Antioxidant enzymes, including heme oxygenase (HO)–1, are an important line of defense against oxidant-mediated liver injury. Because hepatitis C virus (HCV) infection appears to increase the production of oxidants, we evaluated levels of antioxidant enzymes and HO-1 in liver-biopsy samples from HCV-infected patients by immunoblot and semiquantitative reverse-transcriptase polymerase chain reaction. In HCV-infected liver samples, levels of immunoreactive HO-1 and HO-1 mRNA were 3-fold lower than levels in control samples, but levels of superoxide dismutase and catalase were unaffected. Immunohistochemical results confirmed the decreased expression of HO-1 in hepatocytes from liver samples from HCV-infected patients but not in those from patients with other chronic liver diseases. The expression of HO-1 was also reduced in cell lines that stably express HCV core protein, which suggests that core gene products are capable of regulating the expression of HO-1.

Hepatitis C virus (HCV) is a major cause of chronic hepatitis and progressive liver fibrosis leading to cirrhosis [1]. Currently, the mechanism(s) responsible for hepatocellular injury are not understood. However, the initial damage probably results from inflammation and oxidative stress, which lead to chronic liver injury and fibrosis. In response to inflammatory oxidants, hepatocyte defense enzymes are induced that are vital for the maintenance of cellular homeostasis and the prevention of liver disease [2].

The effects of HCV infection on hepatocyte oxidative stress have been difficult to study because of the lack of suitable in vitro models that will support abundant viral replication. Nevertheless, important progress has been made by studying cells and animal models that express individual portions of the HCV genome. Cells transfected with cDNA of the HCV core gene have been shown to have elevated levels of reactive oxygen species (ROS) [3–5], which may cause direct oxidative injury and/or the activation of oxidant-regulated signaling pathways. HCV core protein has also been shown to transcriptionally modulate a variety of human and viral proteins in vitro [6–10], including specific proteins of the cell cycle and apoptosis pathways [11–13]. Recent evidence has also shown that products from the HCV core gene are a mixture of “in frame” and alternate reading frame proteins (ARFP), which suggests multifunctional capabilities of the HCV core gene products [14–16]. Although the function of each translational product of the core gene remains unclear [17], it is apparent that the core protein (we use this term to also include ARFP) likely triggers pro-oxidant events that lead to potential modulation of antioxidant enzymes.
Figure 1.  
A, Immunoblot analysis of hepatic antioxidant enzymes and heme oxygenase (HO)–1 at various stages of hepatic fibrosis. Liver-biopsy samples from patients with histologically matched stages of fibrosis (3–5 patients/group) or histologically normal tissues from liver donors were solubilized in SDS sample buffer, and Western blots were performed as described in Patients, Materials, and Methods by use of specific primary antibodies. Equivalent amounts of solubilized protein from the biopsy samples were analyzed for each enzyme. B, Densitometric analyses of data from 3 separate experiments shown in panel A show marked decrease in the level of expression of HO-1 in hepatitis C virus (HCV)–infected liver-biopsy samples vs. control liver tissue. There were no significant differences in the expression of Mn superoxide dismutase (SOD), CuZnSOD, or catalase (CAT; $P > .05$), and there was a significant decrease in the expression of HO-1 ($P < .01$) in HCV-infected liver samples at different levels of fibrosis or vs. control liver samples. The optical density (OD) is expressed as arbitrary units and, for each determination, was normalized to values obtained from histologically normal liver control samples. Shown is the mean ± SE. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

The effects of HCV infection on hepatic oxidative defense enzymes have not been extensively studied, although the maintenance of redox balance is a crucial response to control inflammation and fibrogenesis [2, 18].

