

Table 1. Two types of insulin resistance

Type	Mechanism
Peripheral	A shortage of insulin action in the muscle due to deficit in the insulin-induced uptake of glucose into muscles
Central	A shortage of insulin action in the liver due to deficit in the insulin-induced suppression of glucose production in hepatocytes

in transgenic mice. For further confirmation, an uptake of glucose into muscle was determined. There were no differences in the uptake in response to administration of insulin between normal and transgenic mice. Therefore, the insulin resistance in mice transgenic for the HCV core gene is central and hepatic.

HCV Core Protein Suppresses the Transduction of Insulin Signaling in Hepatocytes

Next, we evaluated how insulin resistance elicits in mice transgenic for the HCV core gene. For this purpose, liver homogenate was immunoblotted with antiphosphotyrosine and antiphosphoserine antibodies after insulin receptor substrate (IRS)-1 and IRS-2 had been immunoprecipitated. Tyrosines in IRS-1 were weakly phosphorylated both in normal and transgenic mice before they received insulin, with no differences between them. However, after the administration of insulin, the phosphorylation of tyrosines in IRS-1 increased in normal but not in transgenic mice (fig. 5). Obtained results suggested disturbance in tyrosine phosphorylation as one of the factors responsible for insulin resistance in the liver. There were no differences in phosphorylation of serines in IRS-1 or tyrosines in IRS-2 between normal and transgenic mice. Combined, they provided experimental evidence for the development of insulin resistance by the presence of HCV in the liver that would occur by disturbing the transduction of insulin signaling in hepatocytes (fig. 6).

There remains a possibility for the HCV core protein to directly prohibit phosphorylation of tyrosines, or else, it might inhibit tyrosine phosphorylation via certain cytokines. In our extensive searches for the expression of cytokines in the liver of transgenic mice, only TNF- α and IL-1 β have been found with an increased expression [22]. Therefore, for the purpose of evaluating the role of

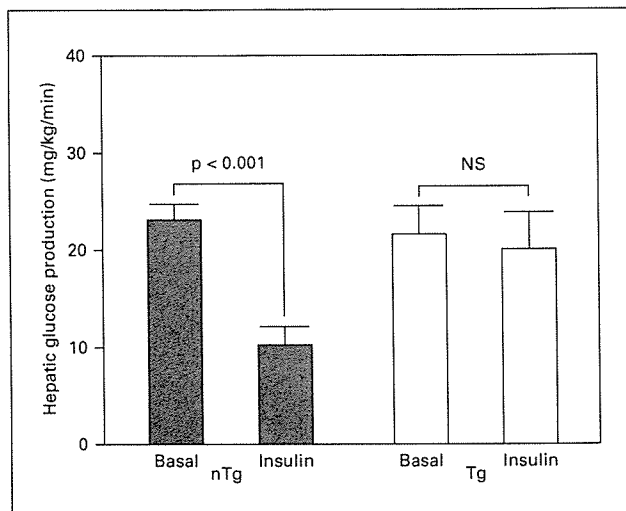


Fig. 4. Characterization of glucose metabolism in transgenic mice. Glucose production in the liver was calculated using the hyperinsulinemic-euglycemic clamp method. NS = Not significant statistically; nTg = nontransgenic mice; Tg = transgenic mice.

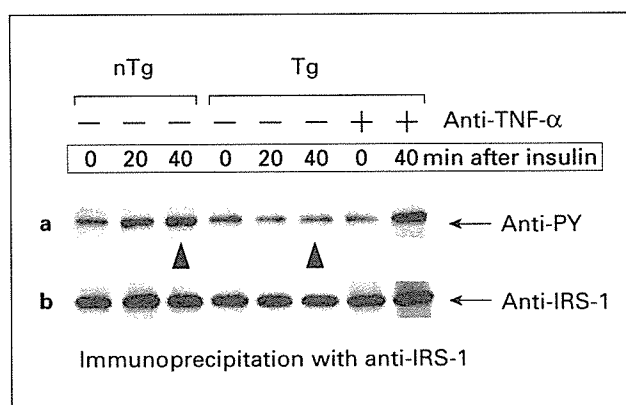


Fig. 5. Phosphorylation of tyrosine in IRS-1 in response to insulin stimulation. Liver tissues from control mice, transgenic mice with or without anti-TNF- α antibody treatment, were analyzed before and 20 as well as 40 min after administration of insulin. Samples were subjected to immunoprecipitation with anti-IRS-1 antibody and then immunoblotted with indicated antibodies. Experiments were performed in triplicate, and a representative picture is exhibited. Immunoblotting with antiphosphotyrosine (PY) antibody (lane a) did not augment phosphorylation of tyrosine in IRS-1 after stimulation with insulin in the core gene transgenic mice (Tg), in contrast to tyrosine phosphorylation markedly enhanced in control mice (nTg). Insulin-stimulated tyrosine phosphorylation was restored 40 min after treatment with anti-TNF- α antibody. Note differences in the intensity of bands 40 min after the administration of insulin (arrowheads). Immunoblotting with anti-IRS-1 antibody (lane b) served as control for the IRS-1 load.

