Sensitization to Fas-Mediated Apoptosis by Hepatitis C Virus Core Protein

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Received October 29, 1996; returned to author for revision November 17, 1996; accepted December 17, 1996

We have characterized viral–cell interactions of hepatitis C virus (HCV) and liver cells to study the pathogenesis of HCV infection. HepG2 cells constitutively expressing HCV core protein showed apoptotic changes in response to stimulation with anti-Fas monoclonal antibody. Cells treated with the antibody showed extensive cell rounding, shrinkage, and cytoplasmic blebbing and finally detached from plates. Fragmentation of the chromatin was observed in the nucleus and DNA ladders were detected. In contrast, cells expressing HCV envelope, nonstructural proteins or normal HepG2 cells did not exhibit such Fas-mediated apoptosis. However, expression of Fas receptor was not upregulated on the surface of the cells expressing HCV core protein. Apoptotic cell death was prevented by pretreatment with a specific inhibitor of the cysteine protease CPP32, while the specific inhibitor of interleukin-1β-converting enzyme did not show the preventive effect. The results suggest (i) that intracellular expression of HCV core protein makes cells prone to apoptotic death without upregulation of surface Fas expression and (ii) that the CPP32 protease plays a part in the apoptosis effector pathway of HCV core-expressing cells. HCV core protein may have a role in immune-mediated liver cell injury.

INTRODUCTION

Hepatitis C virus (HCV) is a positive strand RNA virus, belonging to the Flaviviridae family. It is a major causative agent of parenterally transmitted non-A non-B hepatitis (Choo et al., 1989). A main characteristic feature of HCV infection is its persistent nature and the high frequency of chronicity. Chronic hepatitis may evolve into liver cirrhosis and also into hepatocellular carcinoma (Saito et al., 1990).

Although immunologically mediated and virus-induced mechanisms have been proposed, the pathogenesis of HCV-associated liver damage is not well understood yet. The lack of an efficient replication system for HCV in cell culture has hampered the determination of whether HCV proteins have any direct roles in the immune-mediated liver cell damage and in the viral persistence.

Nucleotide sequences of the gene coding for HCV core protein are well conserved among strains obtained from various parts of the world (Bukh et al., 1994). Antibodies to core protein are most frequently detected in patients and carriers infected with HCV (Chiba et al., 1991). Furthermore, data have been accumulating to suggest that in addition to functioning in the formation of nucleocapsids the HCV core protein has a regulatory function for other gene expression (Kim et al., 1994; Shih et al., 1993; Ray et al., 1995). It is also recently reported that HCV core protein may transform rat embryo fibroblasts in cooperation with ras oncogene (Ray et al., 1996).

Apoptotic cell death plays an important role in the cytopathogenicity induced by several viruses (Hinshaw et al., 1994; Kawanishi, 1993; Laurent-Crawford et al., 1993; Ubol et al., 1996). For example, Jurissen et al. (1992) have shown that the virus-induced apoptosis is responsible for depletion of thymocytes in young chickens infected with chicken anemia virus. In acute human immunodeficiency virus (HIV) infection, low-molecular-weight DNA accumulates in the nucleoplasm of infected cells, indicating that cytopathic effects of HIV are associated with apoptosis (Laurent-Crawford et al., 1991).

In the case of hepatitis, however, involvement of apoptosis in its pathogenicity is not clear. So far only histochemical examinations of biopsy samples from patients with viral hepatitis have been reported to show apoptosis of hepatocytes (Kerr et al., 1979). Fas-mediated apoptosis of hepatocytes has been recently suggested by detecting Fas antigen in the liver tissue with infiltrating mononuclear cells in patients with hepatitis C (Hiramatsu et al., 1994). It is thus important to examine whether apoptotic pathways account for the liver cell damage in acute and chronic hepatitis.

We have established various liver cell lines constitutively expressing HCV proteins to study viral–cell interactions in HCV-infected cells (Harada et al., 1995a,b). When tested in HepG2 cells, the expression of HCV core protein appeared to downregulate the cell growth (Harada and Matsuura, unpublished observations). The HepG2 cells constitutively expressing HCV core protein showed extensive cytoplasmic vacuoles containing apolipopro-

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tein (Barba et al., in press). Hepatitis is considered to be the sum total of phenomena of viral–cell interactions in liver cells, and the above data are suggestive of HCV core protein’s role in the apoptotic pathway.