An enzyme whose role as a cellular antioxidant enzyme in the liver is becoming more appreciated is heme oxygenase (HO)–1. HO enzymatically degrades free heme to biliverdin with the release of CO. Although there are 3 isozymes of HO, HO-1, which is also known as heat-shock protein 32, is the only form that is regulated transcriptionally under conditions of cellular stress, such as hypoxia, cytotoxin exposure, and hyperthermia [19–21]. Recent data have shown that hepatocytes respond to acute injury with an increased expression of HO-1 [22–24]. However, the effects of chronic HCV infection on hepatic HO-1 expression have not been reported.

In the present study, we evaluated the expression of HO-1, Mn superoxide dismutase (SOD), CuZnSOD, and catalase in HCV-infected human liver samples. Because of the known transcriptional regulatory effects of the HCV core protein, we also determined whether this was capable of modulating the same antioxidant enzymes in vitro. Our findings show that the hepatic expression of HO-1 is reduced during HCV infection in vivo and that the expression of HCV core protein causes a similar effect in vitro, whereas the expression of the SODs and catalase is unaffected. The reduced expression of HO-1 during chronic HCV infection, which may be related to the effects of
HCV core protein observed in vitro, may prove to be important for hepatocellular injury caused by the virus, which warrants further study.

PATIENTS, MATERIALS, AND METHODS

**Materials.** Molecular biology–grade phenol (Aramesco), Taq DNA polymerase (Perkin-Elmer Cetus), and moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco/BRL Life Technologies) were used in these studies. Oligonucleotide primers were prepared by the University of Iowa College of Medicine DNA Core Facility (Iowa City, IA) by use of known nucleotide sequences obtained from GenBank. The HCV genotype 1b primers were sense 5'-ATAATTCCATGGCATCAAAGAGT-3' and antisense 5'-TCCTGGCCTGACAGGGTGTGACCGTG-3'.

Commercial antibodies were anti–HO-1 and anti–HO-2 (Stressgen Biotech Serologies), mouse monoclonal antibody to human CD68, macrophage (DakoCytomation), and mouse anti-GAPDH3 (Chemicon). Rabbit anti-catalase, anti–MnSOD, anti–CuZnSOD were gifts from Larry Oberley (University of Iowa, Iowa City, IA). Mouse monoclonal antibody to HCV core protein was a gift from Suresh Desai and George Dawson (Abbott Laboratories). Separate experiments showed that the antibodies to HO-1 and HO-2 were specific for their respective isozymes and did not cross-react (data not shown).

**Human subjects.** The University of Iowa Institutional Review Board approved the study, which was conducted according to the guidelines of the US Department of Health and Human Services, and informed consent was obtained from patients before the collection of clinical samples. Percutaneous liver biopsies were performed for the clinical evaluation of the grade and stage of HCV liver disease during routine patient work-up. HCV-positive patients had positive antibody tests (EIA 3.0; Chiron), HCV RNA present, the cyclic serum elevation of hepatic transaminases (2–10 times normal levels), and characteristic histological findings on liver-biopsy samples [25–27]. Other causes of chronic liver disease, including drugs, alcohol, hereditary hemochromatosis, Wilson disease, autoimmune liver disease, α-1-antitrypsin deficiency, and chronic hepatitis B virus (HBV) infection, were eliminated by appropriate serological studies and findings on liver-biopsy samples. Histologically normal human liver samples were obtained from the Liver Tissue Procurement and Distribution System (Fairview University Hospitals and Clinics, Minneapolis, MN). Six different specimens of normal liver were obtained and used throughout these studies with consistent results. Similar results were also obtained when portions of human donor liver were used as normal controls.

Histopathological determinations were performed on routine 2.0–2.5-cm percutaneous core liver–biopsy samples. Pathologist M.L., who was blinded to the patients’ clinical findings, graded the liver biopsies after the evaluation of fixed, paraffin-embedded sections stained with hematoxylin and eosin or with Masson’s trichrome for collagen. Biopsy samples were scored for grade and stage of chronic hepatitis [25–27] according to the Batts-Ludwig system [28] on a scale of F0–F4, as described elsewhere [29]. Sections of autoimmune hepatitis (AH) and chronic HBV infection were obtained from patients who fulfilled recognized clinical, serological, and histological criteria for these diagnoses.

