Inhibition of Cytochrome c Release in Fas-mediated Signaling Pathway in Transgenic Mice Induced to Express Hepatitis C Viral Proteins*

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Keigo Machida‡‡, Kyoko Tsukiyama-Kohara‡, Eiji Seike††, Shigenobu Tone‡, Futoshi Shibasaki‡‡‡, Masumi Shimizu‡‡, Hiromichi Takahashi‡‡‡, Yukiko Hayashi§§, Nobuaki Funata‡‡, Choji Taya‡, Hiromichi Yonekawa‡, and Michinori Kohara‡

Persistent hepatitis C virus (HCV) infection often progresses to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Numerous viruses have been reported to escape from apoptotic mechanism to maintain persistent infection. In the present study, we characterized the effect of HCV proteins on the Fas signal using HCV transgenic mice, which expressed core, E1, E2, and NS2 proteins, regulated by the Cre/loxP switching system. The transgene expression of HCV transgenic mice caused resistance to Fas antibody stimulated lethality. Apoptotic cell death in the liver of HCV protein expressing mice was significantly reduced compared with non-expressing mice. Histopathological analysis and DNA fragmentation analysis revealed that the HCV proteins suppressed Fas-mediated apoptotic cell death. To identify the target pathway of HCV proteins, we characterized caspase activity. The activation of caspase-9 and -3/7 but not caspase-8 was inhibited by HCV proteins. Cytochrome c release from mitochondria was inhibited in HCV protein expressing mice. These results indicated that the expression of HCV proteins may directly or indirectly inhibit Fas-mediated apoptosis and death in mice by repressing the release of cytochrome c from mitochondria, thereby suppressing caspase-9 and -3/7 activation. These results suggest that HCV may cause persistent infection, as a result of suppression of Fas-mediated cell death.

Hepatitis C virus (HCV) is a positive-strand RNA virus and major causative agent of post-transfusion-associated and sporadic nonA nonB hepatitis. Persistent HCV infection often progresses to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The mechanism involved in the development of persistent infection and the pathogenesis is still unclear.

A number of viruses have been reported to escape from the apoptotic mechanism to maintain persistent infection. The expression of HCV proteins is reported to influence apoptosis. HCV proteins have been variously found to suppress or activate anti-Fas antibody and/or tumor necrosis factor (TNF)-α mediated cell death (1–4). In addition, HCV reportedly activates nuclear factor-κB (NF-κB) and represses Fas and TNF-α-mediated cell death (5–7). The effects of cell death by HCV proteins are not fully understood because several discrepancies have been observed in the activation and repression of Fas or TNF-α-related cell death.

A transgenic mouse model using a stable expression system causes immunotolerance to transgene products. Therefore, an HCV protein switching expression system may be suitable for in vivo assay of HCV protein effects. Using the Cre/loxP system, we developed a transgenic mouse model with efficient conditional transgene activation of HCV cDNA (core, E1, E2, and NS2) (8). HCV proteins were mainly detected in the liver of conditionally expressing transgenic mice. These methods allowed us to investigate the importance of HCV proteins in apoptotic signaling pathways by the conditional expression of HCV proteins in vivo.

Fas-mediated cell death appears to induce hepatic injury (9). Fas-mediated liver injury is likely to play a critical role in some forms of immune-mediated hepatitis. It has become evident that activated T lymphocytes have the ability to kill Fas-bearing targets through membrane expression of Fas ligand (Fas-L).
(10). Experimental liver injury models have demonstrated the important roles of this molecule: intraperitoneal administration of agonistic anti-Fas antibodies damages the liver by massive hepatocyte apoptosis, with (11) or without lethality (12). The involvement of the Fas/Fas-L system has been shown in many injury models (10). In addition, involvement of the Fas/ Fas-L system in liver homeostasis was suggested by a report that Fas-deficient mice show substantial liver hyperplasia (13).

The Fas protein CD95 is a homotrimeric molecule and Fas-L causes clustering of the Fas death domain. An adapter protein called FADD then binds through its own death domain to the clutered receptor death domains (14). Upon recruitment by FADD, caspase-8 is activated through self-cleavage and oligomerization (15). Caspase-8 then activates downstream effector caspsases such as caspase-3/7 (16). Bcl-2 family proteins play a pivotal role in controlling cell life and death, with some members such as Bcl-2 and Bcl-XL, inhibiting apoptosis, and others such as Bax, inducing cell death (17).

