC cleavage activity as described in methods.

**Fig. 4.** FCV infection leads to PARP cleavage. CRFK cells were infected with FCV F9 for the times indicated. Cell extracts were subjected to SDS-PAGE (7 %) and Western blotting for the immunodetection of PARP as described in methods. Cleavage from the 116 kDa native protein to the 85 kDa fragment is shown.

**Fig. 5.** Induction of apoptosis during FCV infection. CRFK cells were mock-infected (panel a) or infected with FCV F9 (panel b) for 9 h, fixed with 4 % formaldehyde and stained with Hoechst 33358. The nuclei were visualized by fluorescence microscopy and photographed. The induction of apoptosis is shown by the appearance of highly condensed chromatin and fragmented nuclei.

**FCV triggers chromatin condensation in infected CRFK cells**

The activation of caspases during FCV infection of CRFK cells suggested that other apoptotic changes might occur during infection. Indeed, CRFK cells infected with FCV F9 showed additional signs of apoptosis beginning at 6 h p.i. Apoptotic cells exhibit characteristic morphology including cell and nuclear shrinkage and condensation of the chromatin. Nuclear integrity can be visualized by staining cells with Hoechst 33358, which penetrates nuclei and binds to DNA. Typical condensation of chromatin was clearly evident in most FCV-infected cells by about 9 h p.i. (Fig. 5, panel b) and was similar to the changes induced by the apoptosis-inducing agent staurosporine (1 μM for 12 h; data not shown).

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**Fig. 5.** Induction of apoptosis during FCV infection. CRFK cells were mock-infected (panel a) or infected with FCV F9 (panel b) for 9 h, fixed with 4 % formaldehyde and stained with Hoechst 33358. The nuclei were visualized by fluorescence microscopy and photographed. The induction of apoptosis is shown by the appearance of highly condensed chromatin and fragmented nuclei.
human caspases to cleave the capsid protein in an *in vitro* assay. \[^{35}S\]Methionine-labelled capsid protein was synthesized in TnT reactions and incubated with a panel of recombinant caspases at 37 °C. Samples were removed at various time points and were subjected to SDS-PAGE and autoradiography. Cleavage of the 62 kDa form and formation of p40 was detected only with caspase-2 and to a lesser extent with caspase-6 (Fig. 6). None of the other caspases tested here cleaved the capsid protein under the conditions used in this study. Of note is the lack of accumulation of p40 despite ongoing cleavage of the native protein at 5 h. This suggests that p40 may be further cleaved. No distinct bands of lower molecular mass were detected (data not shown) suggesting that several additional caspase-2 cleavage sites exist in p40.

**DISCUSSION**

It is widely accepted that viruses can inhibit or activate the apoptotic process. Induction of apoptosis may serve as a mechanism for virus dissemination or may be a host defence mechanism. Conversely, viruses may delay the host responses until replication is complete. The molecular mechanisms responsible for cellular damage and disease induced by caliciviruses have not been widely studied. However, the induction of apoptosis in RHDV infection is thought to play a key role in the pathogenesis of disease (Alonso *et al.*, 1998). Here we demonstrate that FCV also induces apoptosis in cultured cells and propose that apoptosis may be a common feature of calicivirus infections.

FCV infection was shown to induce typical features of apoptosis in CRFK cells within 6–8 h p.i., with nearly all cells having undergone apoptosis by 12 h. This was shown by the activation of caspases, cleavage of PARP, chromatin condensation and DNA fragmentation. A novel target for caspases identified here was the viral capsid protein. A truncated form of the FCV capsid protein corresponding to p40 has been observed previously in infected cells (Carter *et al.*, 1989); however, in this early study the protease responsible for capsid cleavage remained unknown. Here, we found that the time-course of capsid cleavage occurred in parallel with the onset of apoptosis, and two lines of evidence suggest a direct role of caspases in the cleavage process. Firstly, caspase inhibitors such as Z-VAD-FMK and Ac-DEVD-CHO prevented this capsid cleavage. Secondly, we report here that recombinant caspases directly cleaved the capsid protein to generate p40 *in vitro*. This cleavage was specific for caspase-2 and caspase-6, as other caspases were unable to cleave the capsid protein under the conditions tested here.

In contrast to most effector and initiator caspases which have been widely studied, our knowledge of the specific roles played by caspase-2 and caspase-6 is much less extensive. Caspase-2, initially described as Nedd-2/Ich-1 (Wang *et al.*, 1994; Kumar *et al.*, 1994), has the unique feature of being a long prodomain caspase with effector caspase substrate specificity, and hence may act both as an initiator and effector caspase. Despite the evidence for participation in some apoptotic pathways, revealed by the phenotype of caspase-2 null mice (Bergeron *et al.*, 1998), little is known about its activation process and its downstream target polypeptides. Caspase-2 has been suggested to be involved in the apoptosis of neuronal and other cell types in response to a range of different apoptotic stimuli that induce cytotoxic stress (Troy *et al.*, 2000; Lassus *et al.*, 2002; O’Reilly *et al.*, 2002; Robertson *et al.*, 2002). However, an emerging feature is the role of caspase-2 in the cellular response to viral and bacterial infections. He *et al.* (2001) recently showed that apoptosis induction following infection by the paramyxovirus simian virus 5 requires caspase-2. Likewise, caspase-2 needs to be expressed for apoptosis to occur in response to rabies virus infection (Ubol & Kasisith, 2000). Invasive *Salmonella typhimurium* was reported to activate caspase-2 in macrophages and the ensuing apoptotic response was also partially dependent on caspase-2 (Jesenberger *et al.*, 2000). Besides caspase-2 autocatalysis (Butt *et al.*, 1998), the only other cellular polypeptide substrates for caspase-2 thus far known are bid (Guo *et al.*, 2002) and golgin-160 (Mancini *et al.*, 2000). In this study, we found not only that