RNA and immunoblot analyses were performed by use of...
Figure 3. Immunohistochemical staining of control, hepatitis C virus (HCV)–infected, hepatitis B virus (HBV)–infected, and autoimmune hepatitis (AH) liver-tissue samples for heme oxygenase (HO)-1 and/or CD68. Deparaffinized liver-biopsy sections were processed for antigen retrieval, as described in Patients, Materials, and Methods. A, B, and C, Control (normal) liver; D, E, and F, HCV-infected liver; G, HBV-infected liver; and H, AH-infected liver. Sections were stained with anti–HO-1 (A, D, G, and H) and anti-CD68 (B and E). Negative controls (C and F) were stained in the absence of specific primary antibody \( n = 3 \). Sections were counterstained with hematoxylin (blue) nuclear stain. HO-1 is stained brown from peroxidase substrate. Original magnification, \( \times \) 400.

2-mm sections of liver that were excised from the core liver-biopsy sample immediately after the biopsy procedure. These sections were snap frozen in liquid nitrogen and stored at \(-70^\circ C\) until use. Approximately 3–5 2-mm sections were pooled for the biochemical analyses, to minimize variabilities in tissue and patient sampling [30].

Cell lines and culture conditions. HepG2 cell lines stably transfected with the cytochrome p450 enzyme CYP2E1 were a gift from Arthur Cederbaum (Mount Sinai, NY). The E47 cell line is a stable clone of HepG2 cells transfected with and stably overexpressing CYP2E1 [31]. The control for E47 is the C34 cell line stably transfected with the vector alone. The growth and passage of these lines was as described elsewhere [5]. After transfection with PC-DNA3.1 plasmid with a zeocin resistance cassette (Invitrogen) that contained HCV core protein genotype 1b sequences (341–914 nt) or vector control, stable clones were characterized as described elsewhere [5].

RNA isolation and RT-PCR. For liver samples, RNA was isolated by use of TRIZOL Reagent (Gibco BRL). RT was performed on 0.8 \( \mu \)g of RNA by use of the Omniscript Reverse Transcriptase kit (QIAGEN), and cDNA was then used for PCR. Reactions used 1 \( \mu \)L of cDNA incubated in a final volume of
Figure 4. Localization of heme oxygenase (HO)–1 in control and hepatitis C virus (HCV)–infected liver samples by confocal immunofluorescence microscopy. Sections of control liver (A and C) or HCV-infected liver (B and D) were stained with anti–HO-1 (red) and anti-CD68 (green) antibodies and then evaluated by confocal microscopy, as described in Patients, Materials, and Methods. Original magnification: A and B, ×400; C and D, ×800. Exposure times were identical for control and HCV-infected liver samples (n = 3).

Recombinant protein expression and immunoblot analysis.

Cell lines were grown to near confluence, washed with ice-cold PBS, and then collected by scraping and centrifugation. Cells were lysed by sonication in 10 mmol Tris HCl/L (pH 7.5) that contained 0.1 mmol phenylmethylsulfonyl fluoride (PMSF)/L and treated with DNAase I (50 U/mL) for 60 min on ice. The cellular lysates were then mixed with 1 vol of 2× sample buffer that contained 6% SDS and 10% mercaptoethanol, denatured by heating to 95°C for 5 min, and separated on 3% acrylamide stacking and 12% Laemmli running gels for SDS-PAGE. Specific proteins were identified by use of labeled secondary antibodies and appropriate substrates, as described elsewhere [5, 15, 35]. Relative measurements of immunoreactive proteins were obtained by densiometric scanning.