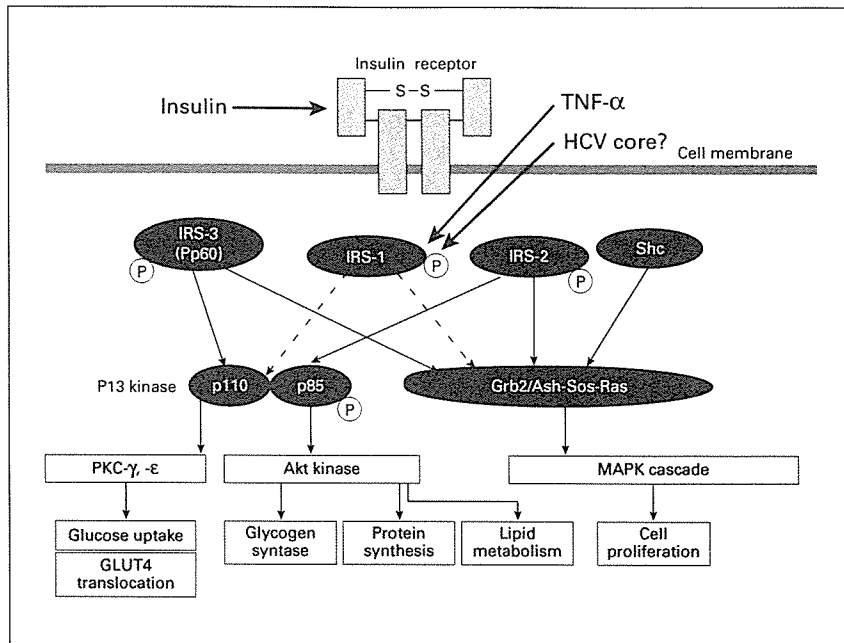


Fig. 6. A proposed mechanism for insulin resistance in HCV infection. HCV itself or elevated levels of cytokines such as TNF- α may inhibit tyrosine phosphorylation of IRS-1 in the liver, suppress intracellular transduction of insulin signal and lead to insulin resistance. PKC = Protein kinase C; MAPK = mitogen-activated protein kinase.

TNF- α in insulin resistance in transgenic mice, serum insulin was determined and an insulin tolerance test performed after they had received anti-TNF- α intraperitoneally. Pretreatment with anti-TNF- α partially improved insulin resistance in mice transgenic for the HCV core gene. Albeit a direct anti-insulin activity of core protein and direct or indirect factors for insulin resistance are not to be excluded, high levels of TNF- α in the liver would be one of the factors for expression of insulin resistance in this mouse model.

Insulin Resistance in Patients with Chronic Hepatitis C

Concurrently with our report in experimental systems, Aytug et al. [23] investigated insulin signaling in biopsied liver specimens from patients with chronic hepatitis C. Specifically, they evaluated changes in IRS-1, IRS-2 and phosphatidyl inositol (PI)3 kinase levels in the liver of patients. With insulin stimulation of biopsied liver samples, insulin receptor proteins and IRS-1 increased, while phosphorylation of tyrosines in IRS-1 decreased to one half of the baseline value, along with a diminished activity for PI3 kinase associated with IRS-1, in patients with chronic hepatitis C. The authors went on to propose a possibility for disturbed transduction of the insulin sig-

naling pathway in the liver to induce insulin resistance in patients with chronic hepatitis C [23]. Their report is quite intriguing in that it opens up the way for evaluating an association between HCV infection and insulin resistance in clinical samples at the molecular level.

The results of Aytug et al. [23] inadvertently coincide with ours in analyzing the mechanism of insulin resistance with the experimental system in mice (vide supra). They unanimously incriminate impaired tyrosine phosphorylation in IRS-1 in the induction of insulin resistance by HCV infection. It struck us as a surprise that the mechanism of insulin resistance induced by HCV infection has been in agreement between clinical samples and experimental animals, in spite of hepatic IRS-2 that was preferred to IRS-1 for its role in development of insulin resistance in former studies [24]. HCV infection is peculiar in that IRS-1 weighs heavier than IRS-2 in the induction of hepatic insulin resistance.