To investigate the effects of intracellular HCV core protein in relation to Fas-mediated apoptosis, we studied the susceptibility of the HepG2 cell line constitutively expressing HCV core protein to anti-Fas antibody.

MATERIALS AND METHODS

Cells and plasmids

To establish HepG2, a human hepatoblastoma cell line, constitutively expressing HCV core protein, cells were transfected with the plasmid pcEF39neo. The plasmid carries the cDNA coding for HCV core protein (amino acid position 1–194) (Takeuchi et al., 1994a; Uetsuki et al., 1994a) under the control of the EF1-α promoter (Uetsuki et al., 1994b). Cells were also transfected with the expression vector pcEF32-1swxneo as a negative control (Kim et al., 1994; Harada et al., 1995b). HepG2 cells constitutively expressing HCV E1–E2 proteins or nonstructural proteins were obtained by transfecting cells with the plasmids pcEF827 and pcEF3269 carrying the HCV coding regions corresponding to amino acid positions 155–810 (E1–E2) and 974–2212 (from the C-terminal of NS2 to the N-terminal half of NS5A), respectively (Harada et al., unpublished, 1995b).

HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM);F12 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 IU/ml of penicillin, and 100 mg/ml of streptomycin and plated on collagen-coated dishes. Cells were transfected by calcium phosphate precipitation method (Pharmacia Biotech, Tokyo, Japan), as previously described (Harada et al., 1995b). After transfection transformants were selected in DM EM-F12 medium containing 1 μg/ml of G418 (Gibco BRL, Gaithersburg, MD). The isolated G418-resistant colonies were designated Hep39 (expressing core protein), Hepswx (transfected with expression vector), Hep827 (expressing E1 and E2 proteins), and Hep3269 (expressing nonstructural proteins; Harada et al., 1995b).

Reagents

A murine anti-human Fas monoclonal antibody, CH-11 clone (anti-Fas mAb) (Yonehara et al., 1989), was purchased from MBL, Nagoya, Japan. The tetrapeptides Ac-YVAD-cho (specific inhibitor of interleukin-1/β-converting enzyme) and Ac-DEVD-cho (specific inhibitor of CPP32/apopain) were purchased from Peptide Institute, Inc., Osaka, Japan.

Immunoblotting analysis

Expression of core protein was detected by Western blotting. Cell lysates from 10^6 cells were separated by 12.5% SDS–PAGE and electrotransferred to polyvinylidene difluoride membrane (Immobilon, Millipore Ltd., Japan). After blocking in nonfat milk solution (Block Ace, Yukijirushi Co., Sapporo, Japan), the membrane was probed with HCV anti-core monoclonal antibody (Suzuki et al., 1995) as primary antibody and incubated for 1 hr at 37°. After being washed, the membrane was further incubated with horseradish peroxidase-conjugated sheep anti-mouse immunoglobulins as secondary antibody. Antigen–antibody complexes were visualized by enhanced chemiluminescence detection system (ECL, Amersham International, Buckinghamshire, England) according to the manufacturer’s protocol.

Indirect immunofluorescence analysis

After being washed with phosphate-buffered saline (PBS), cells were fixed with methanol–acetic acid (3:1 v/v) for 10 min at −20°. Fixed cells were incubated with mouse monoclonal antibodies raised against HCV core E1 and E2 proteins diluted to 1:100 in PBS for 60 min at 20°. The secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (TAGO, Burlingame, CA) diluted to 1:100 in PBS. Cells were incubated for a further 30 min before observation.

Fluorescent ELISA immunoassay

HCV core protein expressed in each cell clone was quantitated by fluorescent ELISA immunoassay (FEIA) (Tanaka et al., 1995). For FEIA 10^5 cells were lysed in TNE buffer [10 mM Tris–HCl (pH 7.8), 150 mM NaCl, 1 mM EDTA, 1% NP-40] and then processed according to the manufacturer’s instruction (International Reagents Co., Kobe, Japan). The relative fluorescence intensity was determined by ELSIA-F3000 reader.

Anti-Fas antibody and proteases inhibitors treatment

To test the cytoidal effect of anti-Fas, each established cell line was treated for 24 hr with 0.25 μg/ml of anti-human Fas mAb in culture medium containing 2% FCS. Three clones of Hep39 cells and one respective clone of Hep827 and Hep3269 cells were tested. Hepswx and untransfected HepG2 cells were also tested as negative controls.