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**Fig. 6.** *In vitro* cleavage of the FCV capsid protein by recombinant caspases. Mature \[^{35}S\]labelled FCV capsid (62 kDa) was produced in a TnT reaction. Equal amounts of capsid protein were incubated at 37°C in the absence (–) or presence of recombinant caspase-1, -2, -3, -6, -7, -8, -9 or -10 as described in methods. At the indicated time-points, samples were removed, subjected to SDS-PAGE and analysed by autoradiography. The cleavage product (p40) is indicated with an arrow.
FCV infection resulted in the activation of caspase-2 but also that caspase-2 was by far the most active at cleaving FCV capsid protein to generate the p40 fragment, and thereby identified FCV capsid as a novel protein substrate for caspase-2.

Caspase-6 plays a major role in nuclear condensation and apoptotic body formation as a result of its ability to cleave nuclear lamin A (Lazebnik et al., 1995; Takahashi et al., 1996; Ruchaud et al., 2002). During apoptosis, lamins A and C are exclusively degraded by caspase-6 (Orth et al., 1996; Takahashi et al., 1996), and although other polypeptide substrates for caspase-6 have been reported, such as cytokeratin-18 (Caulin et al., 1997), focal adhesion kinase (Gervais et al., 1998), NuMA (Hirata et al., 1998), topoisomerase I (Samejima et al., 1999) and vimentin (Byun et al., 2001), these are also cleaved by other caspases, especially caspase-3. Here, we have identified another polypeptide, the FCV capsid, that is cleaved by caspase-6. However, unlike lamins A and C, its cleavage is shared with caspase-2. In both cases the same fragment (p40) appears to have been generated; however, whether both caspases target the same cleavage site needs further investigation. Recently, it was reported that the viral capsid protein of TGEV is cleaved by caspase-6 and caspase-7 during apoptosis following infection of HRT18 cells (Eleouet et al., 2000). We were unable to demonstrate caspase-6 activation in FCV-infected cells because none of the commercially available antibodies tested in this study recognized the Feline form. However, its activation in FCV infection is most likely given that caspase-3 was activated and that caspase-3 is known to process caspase-6 in turn (Srinivasula et al., 1996; Slee et al., 2001).

The monoclonal antibody used in this study to detect p40 has been previously mapped to recognize an epitope consisting of Gly303-Glu-Leu-Ile-Pro-Ala-Gly309 within the FCV capsid protein (Carter, 1989). Another monoclonal antibody directed towards the C-terminal end of the protein and recognizing Pro443-Ile-Phe-Tyr-Lys447 does not react with p40. Consequently, the caspase cleavage site must be located between Asp320 and Asp424. This portion of the FCV capsid protein sequence contains 11 theoretical caspase cleavage sites, including Asp-Thr-Ala-Asp331, Asp-Arg-Ile-Thr-Ala-Asp331-1 Ile. Cleavage at this site would produce a fragment of the size of p40. However, in the absence of any knowledge about possible additional cleavage at the N terminus of the capsid protein required for the generation of p40, further work will be necessary to identify the exact site(s) cleaved by caspase-2 and caspase-6.

The impact of cleavage of the FCV capsid on virus replication and cell survival are presently unclear. Cleavage of viral structural proteins by caspases has been reported for other viruses, namely TGEV and influenza virus. In the case of the coronavirus TGEV the capsid protein is cleaved by caspase-6 and -7 (Eleouet et al., 2000). The authors suggest that this cleavage may affect particle formation, although others have shown that the event does not significantly affect virus yields (Garwes et al., 1984). Similarly, the nucleocapsid protein of influenza virus is also targeted by caspases (Zhirnov et al., 1999) and the authors believe that this may affect virus assembly. It is not known whether the cleaved capsid interferes with assembly and therefore represents a cellular defence mechanism, or if the cleaved product has a role in pathogenesis. For example, cleaved molecules presumably would not be assembled and may actually interfere with the assembly process, or at least lead to reduced yields of virus from infected cells. Conversely, cleaved capsid proteins may elicit non-neutralizing antibodies during infection and so may in fact be beneficial to pathogenesis of the virus (as suggested by Hardy et al., 1995). Of potential importance is the recent finding that the human adenovirus capsid protein, although not cytoxic to the cell by itself, significantly enhances apoptosis induced by the chemotherapeutic drug cisplatin (Duverger et al., 2002). This apoptosis-modulatory role of the viral capsid may have critical implications on the development of the immune response by the host and hence, the pathogenesis of disease (Sasaki et al., 2002).

In conclusion, we report here that FCV infection triggered an apoptotic response in the host cell that was mediated by caspases. Caspases were implicated in the cleavage of the viral capsid protein in infected cells, and studies in vitro with recombinant capsids identified caspase-2 and to a lesser extent, caspase-6 as being able to cleave the FCV capsid protein.

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