Although our data strongly indicate a hepatic character of insulin resistance in HCV infection, they by no means exclude roles of other factors in the induction of this resistance. There is little expression of the HCV core gene in muscles of our animal model; it is not known if HCV infects muscular cells in patients with chronic hepatitis C. Factors not intrinsic to the liver would have to be evaluated to sort this out, including dysfunction of mitochondria for induction of insulin resistance [25].

Insulin Resistance for Advanced Hepatic Fibrosis

Insulin resistance in HCV infection may have an additional significant clinical implication. In 260 patients with chronic hepatitis C, Hui et al. [26] have tried to establish the relationship between liver histology and indicators of glucose metabolism, as well as insulin resistance represented by the homeostasis model assessment of insulin resistance. They have found that insulin resistance already exists in hepatitis C patients with stage 0 or 1 fibrosis in the liver. This indicates that insulin resistance in HCV infection is not attributable to advanced liver disease. In their study, independent predictors of insulin resistance in HCV infection were body mass index, non-response to antiviral treatment, intensity of portal inflammation and infection with HCV genotype 3 [26]. Furthermore, the homeostasis model assessment of insulin resistance was a significant and independent predictor of the stage and velocity of hepatic fibrosis. The results of the study are of much importance, because they implicate a role of insulin resistance and hyperinsulinemia by inference, in promoting the progression of hepatic fibrosis. Insulin has been proven as an aggravating factor not only in atherosclerosis, but also in systemic inflammation and fibrosis. The liver is no exception to this.

Conclusions: Hepatitis C Viewed as a Metabolic Disease and Outlook for Therapeutic Strategies in the Future

We have demonstrated that HCV per se induces insulin resistance in the animal model. Superimposed high-fat diet and obesity may lead to overt diabetes. Since insulin resistance accelerates the progression of chronic hepatitis C, it would naturally influence the development of HCC. Although the association has not been established between nonalcoholic steatohepatitis and HCC, it needs to be energetically pursued in view of the histological homology of nonalcoholic steatohepatitis to chronic hepatitis C. Drugs for improving glucose metabolism and insulin resistance need to be kept in store in the treatment of hepatitis C patients who have failed to respond to antivirals, because they may well prevent progression of fibrosis and development of HCC in such patients. Traditional 'high-protein and high-calorie' diet, especially advocated in Japan after World War II, is obviously detrimental, except in some patients with advanced cirrhosis. Consultation on the dietary habit with hepatitis C patients should include iron restriction [27] as well as weight control, because high-calorie intakes are likely to accelerate hepatic fibrosis by aggravating insulin resistance.

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Review

Oxidative stress and hepatitis C viral infection

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Abstract

The involvement of oxidative stress in the pathogenesis of hepatitis and hepatocellular carcinoma has been strongly suggested. Oxidative stress is produced by inflammatory processes that occur in hepatitis via immunological mechanisms. In addition, in hepatitis C virus (HCV) infectious disease, some role has been assigned to viral proteins in the induction of oxidative stress. In the presence of hepatic steatosis, insulin resistance and increased levels of some cytokines, all of which are also induced by viral protein expression, oxidative stress is enhanced in HCV infection. In this sense, the role of oxidative stress in the progression of chronic hepatitis and hepatocarcinogenesis is greater in hepatitis C than in other types of hepatitis such as hepatitis B or autoimmune hepatitis. The additive effects of oxidative stress caused by the inflammatory process and that induced by HCV proteins may, furthermore, exert synergistic effects with alterations in intracellular signaling systems such as mitogen-activated protein kinases (MAPK), which are also induced by HCV proteins. These synergistic effects may be responsible for rare characteristics, that is, the high incidence and multicentric nature of hepatocarcinogenesis in HCV infection.
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Keywords: Oxidative stress; Hepatitis C virus; Hepatocarcinogenesis; Lipid peroxidation; Steatosis; Insulin resistance

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1. Introduction

There are approximately 200 million people infected with hepatitis C virus (HCV) worldwide, of which about 1.8 million are in Japan. It is one of the most serious causes of liver disease. It was reported that approximately 70% of those with HCV infection suffer from persistent infection, causing active or inactive chronic hepatitis and that about 30% of patients with chronic hepatitis are assumed to develop cirrhosis within their lifetime. Once HCV infection develops into cirrhosis, hepatocellular carcinoma (HCC) develops at an annual rate of 5–7% [1]. The strong association of oxidative stress with HCV infection has been demonstrated recently and it has become possible to explain at least part of the clinical progression of the disease. The pathogenesis of chronic hepatitis C is not merely ascribed to inflammation caused by viral infection, but the role of viral proteins in the pathogenesis was also reported [2–4]. Of proteins constituting HCV, the core protein, in particular, has various functions with respect to host cells [5] and is closely related to oxidative stress. In this overview, the relationship between HCV infection and oxidative stress is reviewed focusing on the pathological effect of the core protein of HCV, and the significance of oxidative stress in the pathogenesis of liver disease will be discussed.