Liver-biopsy samples were separated into 4 different groups according to the level of fibrosis—F0, F1, F2, and F3. To minimize individual patient and tissue variations, each group consisted of a pool of ≥3 samples obtained from different patients at the same level of fibrosis [30]. Samples were homogenized on ice by use of a Disposable Pestle (Fisher Scientific) in lysis buffer and then frozen at −80°C until use.
buffer composed of 1% Nonidet NP40 in PBS, 1 mmol caproic acid/L, and 1 mmol PMSF/L. Protein concentrations were measured in the supernatants by use of Micro BCA protein assay reagent kit (Pierce). Western-blot analysis for specific proteins was then performed as described above.

**Immunohistochemistry.** After routine fixation in 10% buffered formalin, biopsy samples were embedded in paraffin and cut into 6-μm-thick sections. Representative sections were deparaffinized, rehydrated, and treated with either of 2 specific antigen-retrieval methods for HO-1 staining. For the first method, sections were treated with 3 mol urea/L in a pressure cooker for 10 min and then washed in PBS before immunohistochemical testing. In the second procedure, we used microwave irradiation in citrate buffer for 10 min before immunohistochemical testing. Antigen retrieval with a citrate procedure did enhance staining of sinusoidal lining cells for HO-1. However, only minimal hepatocyte staining was observed. In contrast, HO-1 staining after antigen retrieval with 3 mol urea/L and heat was optimal, and the sections revealed bright HO-1 staining in hepatocytes in addition to intense staining of sinusoidal lining cells. The second method was used in all our immunohistochemical studies (figures 3 and 4 below).

After antigen retrieval, sections were incubated with antibody to HO-1 (1:1000 dilution for 30 min), washed, and then incubated with labeled second antibody. Immunoreactivity was visualized with the Vectastain ABC kit and with 3-amino–9-ethylcarbazole or 3,3′-diaminobenzidine tetrahydrochloride substrate as the final chromogen (Vector Laboratories). Sections were counterstained with Vector hematoxylin. Negative controls included sections stained after omission of the first antibody or after incubation with mouse immunoglobulin as the primary antibody.

**Immunofluorescence.** After deparaffinization, rehydration, and antigen retrieval, samples were incubated with primary antibody overnight at 4°C and washed in PBS (pH 7.4). Secondary antibodies were a mixture of rhodamine red-X–AffiniPure donkey anti–rabbit IgG and fluorescein isothiocyanate AffiniPure donkey anti–mouse IgG (1:100; both Jackson ImmunoResearch Laboratories) for HO-1 and CD68, respectively. Sections were incubated with the secondary antibodies overnight at 4°C and then studied using confocal microscopy.

**Data analysis.** Data from individual experiments, as well as combined data from separate experiments, are expressed as mean ± SE. The statistical significance of observed differences between means was determined using Student’s t test or analysis of variance. P < .05 was considered to be significant.

**RESULTS**

**Evaluation of antioxidant enzymes and HO-1 in HCV-infected liver samples.** We first examined the expression of several major antioxidant enzymes (MnSOD, CuZnSOD, and catalase) in liver-biopsy samples from patients infected with HCV at different stages of fibrosis. Each group of liver-biopsy samples consisted of pooled samples from 3 different patients with the same level of fibrosis, to minimize variations in patients and tissue sampling [30]. Liver tissue from healthy donors was used as a control. The protein levels of antioxidant enzymes were determined by immunoblotting (figure 1). We found no significant differences in the expression of MnSOD, CuZnSOD, or catalase as the final chromogen (Vector Laboratories). Sections were counterstained with Vector hematoxylin. Negative controls included sections stained after omission of the first antibody or after incubation with mouse immunoglobulin as the primary antibody.

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**RESULTS**

**Evaluation of antioxidant enzymes and HO-1 in HCV-infected liver samples.** We first examined the expression of several major antioxidant enzymes (MnSOD, CuZnSOD, and catalase) in liver-biopsy samples from patients infected with HCV at different stages of fibrosis. Each group of liver-biopsy samples consisted of pooled samples from 3 different patients with the same level of fibrosis, to minimize variations in patients and tissue sampling [30]. Liver tissue from healthy donors was used as a control. The protein levels of antioxidant enzymes were determined by immunoblotting (figure 1). We found no significant differences in the expression of MnSOD, CuZnSOD, or catalase...
in the livers from patients with chronic HCV infection, compared with control liver samples \((n = 3)\), nor were there quantitative differences among the stages of fibrosis. These results suggest that HCV infection does not induce the up-regulation of MnSOD and catalase in the liver that is observed in other forms of oxidative stress \([36]\).