To examine the effect of the apoptosis inhibitors, Hep39 cell lines were pretreated for 2 hr with 100 μM either Ac-YVAD-cho or Ac-DEVDD-cho. Cells were further incubated at 37° with anti-Fas mAb (0.25 μg/ml) for 24 hr in the presence of each inhibitor.

Nuclear staining

For detection of changes in nuclear chromatin, cells were fixed in methanol–acetic acid (3:1 v/v) for 10 min at −20°. The cell nuclei were stained with 4′,6′-diamidino-2-phenylindole (DAPI) (0.1 μg/ml in PBS) for 5 min in the dark. After washing in PBS, nuclear samples were
mounted in Mowiol (Calbiochem-Novabiochem Co., La Jolla, CA) and visualized under a fluorescence microscope with a uv filter.

DNA fragmentation assay

For DNA fragmentation analysis, adherent and floating cells were harvested and washed in PBS. Total DNA was extracted by incubating cells in 10 mM Tris - HCl (pH 8.0) containing 10 mM EDTA and 0.5% Triton X-100, for 5 min on ice. After centrifugation at 12,000 rpm for 15 min at 4°C, the supernatants were incubated with RNase A (5 μg/ml, Sigma, Miyata Kagaku, Tokyo, Japan) overnight at 37°C, followed by 2 hr incubation at 50°C with proteinase K (200 μg/ml, Sigma) and 0.5% SDS. After extraction with phenol/chloroform, fragmented DNA was precipitated with ethanol and 5 M NaCl and analyzed by 1.5% agarose gel in TBE buffer. DNA fragmentation was analyzed for three clones of both Hep39 and Hepswx cell lines and for HepG2 cells under identical conditions, after 24 hr incubation with anti-Fas antibody.

Analysis of apoptosis by flow cytometry

For flow cytometry analysis of cells undergoing apoptotic death after treatment with anti-Fas antibody, adherent and floating cells were pooled and washed with cold citrate buffer (250 mM sucrose, 40 mM trisodium citrate, 20% DMSO). Cell pellets were incubated for 10 min at room temperature (RT) with 0.03% trypsin solution containing 3.4 mM trisodium citrate, 1.5 mM spermene, 0.5 mM Tris, and 0.01% NP-40 (pH 7.6). The samples were further incubated for 10 min after addition of trypsin inhibitor (0.5 mg/ml, Sigma) and RNase A (0.1 mg/ml, Sigma). Cells were then stained with propidium iodide (0.4 mg/ml) for 10 min at RT. The samples were analyzed by a FACScan flow cytometer (Becton-Dickinson, USA). Apoptotic cells were analyzed by Lysis II program and the data from 10,000 cells for each sample are shown in tridimensional view.

Analysis of Fas expression on cell surface

The expression of Fas antigen on HepG2, Hepswx, and Hep39 cells was analyzed by flow cytometry using the mouse anti-human Fas mAb (CH-11) as primary antibody. Adherent cells were detached and washed with DMEM-F12 medium containing 2% FCS. Aliquots of 10^6 cells were incubated with 20 μg/ml of CH-11 mAb for 30 min on ice. After washing, cells were incubated with FITC-conjugated goat anti-mouse immunoglobulins (Biosource International, Inc., Tago Products, USA) as secondary antibody for further 30 min on ice. After final washing cells were analyzed by a FACScan flow cytometer (Becton-Dickinson).

RESULTS

Expression of HCV proteins in stable transfectants

Expression of HCV core protein in stable transfectants was examined by immunoblot analysis. Figure 1A shows the expression of 22-kDa HCV core protein detected in three different clones of established Hep39 cells. The molecular weight of the detected protein is in agreement with that reported for HCV capsid protein in mammalian and insect cells (Harada et al., 1991; Suzuki et al., 1995). HCV core protein was localized in the cytoplasm of all Hep39 clones examined, as determined by immunofluorescence (Fig. 1B, a). We further quantitated HCV core protein expressed in each cell line by FEIA (Tanaka et al., 1995). Expression levels of HCV core protein in cell clones were almost the same (Fig. 1A). Expression levels of other HCV proteins in other stable transformants were also examined by immunofluorescence analysis. Although the staining was not as bright as that of core protein in Hep39 cells, both E1 and E2 proteins were detected in almost all Hep827 cells (Fig. 1B, b and c). Immunofluorescence analysis of nonstructural proteins in Hep3269 was reported previously (Harada et al., 1995b).