2. Oxidative stress, reactive oxygen, and the liver

2.1. Oxidative stress and reactive oxygen

The main source of reactive oxygen species (ROS) in hepatocytes is the mitochondria. Outside of hepatocytes, ROS also originate from nicotinamide adenine dinucleotide phosphate

(NADPH) oxidase and xanthine oxidase in Kupffer cells and inflammatory cells. Several percent of consumed oxygen is constantly converted into ROS in the mitochondria accompanied by oxygen consumption in the electron transport system (ETS, Fig. 1). Hepatocytes contain many mitochondria and therefore have a high ROS production. Generated ROS are very unstable and highly reactive, and attack biomolecules such as DNA, lipids, and proteins. The liver not only produces much ROS but is also the center of the anti-oxidative effect in the form of protein synthesis. Oxidative stress refers to the oxidation-reaction-dominant state of the living body induced by an imbalance between the oxidation reaction caused by ROS and the anti-oxidation reaction. Main ROS include superoxide ($\text{}^{\circ}\text{O}_2^-$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO°). ROS are mainly produced from $\text{}^{\circ}\text{O}_2^-$ and converted into stable H_2O_2 through dismutation reaction. H_2O_2 is converted into highly reactive HO° in the presence of a transition metal.

2.2. Antioxidation system and oxidative stress markers

Antioxidants include glutathione (GSH), thioredoxin (TRX), vitamin E, vitamin C, and β -carotene. Reactive oxygen elimination enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase. SOD is induced by oxidative stress and dismutates $\text{}^{\circ}\text{O}_2^-$ to H_2O_2 and oxygen. GSH is a compound belonging to the SH group and is highly abundant in the living body, and the SH group provides electrons to free radicals to stabilize the radicals. GSH exists in a reduced form in cells. Because it is converted into dimeric oxidized glutathione (GSSG) and becomes stable after donating electrons, GSSG prevents free radicals from continuously scrambling for electrons. GPx decomposes H_2O_2 into water

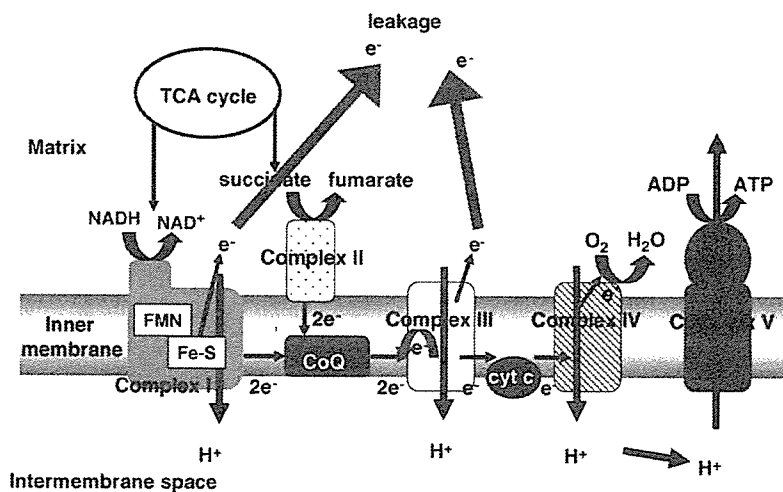


Fig. 1. The electron transfer system (ETS) of the mitochondrion. Most of the oxygen, consumed by mammalian cells, is converted to water via the mitochondrial ETS. However, up to 5% of the electrons entering the mitochondrial ETS can become uncoupled and singly leak out onto oxygen to form superoxide. Therefore, if there is impairment in the mitochondrial ETS function, it can be a cause of the overproduction of reactive oxygen species (ROS). TCA, tricarboxylic acid; NADH, nicotinamide adenine dinucleotide phosphate; FMN, flavine mononucleotide; CoQ, coenzyme Q; cyt c, cytochrome c.