In the present paper, we establish an experimental system in which the effects of HCV protein on Fas-mediated apoptosis can be examined. Some of the potential target pathways of HCV proteins are also characterized.

**EXPERIMENTAL PROCEDURES**

**Expression of HCV Transgene in Vivo—**HCV transgenic mice CN2 (BALB/c, 9–13 weeks old) were used in experiments. The CN2 transgenic mouse is the HCV genotype 1b transgenic mouse regulated by the CRE/loxP conditional switching system (8). AxCANCre and AxCAw1 recombinant adenoviruses were replication-deficient, lacking the E1A, E1B, and E3 regions (18). AxCANCre expressed recombinase Cre, and AxCAw1, which was used as the control adenovirus, lacked the inserted cre gene. AxCANCre or AxCAw1 (2 × 10^10 PFU) were intravenously injected into transgenic or nontransgenic female mice between 9 and 13 weeks of age. The CN2 mice were intravenously injected with AxCAw1 and AxCANCre virus and killed at 1, 2, 3, 4, 5, and 6 days after the injection. The liver tissue was stained with anti-core polyclonal antibody R85 using TSA direct method (PerkinElmer Life Sciences). Four days after the inoculation of recombinant adenovirus, mice were administered anti-Fas antibody (clone Jo2; Pharmingen) intravenously, with monoclonal antibody 5F11. The tube was incubated for 10 min at 37 °C. Monoclonal antibody 5E3 conjugated to Alexa-488 or Alexa-594 was added after washing with buffers, and the mixture was incubated for 90 min at 37 °C. The fluorescence of released 7-amino-4-trifluoromethylcoumarin (AFC) was detected by a fluorescence spectrometer (model P2000; Hitachi). Excitation wavelength was 400 nm and emission wavelength was 505 nm. Specific caspase-3/7 or -8 activity was determined by subtracting the values obtained in the presence of 10 μM Ac-DEVD-CHO or Ac-IETD-CHO (Peptide Institute, Osaka, Japan), inhibitors of caspase-3/7 or -8, respectively. One unit corresponds to the activity required to cleave 1 pmol of the substrate in 60 min.

**Western Blotting of Caspase-8 and Bcl-2 Family Members—**The liver was perfused with phosphate-buffered saline (−) at 37 °C through the portal vein to remove monocytes, sectioned, and rapidly frozen in liquid nitrogen. Liver tissue was homogenized in a Dounce glass homogenizer using five complete up and down cycles of a glass loose-type pestle in a homogenizing buffer composed of 1% SDS, 0.5% Nonidet P-40, 0.15 M NaCl, 10 mM Tris (pH 7.4), and 1 × complete protease inhibitor mixture (Roche Molecular Biochemicals). The lysates were centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant was centrifuged and used as liver lysate. Lysates (30 or 50 μg) were separated via 15% SDS-polyacrylamide gel electrophoresis using Tris Tricine buffer (Daichi Pure Chemicals) and transferred onto polyvinylidene difluoride membrane (Amersham Pharmacia Biotech). The membrane was incubated in blocking buffer composed of 5% skim milk in TBST (20 mM Tris (pH 7.4), 137 mM NaCl, 0.5% Tween 20) followed by primary antibody; anti-Caspase-8 (T-16, Santa Cruz Biotechnology), anti-Bcl-XL (S-18, Santa Cruz Biotechnology), Bid (R&D Systems), Bax (N-20, Santa Cruz Biotechnology), Bel-2 (4C-11, Santa Cruz Biotechnology), and Bad (New England Biolabs, Inc.).