We next extended our study to HO-1, an enzyme that is induced rapidly after oxidative stress in numerous systems \([20, 21]\) and in rat hepatocytes after acute injury \([22–24]\). According to the results of immunoblot analysis (figure 1), there was a significant decrease (70%), as determined by densitometric scanning, in the level of expression of HO-1 in HCV liver-biopsy samples, compared with control liver tissue \((n = 3; P < 0.01)\) (figure 1B). It is of interest that no significant differences were seen in HO-1 expression among the different fibrosis levels, which suggests that the reduction in immunoreactive HO-1 is a result of HCV infection, rather than progressive liver fibrosis. In contrast, we found no significant difference in the expression of HO-2 between control liver tissue and liver samples from patients with chronic HCV infection at any level of fibrosis (data not shown).

To determine whether the effect of HCV infection on the protein expression of HO-1 is apparent at the mRNA level, a relative quantification of HO-1 mRNA was performed on RNA extracts from both HCV-infected biopsy samples and control liver samples. This analysis revealed a marked decrease in the level of HO-1 mRNA in HCV-infected liver-biopsy samples at all stages of fibrosis, compared with control liver tissue (figure 2). This result is consistent with the results of immunoblot studies (figure 1) and suggests that HCV infection reduces the expression of HO-1 at least in part via a reduction in steady-state HO-1 mRNA levels.

### Table 1. Relative quantitation of heme oxygenase (HO)--1 mRNA by real-time reverse-transcription polymerase chain reaction.

<table>
<thead>
<tr>
<th>Source of RNA</th>
<th>Fold difference (\pm SE)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control liver</td>
<td>33.0 (\pm) 1.5</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HCV-infected liver</td>
<td>1.0 (\pm) 1.4</td>
<td>...</td>
</tr>
<tr>
<td>C34</td>
<td>4.4 (\pm) 1.2</td>
<td>&lt;.025</td>
</tr>
<tr>
<td>C34 core</td>
<td>1.0 (\pm) 1.5</td>
<td>...</td>
</tr>
<tr>
<td>E47</td>
<td>6.2 (\pm) 2.3</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>E47 core</td>
<td>1.0 (\pm) 1.2</td>
<td>...</td>
</tr>
</tbody>
</table>

**NOTE.** Relative quantitative analysis was determined by use of the competitive cycle threshold method, which used separate reaction tubes for target (HO-1) and reference (18S rRNA) sequences, as described elsewhere \([39]\). Comparisons were paired between hepatitis C virus (HCV)--infected and control liver, C34 and C34 core cell lines, and E47 and E47 core cell lines. Paired comparison between C34 and E47 lines showed an \(\approx\)4-fold difference \((E47>C34)\) between these clonal lines (data not shown). Samples were run in quadruplicate, and variability is expressed as the SE of the difference between the paired comparisons, calculated as described elsewhere \([39]\). Data comparing control and HCV-infected liver are the results of 3 separate determinations. C34 and C34 core are the pool of 8 experiments, and E47 and E47 core are the pool of 3 experiments.