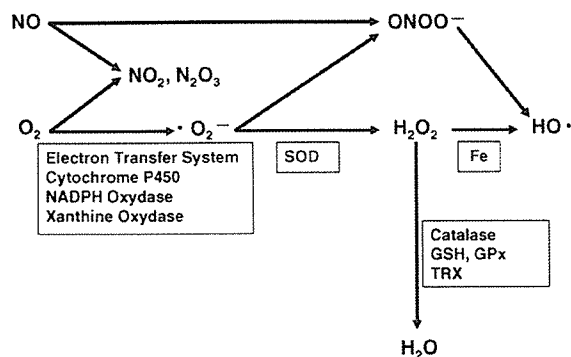


Fig. 2. Generation and scavenging of oxidative stress. SOD, super-oxide dismutase; GSH, reduced glutathione; GPx, glutathione peroxidase; TRX, thioredoxin.

and oxygen with GSH as an electron donor and reduces lipid peroxide to become neutralized. GSSG is converted back to GSH when glutathione reductase transfers an electron from NADPH to GSSG. Catalase in peroxisomes also decomposes H₂O₂ to water and oxygen. TRX is also a protein induced by oxidative stress, and is reduced via the S–S binding of the substrate protein by two SH groups in TRX and acts on the H₂O₂ elimination system via peroxiredoxins (Fig. 2).

ROS cause various forms of cellular damage. 4-Hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) are the peroxidation reaction products of lipids, and 8-hydroxydeoxyguanosine (8-OHdG) is the product of DNA base modification (Fig. 3). These products serve as oxidative stress markers.

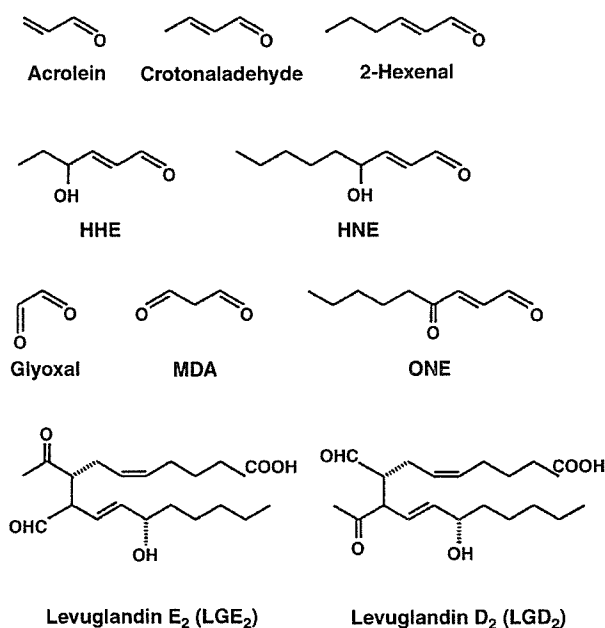


Fig. 3. Representative aldehyde species generated via lipid peroxidation reaction.

3. Viral infection and oxidative stress

3.1. ROS production associated with viral infection

Upon viral infection, ROS are produced by NADPH oxidase and xanthine oxidase in neutrophils and macrophages. In particular, NS3, one of the non-structural proteins of HCV, was reported to induce ROS production by NADPH oxidase in neutrophils [6]. Furthermore, in viral hepatitis, ROS are also produced in hepatocytes through the release of inflammatory cytokines such as TNF- α and IL-1 β from inflammatory cells. Increased hepatic or serum 8-OHdG, HNE and MDA levels are observed in chronic hepatitis C, indicating an increase in ROS production [7–13]. Findings that indicate an increase in the activity of the ROS elimination system including decreased hepatic and blood GSH levels, an increased GSSG/GSH + GSSG ratio, and an increased serum TRX level have been reported [13–16]. The findings of markedly decreased HNE level following viral eradication with interferon [12] and decreased serum ALT and TRX levels following the administration of vitamin E, an antioxidant [17], also demonstrated that oxidative stress plays an important role in chronic hepatitis C.

3.2. Nitric oxide production associated with viral infection

In the presence of an inflammation, inducible nitric oxide synthase (iNOS) is induced in macrophages and hepatocytes by TNF- α and IFN- γ [18–20]. Other investigators reported that protein kinase (PKR) activated by double-stranded RNA formed during virus reproduction in turn activates the transcripts of NF- κ B and IRF-1 to induce iNOS [21]. In the case of HCV, it was reported that its constituent proteins (E2 and non-structural (NS) protein 5A) inhibit PKR activity [22,23], but iNOS induction by viral RNA via PKR is also suspected. Indeed, iNOS synthesis correlates with intrahepatic viral load in chronic hepatitis C [24].