**Western Blotting of Cytochrome c—**Liver tissue was minced and washed once in ice-cold homogenization buffer composed of 0.1% bovine serum albumin, 1 mM EDTA, 300 mM sucrose, 5 mM MOPS, 5 mM KH2PO4 (pH 7.4), and 1 × complete inhibitor mixture (Roche Molecular Biochemicals). The minced liver was gently homogenized in the homogenate buffer in a Dounce glass homogenizer using five complete up and down cycles of a glass loose-type pestle in a homogenizing buffer composed of 1% SDS, 0.5% Nonidet P-40, 0.15 M NaCl, 10 mM Tris (pH 7.4), and 1 × complete protease inhibitor mixture (Roche Molecular Biochemicals). The cytosolic fraction of liver tissues was isolated as previously reported, with partial modification (20). Briefly, liver tissue was homogenized in a Dounce glass homogenizer (loose type) with lysis buffer (0.3 M mannitol, 5 mM MOPS, 1 mM EGTA, 4 mM KH2PO4, 20 μg/ml leupeptin, 10 μg/ml pepstatin A, 10 μg/ml aprotinin, 2 mM phenylmethylsulfonyl fluoride) and fractionated into pellet, heavy membrane, light membrane, and cytosolic fractions. The lysates were centrifuged at 10,000 × g for 10 min at 4 °C to remove debris or fiber. The supernatants were centrifuged at 10,000 × g for 15 min at 4 °C and the pellets collected as the heavy membrane fraction. The supernatants were again centrifuged at 100,000 × g for 45 min at 4 °C and collected as the cytosolic fraction. The activities of caspase-8, -9, and -3/7 were measured using Ac-LETD-ACF, Ac-LQTD-ACF, Ac-LEHD-ACF, or Ac-DEVD-ACF (Enzyme Systems Products) as substrates, respectively. Substrates were preincubated for 10 min at 37 °C in reaction buffer composed of 20 μM substrate, 100 mM Hepes (pH 7.5), 10% sucrose, 10 mM dithiothreitol (pH 7.5), and 0.1% CHAPS (21). After preincubation, 100 μg of lysate was added to 1.25 ml of reaction buffer and incubated for 15 min at 37 °C. The fluorescence of released 7-amino-4-trifluoromethylcoumarin (AFC) was detected by a fluorescence spectrometer (model P2000; Hitachi). Excitation wavelength was 400 nm and emission wavelength was 505 nm. Specific caspase-3/7 or -8 activity was determined by subtracting the values obtained in the presence of 10 μM Ac-DEVD-CHO or Ac-IETD-CHO (Peptide Institute, Osaka, Japan), inhibitors of caspase-3/7 or -8, respectively. One unit corresponds to the activity required to cleave 1 pmol of the substrate in 60 min.

**Histopathological Analysis—**Liver tissues were frozen with O.C.T. compound (Tissue Tech) for immunohistochemical analysis. The sections were fixed with a 1:1 solution of acetone:methanol at -20 °C for 10 min and then washed with phosphate-buffered saline (−). Subsequently, the sections were incubated with the IgG fraction of an anti-HCV core rabbit polyclonal antibody (RRS (8)) in blocking buffer for 16 h at 4 °C. The sections were incubated with secondary antibody, horseradish peroxidase-conjugated anti-rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, PA), for 1 h at room temperature. Immunohistochemical staining was completed using tyramide signal amplification (PerkinElmer Life Sciences).

For cytochrome c staining, the sections were incubated in blocking buffer for 30 min at room temperature and incubated with rabbit 2 P. Shibasaki, manuscript in preparation.
anti-cytochrome c antibodies for 3 h at room temperature. The sections were washed with phosphate-buffered saline (−). Primary antibodies were detected by Texas Red-conjugated anti-rabbit IgG (CAPPEL). Fluorescently labeled sections were stained with 0.5 μg/ml Hoechst 33342 dye (Molecular Probes) for 1 min at room temperature before being washed with phosphate-buffered saline to stain cell nuclei. Fluorescence was observed under a fluorescence microscope (Carl Zeiss).

**FACS Analysis—**Cytofluorometry was performed by FACS Calibur (Becton-Dickinson, San Jose, CA). Primary hepatocytes were stained with an optimal dilution of anti-Fas-FITC antibody (06134D, Pharmingen, San Diego, CA), and examined by FACScan.

**Statistical Analysis—**Data represent the mean ± S.D. All statistical analysis was performed using Student’s t test. Statistical significance was established at p < 0.05.

**RESULTS**

**HCV Protein Expression in the Liver of HCV Transgenic Mice—**HCV proteins were expressed in the liver of HCV transgenic mice. Expression of core protein in AxCANCre adenovirus-injected CN2-8 transgenic mice liver was confirmed by immunofluorescence staining. Core protein was expressed in 50–60% of hepatocytes in the lobule of the liver section at day 4, and 70–80% of hepatocytes at 7 days after AxCANCre injection (Fig. 1A). The genomic DNA was prepared from liver 4 or 5 days after the injection of adenovirus and analyzed by Southern blot analysis. Transgene recombination occurred in the livers of the transgenic mice (Data not shown). In contrast, core protein was not expressed in liver sections from transgenic mice without AxCANCre injection or in AxCaw1-injected CN2 mice (Fig. 1A). The core protein levels in hepatocytes were measured by FEIA. The mean level of core protein was 5.7 ng/mg total protein in the HCV transgenic mice liver 4 days after the administration of AxCANCre adenovirus (Fig. 1B). The expression of core, E1 and E2 proteins was detected in AxCANCre-injected mouse liver by Western blot analysis (data not shown).