**HO-1 localization by immunohistochemistry.** To determine the cell types affected by the reduction in HO-1 protein in HCV-infected livers, immunohistochemical staining with specific HO-1 antibody was performed on standard sections of fixed liver-biopsy samples from control subjects and HCV-infected patients (figure 3A and 3D). HO-1 staining in control liver samples localized intensely to sinusoidal lining cells but with more diffuse and occasional granular staining of hepatocytes (figure 3A). Serial sections were also stained for Kupffer cells with anti--CD-68 antibody (figure 3B and 3E) \([37]\). In contrast to results with control liver samples, samples from HCV-infected liver revealed little HO-1 staining in hepatocytes, whereas staining of sinusoidal lining cells was grossly unaffected (figure 3D). To assess the specificity of this effect for chronic HCV infection, HO-1 immunostaining was performed on biopsy samples from patients with HBV infection and AH (figure 3G and 3H). In contrast to the results with HCV-infected livers, hepatocytes from both HBV- and AH-infected livers stained intensely with anti--HO-1 antibodies. This result suggests that reduced HO-1 expression in chronic HCV infection is not a generalized response to hepatic inflammation.

The decrease in hepatocyte HO-1 expression that we observed in HCV-infected hepatocytes was also confirmed with confocal immunofluorescence microscopy by use of both red (anti--HO-1) and green (anti--CD68) fluorescence (figure 4). Again, the HCV-infected sections revealed reduced hepatocyte HO-1 staining, whereas sinusoidal-cell staining for CD68 was grossly unaffected, compared with control liver sections (figure 4). Collectively, these data suggest that HO-1 is down-regulated in hepatocytes from HCV-infected livers, whereas the expression of HO-1 in Kupffer cells is not decreased.

**In vitro HO-1 expression in clonal cell lines expressing HCV core protein.** Because of the known transcriptional regulatory properties and pro-oxidant behavior of HCV core protein, we hypothesized that the protein may also affect the expression of HO-1 in hepatoma cell lines in vitro. We previously reported the development and characterization of stable cell lines that overexpress HCV core protein \([5]\). Using immunoblot analysis, we compared the expression of various antioxidant enzymes in these lines. Equivalent amounts of solubilized cellular protein were electrophoresed for each cell line. HO-1 was identified as a 32-kDa band reacting with specific anti--HO-1 monoclonal antibody in the cell lines (figure 5). In E47 or C34 lines that expressed HCV core protein, HO-1 expression was considerably reduced (45%, by densitometry), compared with control parental line. Immunoblots stained for HO-2 did not show appreciable differences between core protein expression and control cell lines. Likewise, the expression of actin, MnSOD, and Cu/ZnSOD was unchanged by HCV core protein. In contrast, the expression of catalase was increased in the E47 line,
regardless of core expression, ~2-fold (by densitometry), in agreement with the findings of Mari and Cederbaum [38].

Further evidence for reduced HO-1 expression in the cell lines that stably overexpress HCV core protein was obtained by the assessment of HO-1 mRNA (figure 6). By use of equivalent amounts of RNA for each RT-PCR reaction, HO-1 mRNA was decreased in lines that expressed core protein, whereas mRNA for GAPDH was unaffected. These experiments also confirmed the expression of HCV core protein and CYP2E1 in their respective cell lines, as we demonstrated elsewhere [5].

Relative differences in the amount of HO-1 mRNA in the clonal cell lines and liver-biopsy samples were studied by use of real-time detection RT-PCR (TaqMan). We used the comparative cycle threshold (Ct) method with separate reaction tubes for target (HO-1) and reference (18S rRNA) sequences, as described elsewhere [39]. Preliminary validation experiments demonstrated that the efficiencies of target and reference signal amplifications were approximately equal, with plots of log input RNA versus ΔCt showing slopes <0.1 (data not shown). We compared the relative differences in HCV-infected versus control liver samples as well as the relative differences in the parental clonal lines C34 and E47 with their respective clones that overexpress HCV core protein (table 1). These experiments revealed that control liver expresses ~30 times as much HO-1 mRNA as does HCV-infected liver. Moreover, the parental C34 and E47 cell lines contained 4.4- and >6-fold more HO-1 mRNA, respectively, than did their clonal lines that overexpress HCV core protein. We also confirmed the up-regulation of HO-1 in the E47 line, compared with C34, as reported elsewhere [40]. Collectively, these data show that HCV infection down-regulates HO-1 expression in the liver and suggest a role for HCV core protein in the process.