NO is generally synthesized as a non-specific defense reaction to infectious diseases; however, in viral infection, antiviral activity may be present or absent in various viral types [20]. NO is reported to exhibit no antiviral activity against a tick-borne encephalitis virus (TBE-V), flavivirus [25], and NO may also have no antiviral activity against HCV. On the contrary, NO causes cellular damage upon its reaction to O₂ or simultaneously produced O₂•⁻ (reactive nitrogen species, RNS). Upon reaction to O₂•⁻, in particular, NO acts as a strong oxidant with the generation of peroxynitrous acid (ONOO⁻), and ONOO⁻ also produces nitrotyrosine through the nitration of aromatic amino acid residues in the presence of a transition metal. Nitrotyrosine accumulation was observed in correlation to inflammation severity in chronic hepatitis C tissue [26]; suggesting that the production of both NO and ROS increased. ROS and RNS are produced as defense factors for biological viral clearance, but these factors also have cytotoxic effects that

are assumed to contribute to the exacerbation of the disease state.

4. Oxidative stress caused by viral protein

The HCV genome comprises the genes of four structural proteins and six non-structural proteins (Fig. 4), and it has been reported that at least two viral proteins cause oxidative stress in cells. The core protein, a structural protein, was found to have various actions, including the induction of oxidative stress and accumulation of lipids, in experimental studies using cultured cells and transgenic mice [2,27]. Experiments using mice transgenic for the core gene showed an increased ROS production, an increased intrahepatic catalase activity, a decreased intrahepatic GSH level and a decreased GSH/GSH – GSSG ratio indicating an anti-oxidation effect inhibition, although there was no increase in serum ALT level nor a histological finding of hepatitis. Increased levels of intrahepatic peroxide lipids in the core gene transgenic mice with aging as compared with those in the control mice also indicate increased oxidative stress. As a mechanism underlying oxidative stress induction by the core protein, mitochondrial damage is considered. Morphological abnormalities of the mitochondria were observed in the core gene transgenic mouse liver [2], and an increased ROS production caused by damage of the mitochondrial electron transport system was noted in core-protein-expressing cells [27]. Mitochondrial DNA, which has no protective proteins such as histone, is susceptible to damage by ROS [28,29]. Mitochondrial DNA in the core gene transgenic mice showed damage as early as 3-months old. This mitochondrial damage disrupts the synthesis of proteins constituting the electron transport system complex and could also increase oxidative stress caused by damage of the electron transport system.

A study using a cell culture system demonstrated that non-structural protein 5A (NS5A) also causes oxidative stress. NS5A induces endoplasmic reticulum calcium release via

endoplasmic reticulum stress, and this leads to an increased ROS production in the mitochondria [4]. Although the effect of NS5A has not been confirmed yet by other study groups, HCV has the direct action of increasing intracellular ROS production via its proteins, separate from oxidative stress induction by inflammation caused by viral infection. A report that oxidative stress was also observed in HCV carriers with a normal ALT level [13] indicates that it is caused by a direct oxidative stress induction without being mediating inflammatory reactions.

5. Relationship of HCV infection with insulin resistance

The relationship of HCV infection with insulin resistance and type 2 diabetes has been suggested epidemiologically [30–32]. Insulin resistance was also observed in core gene transgenic mice before the onset of hepatic steatosis [33]. A disrupted tyrosine phosphorylation of the insulin receptor substrate (IRS-1) was observed in the liver of these transgenic mice. The analysis of hepatic tissues in patients with chronic hepatitis C not complicated by diabetes showed that insulin receptor and IRS-1 expression levels are elevated in patients with HCV infection, whereas the tyrosine phosphorylation of IRS-1 induced by insulin is inhibited. An excessive oxidative stress may be another potential cause of this insulin resistance. Oxidative stress indirectly blocks the phosphorylation of tyrosine residues of insulin receptors and IRS-1 and inhibits insulin signaling [34].