Sensitivity to Fas-mediated apoptosis of Hep39

To examine the susceptibility of HepG2 cells expressing HCV core protein to Fas-mediated apoptosis, cells were treated with anti-Fas mAb, as described under Materials and Methods. Apoptotic cell death was evaluated on the basis of morphological and nuclear changes of cells, nuclear DNA fragmentation, and flow cytometry analysis. We found that interaction of Fas with anti-Fas antibody induced marked apoptosis in HCV core-expressing HepG2 cell lines, while it failed to induce apoptotic cell death in control Hepswx and HepG2 cells. From 5 to 8 hr after treatment with anti-Fas antibody, cells expressing core protein started to round up and lose contacts with neighboring cells. After 24 hr cells displayed severe shrinkage together with cytoplasmic blebbing. Most of the apoptotic cells were detached from the plate and were floating in the medium (Fig. 2). We also stimulated HepG2 transformant cell lines with various monoclonal antibodies to examine their interactions. We used antibodies to MHC class I, IL-6 receptor and CD54 which were all well-expressed on the surface of those cell lines. No apparent apoptosis was observed in cells treated with those antibodies after 24 hr of incubation (data not shown).

Nuclear staining with DAPI showed condensed chromatin at the periphery of the nuclear membrane and fragmentation of chromatin into small round bodies, characteristic of apoptotic nuclei (Fig. 3). In contrast, cells transfected with the expression vector alone or untransfected HepG2 cells did not show any signs characteristic
FIG. 1. Expression of HCV proteins in stable transformants. (A) Detection of HCV core protein in stable transformants by immunoblot analysis. 22-kDa HCV core protein detection is shown in three clones of Hep39 cells (clone Nos. 1, 2, and 3); three clones of Hepswx (clone Nos. 1, 2, 3) are shown as negative control. The numbers at the bottom indicate the expression levels of HCV core protein (ng/10^6 cells) in each clone of Hep39 cells detected by FEIA. (B) Indirect immunofluorescence of HCV structural proteins. Hep39 (a), Hep827 (b, c), and Hepswx cells (d, e, f) were fixed and incubated with monoclonal antibodies against core (a, d), E1 (b, e), and E2 (c, f) proteins. HCV proteins were visualized with FITC-conjugated anti-mouse IgG.

FIG. 2. Representative morphology of apoptotic Hep39 cells (A) and control Hepswx cells (B) 24 hr after incubation with anti-Fas antibody (CH-11).
of apoptotic morphology after anti-Fas treatment (Fig. 2B and Fig. 3B). Detection of apoptotic changes in all three clones of Hep39 cells examined indicated that sites of integration in stable transformants of Hep39 cells did not influence their response to anti-Fas mAb.

Although some exceptional cases have been described where apoptotic death occurs in the absence of DNA fragmentation (Oberhammer et al., 1993), internucleosomal degradation of DNA, characterized by the appearance of DNA ladders due to activation of nuclear endonucleases, is considered a hallmark of cells undergoing apoptosis (Willie et al., 1982). In Hep39 cells, degradation of DNA produced typical oligonucleosomal DNA ladders after anti-Fas treatment in all clones (Fig. 4). In contrast, control and untransfected HepG2 cells did not show any degradation of the DNA under identical conditions.

Apoptosis rates on flow cytometry analysis

Rates of apoptosis after anti-Fas treatment were determined by the appearance of hypodiploid DNA peaks on propidium iodide (PI) staining and flow cytometry analysis (Fig. 5). Apoptotic cells in the sub-G0/G1 region were detectable only in HCV core-expressing cells, whereas Hep827 and Hep3269 cells, as well as controls, did not show any clear apoptotic population above background level. Hep39 cell culture exhibited high levels of apoptotic cells (44–55%), whereas the control Hepswx cell lines showed only background levels of apoptosis (11%) under identical conditions. Cells expressing core protein in the absence of stimulation with anti-Fas antibody did not show any apoptosis. HepG2 cells constitutively expressing HCV envelope proteins, Hep827, and nonstructural HCV proteins, Hep3269, indicated low levels of apoptosis (5 and 13%, respectively), not significantly different from controls. This is consistent also with the lack of morphological and nuclear apoptotic changes in HepG2 cells.
TABLE 1

Expression of Surface Fas Antigen in HepG2 Cells Constitutively Expressing HCV Core Protein

<table>
<thead>
<tr>
<th>Fas-positive cells (%)</th>
<th>Intensity of surface Fas expression (mean)</th>
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<tbody>
<tr>
<td>HepG2</td>
<td>88</td>
</tr>
<tr>
<td>Hepswx</td>
<td>81</td>
</tr>
<tr>
<td>Hep39</td>
<td>92</td>
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*The values shown are representative of three experiments and of three different clones of Hep39 and Hepswx cell lines.