DISCUSSION

Free-radical species and other oxidants have been implicated as likely mediators of liver damage resulting from chronic infection with HCV [41–43]. Although the sources of oxidative stress during chronic HCV infection are not understood, the cellular expression of individual hepatitis C viral proteins, such as HCV core and NS5A, increases the cellular generation of ROS [3–5, 44]. Further investigation into the role of HCV proteins in cellular oxidative stress and the induction of antioxidant enzymes is important to fully understand how the virus causes liver injury.

The overall aim of our experiments was to evaluate the relative expression of several key antioxidant enzymes in liver samples from HCV-infected patients and to determine whether similar patterns of enzyme expression also occur in cells stably transfected with HCV core protein in vitro. HCV-infected liver-biopsy samples showed no detectable changes in the expression of MnSOD, CuZnSOD, or catalase, compared with control liver samples (figure 1). These findings confirm an earlier study of MnSOD in HCV-infected liver-biopsy samples, compared with control liver samples [36].

HO-1 is rapidly induced in response to inflammation, hypoxia, and other kinds of cellular stress [20, 21]. The protein also plays a role in the regulation of cellular iron content [45], and human genetic HO-1 deficiency leads to an accumulation of iron in the liver [46]. Consequently, it might be anticipated that the enzyme would be up-regulated in response to HCV infection. In contrast, our findings revealed that HO-1 expression is reduced in both HCV-infected liver samples and cultured hepatoma cells that stably overexpress HCV core protein. Furthermore, we recently reported that classic inducers of HO-1—such as heme, cadmium, and hypoxia—showed an attenuated induction of HO-1 in our clonal cell lines that overexpress core protein [47]. These findings suggest that the ability of core-expressing cells to respond to toxic stimuli may also be significantly impaired.

The results of immunohistochemical and confocal microscopy studies indicated that the reduction of HO-1 protein in the liver appears to represent a selective loss of the enzyme from hepatocytes. This does not appear to be a generalized response to hepatic inflammation, because samples from patients with HBV infection and AH, both of which are inflammatory diseases with histological similarities to chronic HCV infection [25], failed to show reduced staining of HO-1 in hepatocytes.

HO-1 is transcriptionally induced in response to a wide variety of cellular stressors [20, 21]. Increased cellular levels of HO-1 provide cytoprotection from oxidants in animal and cell-culture models [48, 49], although the enzyme’s antioxidant protective mechanisms remain unclear. HO-1 may reduce the availability of cellular heme, a pro-oxidant. The reaction products of heme breakdown (biliverdin and CO) also behave as antioxidants [50]. It is of interest that the genetic deficiency of HO-1 in humans [46], as well as HO-1 knockout mice [45], is associated with the increased deposition of iron in the liver and increased sensitivity to cellular oxidative stress. Furthermore, the severity of HCV liver disease correlates with the amount of siderosis present in liver-biopsy samples [51]. Thus, the reduction of HO-1 by HCV infection may contribute to the occasional siderosis seen in HCV-infected liver-biopsy samples [52] and may also participate in hepatocellular injury.

The mechanism(s) responsible for the reduction of HO-1 by HCV core protein in vitro and HCV infection in vivo require additional study. Nevertheless, our data suggest that the in vitro transcriptional activities of HCV core protein also may extend to in vivo conditions and raise the possibility that the protein influences pathways that are important for the prevention of cellular injury and oxidative stress. In normal liver, HO-1 is predominantly expressed in Kupffer cells. However, in cases of cirrhosis and portal hypertension, the increased expression of HO-1 occurs in hepatocytes [53, 54]. Our immunohistochemical results of HO-1 staining in HBV- and AH-infected samples are
consistent with these reports. In contrast, chronic HCV infection appears to down-regulate the expression of HO-1 and may render hepatocytes more susceptible to the injurious effects of oxidative stress that occur as a result of inflammation or because of pro-

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