These reported results thus indicate an insulin signaling disorder in the liver infected with HCV [35]. There has been no report to date directly proving that hepatic insulin signaling disorder in patients with HCV infection is attributable to oxidative stress. However, because diabetes, which is the state of having abnormally high blood sugar levels that cannot be self-regulated by individual organisms, also induces oxidative stress [34], the close relationship between insulin resistance or diabetes and oxidative stress as the cause and the

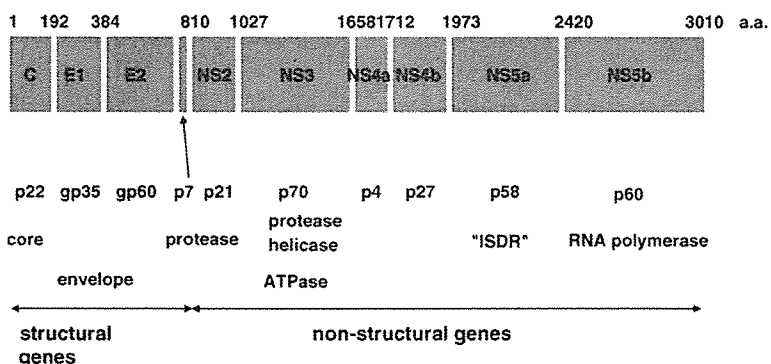


Fig. 4. Structure of hepatitis C virus genome. The genome of HCV consists of two parts, structural and non-structural regions. The former comprises the core and envelope regions, and the latter consists of NS2 to NS5a, which regions chiefly code enzymes necessary for viral replication. NS, non-structural; ISDR, interferon sensitivity-determining region.

result, respectively, is a very interesting issue to investigate in the future.

6. Relationship of HCV infection with hepatic steatosis

Hepatic steatosis is frequently observed in patients with HCV infection. The relationships of HCV infection with intrahepatic viral loads and core protein levels, different prevalence of hepatic steatosis by viral genotype [higher incidence for genotype 3a], and improved steatosis following viral eradication were reported [36–38]. It is presumed from these reports that HCV itself causes hepatic steatosis. A similar hepatic steatosis caused even by the core protein alone was observed in a study using an expression system in cultured cells and transgenic mice, and it was thus suggested that the core protein plays a significant role in hepatic steatosis as the direct action of HCV [39,40]. Hyperinsulinemia induced by insulin resistance mentioned above causes the overloading of the liver with fatty acids from fat cells, and mitochondrial damage inhibits the β -oxidation of fatty acids [41]. Furthermore, the core protein was reported to inhibit microsomal triglyceride transfer protein (MTP) activity that is required when neutral fat is released as very low-density lipoproteins (VLDLs) [42]. All these actions could cause hepatic steatosis. In the liver of non-alcoholic steatohepatitis (NASH) patients, it was reported that β -oxidation in the mitochondria and peroxisomes or the metabolism of fatty acids by cytochrome P450 2E1 (CYP2E1) in microsomes is promoted under an excessive load of fatty acids, resulting in ROS production [43,44]. In HCV infection as well, intrahepatic fat accumulation possibly increases ROS production as in NASH. Because hepatic steatosis in chronic hepatitis C was reported to be a factor for disease progression [45–47], increased oxidative stress associated with hepatic steatosis is presumably involved in disease progression.

7. Iron and reactive oxygen

The iron content in the liver and spleen is high, and transition metals facilitate electron transfer and play an important role in the production of free radicals. Iron in combination with transferrin and ferritin is stable, but an unstable iron ion is freed when ferritin is decomposed by lysosomes [48]. ROS additionally promote iron release from ferritin [49]. A free iron ion catalyzes changes from relatively poor reactive O_2^- and H_2O_2 to a highly reactive HO^\bullet (Fenton reaction) [50,51]. HO^\bullet oxidizes membrane phospholipids, which compose cells and intracellular organelles, and iron forms radicals from produced peroxide lipids, thereby enhancing lipid peroxidation. Iron site-specifically combines with DNA and promotes DNA damage caused by ROS. Iron also increases ROS production by CYP2E1 [52]. A report that an enhanced peroxidation of intrahepatic lipids is attenuated by exsan-

guination in hemochromatosis also supports the involvement of iron in oxidative stress [53].

An excessively high iron content in the liver was observed in chronic hepatitis C [8,54]. Other investigators reported that iron removal therapy by exsanguination of chronic hepatitis C patients significantly improves serum ALT level without affecting viral load [55–57]. Another study showed that hepatic impairment is exacerbated following the administration of iron to chimpanzees with chronic hepatitis C [58]. Furthermore, oxidative stress is decreased by the iron removal therapy for chronic hepatitis C using intrahepatic 8-OHdG level as an index [57]. The above-mentioned reports show the close relationships of chronic hepatitis C with iron metabolism and oxidative stress.