The livers of CN2 mice, 5 days after administration of AxCANCre and AxCaw1, did not show infiltration of mononuclear cells or an increase in serum ALT levels (Fig. 1C). A significant liver injury was not evoked until day 5. Therefore, 4 days after the administration of AxCANCre or AxCaw1 was a suitable time point to characterize the effect of HCV proteins on Fas-mediated cell death.

**Inhibition of Fas-mediated Death by HCV Proteins—**Mice not expressing HCV were treated with 0.14 μg/g mice body weight of anti-Fas antibody, which killed 50% of the mice over the initial 24 h (n = 6). In contrast, all of the HCV protein expressing transgenic mice were resistant to Fas stimulated lethality over the initial 24 h (Fig. 2A). Another transgenic mice strain, CN2-29 (8) was also analyzed to better understand the effects of the transgene integration site. CN2-29 mice expressed an average of 0.54 μg/mg core protein day 4 after AxCANCre administration. Ten of the AxCaw1 adenovirus-injected CN2-29 mice were administered 0.14 μg/g anti-Fas antibody and observed for 24 h (Fig. 2B). Half of the AxCaw1 adenovirus-injected CN2-29 mice died. In contrast, 80% of the AxCANCre adenovirus-injected CN2-29 mice survived after the administration of anti-Fas antibody (n = 10). To investigate the effects of adenovirus, AxCANCre and AxCaw1 adenovirus-injected nontransgenic BALB/c mice were administered 0.14 μg/g anti-Fas antibody. Within 24 h of administration, 50% AxCaw1 and 50% AxCANCre injected nontransgenic BALB/c mice died (n = 10) (Fig. 2C). Expression of HCV proteins appeared to inhibit the progression of death after the injection of anti-Fas antibody.

**Inhibition of Hepatic Injury in Transgenic Mice with HCV Protein Expression—**Serum ALT levels dramatically increased within 3 h of injection of the anti-Fas antibody in AxCaw1-injected CN2-8 mice (Fig. 3A). In contrast, significantly lower ALT levels were observed in HCV protein expressing CN2-8 transgenic mice 3 and 6 h after injection of the anti-Fas antibody (p = 0.0352, and p = 0.0266 respectively).

Liver tissue of anti-Fas antibody-treated mice was sampled from 0 to 4 h after the intravenous injection for histological examination (Fig. 3B). Four h after the anti-Fas antibody injection, hepatic injury was observed predominantly in the liver of mice not expressing HCV proteins (Fig. 3B) and fragmented nuclei were observed in over 80% of the hepatocytes. In contrast, histological analysis of livers from HCV protein expressing transgenic mice revealed that liver injury was suppressed and cell death was far less prevalent than in the liver of mice not expressing HCV proteins (Fig. 3B). The nuclear shrinkage
and fragmentation observed in hepatic cell death resembled apoptotic cell death. Therefore, DNA fragmentation was assessed in the livers of anti-Fas antibody injected mice by genomic DNA laddering. Ladder formation was much more moderate in the HCV protein expressing transgenic hepatocytes than in the hepatocytes in the HCV-nonexpressing mice at 5, 6, 8, and 12 h after the injection of anti-Fas antibody (Fig. 4). These findings indicate that expression of HCV proteins inhibited the progress of apoptotic hepatocyte death caused by anti-Fas antibody injection.