Analysis of apoptotic effector pathway in Hep39 cells

It has recently been demonstrated that interleukin-1β-converting enzyme (ICE) (Kuida et al., 1995) and several other identified ICE-like cysteine proteases are involved in the effector pathway of apoptosis (Fernandes-Alnemri et al., 1994; Tewari et al., 1995; Nicholson et al., 1995). To investigate the possible involvement of ICE or an ICE-like protease, CPP32/apopain, in Fas-mediated apoptotic death of Hep39 cells, we examined the effect of two tetrapeptides, Ac-YVAD-cho and Ac-DEVD-cho, which specifically inhibit ICE and CPP32 proteases, respectively. In the presence of Ac-DEVD-cho, the level of apoptotic cells in the sub-G1 region population was of the same order as the background level (7%), whereas in the presence of Ac-YVAD-cho, the percentage of hypodiploid cells was much higher than the background level (20%) (Fig. 6). It was close to the levels of apoptosis in Hep39 cells treated with anti-Fas in the absence of any protease.

FIG. 5. Flow cytometry analysis of apoptotic cells. The samples were analyzed by a FACSscan flow cytometer (Becton–Dickinson Immunocytometry Systems) after propidium iodide staining as described under Materials and Methods. The percentage of apoptotic cells in the sub G0/G1 region, after incubation with anti-Fas antibody, was analyzed for untransfected HepG2 cells, Hepswx cells, Hep39 cells not treated with anti-Fas (control), three clones of HepG2 cells expressing core protein (Hep39 Nos. 1, 2, and 3), cells expressing HCV envelope proteins (Hep827), and cells expressing HCV nonstructural proteins (Hep3269). The numbers in parentheses indicate the percentage of apoptotic cells, detected by hypodiploid DNA peaks.

Expression of surface Fas antigen

We then examined the association between Fas antigen expression on the cell surface and intracellular presence of HCV core protein by flow cytometric analysis using anti-Fas mAb. The number of cells expressing Fas antigen was not significantly different in core-expressing cells (92%) compared to those of the controls, HepG2 and Hepswx (88 and 81%, respectively) (Table 1). In addition, the expression levels of surface Fas did not show significant changes between Hep39 cells (intensity of surface Fas: 30) and controls (intensity of surface Fas: 26). This means that the levels of Fas antigen on the cell surface are not influenced by the presence of intracellular HCV core protein. It also indicates that neither the number of Fas antigen-positive Hep39 cells nor the expression levels of surface Fas antigen correlated with anti-Fas-induced apoptosis in Hep39 cells.

FIG. 6. Inhibition of Fas-mediated apoptosis by Ac-YVAD-cho or Ac-DEVD-cho. Adherent and detached cells were pooled and processed for flow cytometry analysis. Histograms are presented for untreated Hep39 cells (control), Hep39 cells incubated with anti-Fas antibody in the absence of any inhibitor, and Hep39 cells treated with anti-Fas in the presence of either Ac-DEVD-cho or Ac-YVAD-cho. The histograms are representative of analyses performed in triplicates.
inhibitor (30%). Fas-mediated apoptosis of HepG2 cells harboring HCV core protein was prevented by Ac-DEVD-cho, but not by Ac-YVAD-cho.

**DISCUSSION**

In spite of the lack of an in vitro cell system to support the efficient replication of HCV, the constitutive expression of viral proteins in human hepatoma cell lines provides useful information for studying the effects induced by expression of HCV proteins. Here we report the effect of intracellular HCV core protein in relation to Fas-mediated apoptosis.

Data have been accumulating to suggest that HCV core protein is a trans-acting regulatory protein. The 22-kDa core protein trans-suppressed CAT gene expression under the control of various promoters (Kim et al., 1994). It was reported to regulate the replication and the expression of the HBV genome (Shih et al., 1993) and to trans-activate c-myc oncogene (Ray et al., 1995). HCV core protein enhanced the H-ras oncogene activity in immortalizing rat embryo fibroblast (Ray et al., 1996). These observations suggest that HCV core protein functions not only as a structural nucleocapsid protein but also as a regulatory protein of gene expression.