Expression of HCV Proteins Inhibited Activation of Caspase-3/7 and Caspase-9—To clarify the mechanism of Fas-mediated apoptosis inhibition by HCV proteins, expression of Fas antigen on hepatocytes and activation of caspase-8, -9, and -3/7 proteases were examined in liver samples. Four h after the administration of anti-Fas antibody, Fas protein levels in hepatocytes and expression on the cell surface did not differ significantly between HCV-positive and HCV-negative mice (Fig. 5, A and B). Caspase-8 was similarly activated within 1 h in both HCV-negative and HCV-positive transgenic mice treated with anti-Fas antibody (Fig. 6). The active form of the caspase-8 subunit (p18) appeared within 1 h after anti-Fas antibody injection (Fig. 6A). No significant difference was observed in p18 patterns between HCV protein expressing and nonexpressing CN2-8 mice (Fig. 6A). To determine the activity of caspase-8, -9, and -3/7, the fluorogenic substrates with the cleavage site by each caspase were used. The results were consistent with the results from Western blot analysis (Fig. 6A). Caspase-8 was similarly activated in the liver tissue of HCV protein expressing and nonexpressing mice within 1 h after anti-Fas antibody injection (Fig. 6B). HCV proteins expression did not repress substrate cleavage activity by caspase-8 as shown by the substrate cleavage assay using both Ac-LETD-AFC and Ac-LQTD-AFC, which are the known cleavage sites of Bid (22). After 3 and 4 h of Fas antibody administration, caspase-8 activity decreased in liver tissues from mice not expressing HCV proteins (Fig. 6B). The results suggested that the greater damage to liver tissue observed in mice not expressing HCV proteins contributed to the decrease in caspase-8 activity itself in HCV-negative livers. In contrast, activation of caspase-3/7 and -9 was suppressed in HCV protein expressing liver tissue, 3 and 4 h after antibody administration (Fig. 6B).

Immunoblot Analysis of Bcl-2 Family Proteins in Hepatocytes of Mice Injected with Anti-Fas Antibody—The inhibitory mechanism of Fas-mediated cell death by HCV genome expression is unclear. However, the expression of the Bcl-2 family proteins were examined in the liver of mice injected with anti-Fas antibody. The results showed that the expression of Bcl-2, Bcl-xL, and Bcl-2L1 was decreased in HCV protein expressing liver tissue compared to nonexpressing liver tissue (Fig. 7). The decreased expression of Bcl-2 family proteins may contribute to the inhibition of Fas-mediated apoptosis by HCV proteins.
was analyzed further. We examined expression levels of the Bcl-2 protein family, which localize in the mitochondria and regulate the progression of apoptotic cell death. Bcl-2 family protein level, including Bid, Bcl-X$_L$, Bcl-2, Bad, and Bax was analyzed by immunoblot (Fig. 7, A and B). Caspase-8 activity of Bid cleavage was not inhibited by HCV proteins. Translocation of the C-terminal of Bid protein into mitochondria was not repressed by HCV proteins 4 h after the administration of anti-Fas antibody (HCV not expressed, open bars; HCV expressed, black bars). Caspase and Bid cleavage activities were measured by release of AFC from peptide substrates. Units represent the mean and S.D. of three individual experiments.

Inhibition of Fas-mediated Apoptosis by HCV

The quantity of cytochrome c in the heavy membrane fraction of HCV protein expressing mice liver after 4 h of Fas antibody administration was not significantly reduced compared with HCV nonexpressing mice (Fig. 8B). Hence, the cytoplasmic fraction after both 3 and 4 h in HCV protein expressing mice liver had 3–4-fold decrease of cytochrome c than that of hepatocytes in HCV nonexpressing mice (Fig. 8C).
and relative intensity of each band was indicated. The quantification of detected cytochrome c was analyzed by Western blotting of the heavy membrane fractions (HM) from transgenic mice livers. Cytochrome c was analyzed by Western blotting with chemiluminescence assay was performed with a Chemi Doc (Bio-Rad) and relative intensity of each band was indicated.

The results indicated that the release of cytochrome c from mitochondria to cytoplasm in hepatocytes was suppressed by HCV protein expression.

**DISCUSSION**

A part of the defense mechanism against virus infection is induced to initiate apoptotic cell death by signals delivered from CTL. On the other hand, numerous viruses have been reported to escape from apoptotic mechanism to maintain persistent infection (24–28). In this study, we investigated that HCV might cause persistent infection, as a result of suppression of Fas-mediated cell death and inhibition of HCV-infected hepatocyte rejection in the liver (Fig. 9).

Some reports indicated that the HCV core protein has anti-apoptotic (1, 2, 5–7) and proapoptotic (3, 4) functions. The reason for the discrepancy among these reports is still unclear. Moreover, NF-κB has an antiapoptotic function against anti-Fas antibody and TNF-α-induced cell death. It was recently shown that HCV core protein activated NF-κB-associated signal (5–7). It may be that the core has multifunctional roles in the apoptotic signaling. This discrepancy may be explained by the possibility that it was caused by using established cell lines and clonically selected permanent transfectant cells in the previous studies. To avoid the artificial effects in cell lines and transgenic mouse with persistent expression of HCV protein, we examined the transgenic mouse using conditional transgene expression system (8). These hepatocytes of transgenic mouse retain original sensitivity to the apoptosis mediated by Fas signal. The conditional transgene expressed mouse makes it possible to examine native effect of HCV protein to Fas-mediated apoptosis.

The results of the present study revealed that Fas-mediated apoptotic cell death was suppressed by the expression of HCV proteins (core, E1, E2, and NS2) in vivo. In the presence of HCV proteins, lethality after the administration of anti-Fas antibody significantly decreased (Fig. 2). The survival rate correlated to HCV protein expression levels in transgenic mice (CN2-8 and 2-29) (Fig. 2, A and B).

These results revealed that apoptosis was inhibited by HCV protein expression, as shown by DNA ladder examination of mouse liver (Fig. 4). Expression of Fas on the surface of hepatocytes did not differ significantly among AxCANCre- or AxCaw1-injected mice and untreated mice (Fig. 5). Therefore, the downstream target of Fas signaling pathway was characterized. Many caspases were involved in the downstream of Fas signaling pathway (Fig. 9). Each caspase has a specific function, for example, as an initiator or effector. The initial caspase-8 cascade was activated in both HCV protein expressing mice and nonexpressing mice (Fig. 6, A and B). Caspase-9 and -3/7 activities were down-regulated in liver tissue of HCV protein expressing mice (Fig. 6). Apoptosis of hepatocytes requires effector caspases such as caspase-3/7 (16, 29). These results suggest that caspase-9 and -3/7 activation were inhibited by HCV proteins and/or that the mitochondria amplification loop of the Fas-mediated signaling pathway is inhibited by HCV proteins (Fig. 9).

Bcl-2 family proteins are involved in progression of apoptotic cell death in response to Fas signal (30). Bid is likely the activator of the mitochondrial pathway through cleavage by caspase-8 and translocation into the mitochondria. Bid then interacts with Bcl-2 and/or Bax protein causing the release of cytochrome c from mitochondria into the cytoplasm (31, 32). In the present study, distribution of Bid protein in the cytosol and heavy membrane fraction did not differ significantly between HCV protein expressing and nonexpressing mice (Fig. 7A). Therefore, the Bid pathway was not influenced by HCV pro-
tein. Bcl-2, Bad, and Bax protein quantities did not differ between HCV protein expressing and nonexpressing mice (Fig. 7, A and B). On the other hand, in the presence of HCV protein, Bcl-XL protein did not decrease 3 and 4 h after the administration of Fas antibody. This may have resulted from the down-regulation of caspase-3/7 activity (Fig. 7A) (16, 29). Therefore, the Bcl-2 family may not be significantly influenced by HCV proteins.

Immunohistochemical analysis of affected liver regions confirmed the release of cytochrome c from the mitochondria of HCV nonexpressing mice and expressing mice by anti-Fas antibody administration. The release of cytochrome c from the mitochondria to cytoplasm was suppressed in the presence of HCV after 3 and 4 h of Fas antibody administration (Fig. 8A). Consistent with this result, cytochrome c released 5.2–6.6-fold increase from heavy membrane fraction to cytoplasmic fraction in the HCV nonexpressing liver tissue after 3 and 4 h of Fas antibody administration (Fig. 8, B and C). In contrast, cytochrome c was mainly present in the heavy membrane fraction, and the release of cytochrome c from the mitochondria to cytoplasm was suppressed in the presence of HCV after 3 and 4 h of Fas antibody administration (Fig. 8, B and C). These results indicated that the release of cytochrome c from mitochondria to cytoplasm was inhibited by the expression of HCV proteins in transgenic mice. The inhibition of the activation of effector caspase-3/7 resulted from the suppression of the cytochrome c/Apaf-1/caspase-9 amplification loop by HCV and decreased lethality after Fas antibody administration. Further experiments are needed to determine whether the inhibition of cytochrome c from mitochondria is due to the direct interaction of particular HCV proteins with a cellular protein. Or, it is possible that the interaction of expression of HCV E1 and E2 glycoproteins may induce a more general cellular response, stimulating chaperones and delaying the entry into apoptosis (33, 34). Further elucidation of the apoptotic signaling functions of HCV will not only advance the understanding of molecular mechanisms of HCV pathogenesis, but also shed light on the basic mechanism of apoptosis.

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