Apoptosis, a cellular genetic program leading to cell shrinkage, cytoplasmic blebbing, and cleavage of chromosomal DNA, is a cellular response to various stimuli. Both physiological and pathological stimuli including viral infections induce apoptosis (see Vaux and Strasser, 1996, for review). The mechanisms by which a virus activates the apoptotic pathway in infected cells are not known. Although intracellular synthesis of viral proteins is generally required, expression of single viral protein may be sufficient. Apoptosis, for instance, is associated with chicken anemia virus VP3 protein (Noteborn et al., 1994) and with human T-cell leukemia virus Tax protein (Yamada et al., 1994). Fas agonists induced typical apoptosis in primary T lymphocytes from HIV-infected patients and in CD4+ cell lines expressing viral gp160 (Estaquier et al., 1996; Lu et al., 1994).

In addition to direct viral cytotoxicity, apoptotic cell death during viral infection can occur as a result of an immune response mediated by cytotoxic T lymphocytes (CTL). Since liver cells were shown to be sensitive to induction of apoptosis by agonists of Fas antigen both in primary human hepatocytes in vitro (Galle et al., 1995) and in murine model in vivo (Ogasawara et al., 1993), the release of soluble Fas ligand into systemic circulation from activated CTLs may contribute to the hepatocyte injury (Que et al., 1996). In fact, the expression of Fas antigen was shown in the liver tissue in patients with hepatitis C, with a higher prevalence in regions of active inflammation (Hiramatsu et al., 1994). Moreover, expression of Fas ligand was reported in liver-infiltrating mononuclear cells in patients with hepatitis C (Mita et al., 1994). From the observations described above, apoptotic cell death may account for the liver cell damage observed in acute and chronic hepatitis. Apoptosis of hepatocytes in viral hepatitis may also give clues to understand the discordance between serum transaminase values and the degree of histological injury (Que et al., 1996).

Apoptosis under physiological cell conditions is a two-phase process. In the first phase, even if they look normal, cells are committed to undergo apoptotic death in response to various stimuli. In the second phase of apoptosis cells undergo dramatic morphological and physiological changes, culminating in cell death (Steller, 1995). With respect to this concept, the sensitivity to anti-Fas-triggered apoptosis in Hep39 cells described here is considered in that the Fas transduction pathway is primed to induce apoptosis in HepG2 cells harboring certain intracellular HCV protein. The results, together with the lack of apoptotic cell death in HepG2 cells constitutively expressing other HCV proteins like envelope protein is not associated with the upregulation of surface Fas antigen. The independence of apoptosis priming from Fas antigen expression level in Hep39 cells suggests that the apoptosis effector pathway located downstream of the surface Fas is affected by HCV core protein. Such an effect of HCV core protein makes the cells highly susceptible to anti-Fas antibody.

Protease activity has been described to have a prominent role in apoptotic cell death (see Seamas and Douglas, 1995, for review). In mammalian cells, the ICE family of proteases includes at least seven recently identified members, whose prototypes are ICE, CPP32, and ICh-1 (Duan et al., 1996). Inhibition by Ac-DEVD-cho peptide indicates that Fas-mediated apoptosis in Hep39 cells involves the preferential activation of CPP32. Moreover, this result suggests that the apoptotic machinery of Hep39 cells shares the effector pathway of the Fas-induced death signal reported for other cell systems, such as some T lymphoid cells. In addition, as CPP32 activation has been shown to be a downstream event in the apoptotic pathway (Greideringer et al., 1996), commitment to apoptosis mediated by HCV core protein may involve an upstream event(s) which is not known yet.

In conclusion, by stimulation with anti-Fas antibody, we could demonstrate apoptotic cell death in a human hepatoma cell line constitutively expressing HCV core protein. This may help to explore the new aspects of
virus - cell interaction to understand the pathogenicity of HCV infection.

ACKNOWLEDGMENTS

We thank Dr. Maria Rapicetta for helpful discussion and support. We also thank Y. Hira and A. Suzuki for technical assistance and T. Mizoguchi for preparation of the manuscript. This work was supported in part by grants for non-A, non-B hepatitis research from the Ministry of Health and Welfare and for the Interministerial Cooperative Basic Research Core System from the Agency of Science and Technology, Japan. A. Ruggieri is a Science and Technology Agency Fellow from the Japan International Science and Technology Exchange Center.

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