8. Interactions with alcohol

Alcohol metabolism plays an important role in ROS production. Mainly alcohol dehydrogenase (ADH) in the cytosol and CYP2E1 (microsomal ethanol-oxidizing system) in microsomes are responsible for alcohol metabolism in the liver. When alcohol dehydrogenase oxidizes ethanol to acetaldehyde, the reduction from NAD^+ to NADH simultaneously occurs. NADH accumulation causes stress on the mitochondrial electron transfer system, leading to an increased production of ROS [59]. NADH also inhibits xanthine dehydrogenase activity, and xanthine is thereby oxidized by xanthine oxidase with the production of ROS [60]. CYP2E1 is induced by chronic alcohol intake and ROS are produced when CYP2E1 oxidizes ethanol to acetaldehyde [52,61].

There is no significant difference in hepatic peroxide level between core gene transgenic mice at 3–6-months old and control transgenic mice, but hepatic peroxide level significantly increases following the administration of a low dose of alcohol in the core gene transgenic mice [2]. ROS production increases upon glutathione reduction in HepG2 cells, with the co-expression of the core protein and CYP2D1, the latter of which is induced by alcohol [62]. These findings show that the core protein and alcohol in combination increase oxidative stress. Indeed, it was reported that alcohol intake plays a role in promoting the progression of chronic hepatitis C [63,64] and that increased levels of oxidative stress markers such as HNE and lipid hydroperoxide also support these findings [65]. From the viewpoint of oxidative stress also, HCV infection and alcohol intake are both considered to promote hepatic impairment.

9. Hepatocarcinogenesis and oxidative stress

It has been demonstrated that oxidative stress plays a key role in carcinogenesis [66,67]. Animal experiments using hepatocarcinogenesis models with the administration of a chemical substance (diethyl-nitrosamine, peroxisome proliferators) and with the administration of a choline-deficient

amino acid diet also indicates the involvement of oxidative stress [68–72]. In Long Evans Cinnamon (LEC) rats, an animal model that spontaneously develops heritable hepatitis and HCC caused by an abnormal copper accumulation, a congenitally decreased glutathione peroxidase expression level was reported, and the close relationship between oxidative stress and hepatocarcinogenesis was indicated [73].

The epidemiological relationship between HCV infection and HCC is evident [74,75], but the mechanism underlying this relationship has not been fully elucidated yet. Among postulated hypotheses on the mechanism of HCV-associated hepatocarcinogenesis, that of the involvement of the viral protein, in particular, the core protein of HCV is attractive: HCC develops in core gene transgenic mice, and carcinogenesis starts with well-differentiated carcinoma with an excessively high fat content, similar to hepatocarcinogenesis in human chronic hepatitis C, and poorly differentiated carcinoma with a low fat content develops in the form of “nodules in nodules” [76]. Because oxidative stress is increased in the core gene transgenic mice as mentioned above, it is assumed that oxidative stress plays an important role in hepatocarcinogenesis in chronic hepatitis C. Because the development of HCC is also observed in transgenic mice carrying the full-length HCV protein gene, the non-structural protein may have an additive effect to the effect of the structural proteins including the core protein, contributing to hepatocarcinogenesis [77]. NS5A, which was also reported to induce ROS production [4], may also contribute to hepatocarcinogenesis, although ROS induction by NS5A is not unequivocally confirmed yet.

Mitochondrial DNA has no potent protective proteins such as histone and is near the electron transport system, the major ROS production site. Hence, it is 10 to 15 times more susceptible to mutation caused by ROS than nuclear DNA [28,29]. In an investigation of mitochondrial DNA mutation in the human normal liver, both cancerous and non-cancerous liver tissues in patients with HCC showed very high incidences of DNA mutations [78]; thus, a relationship between oxidative stress persistence and hepatocarcinogenesis is suggested.

In the core protein expression system in the hepatic tissue and cultured cells of core gene transgenic mice, the activation of transcription factor AP-1 via mitogen-activated protein (MAP) kinase was observed [79–83]. The activation of the transcription factors AP-1, NF- κ B, and signal transducer and activator of transcription (STAT) 3 by NS5A were also reported [4,84]. The activation of these transcription factors may facilitate cell proliferation, contributing to tumorigenic transformation.

It was also reported that ROS facilitate apoptosis via c-Jun N-terminal kinase (JNK)/p38 MAP kinase or by directly attacking the mitochondria. Apoptosis is a protective mechanism of the host against viral infection and carcinogenesis. Some reports stated that the core protein facilitates apoptosis [85–88], whereas other reports stated that the core protein inhibits apoptosis [89–92]; thus, no fixed view has yet been established. If it indeed inhibits apoptosis, it is assumed that this inhibition proceeds by maintaining oxidative stress and

that the core protein has a beneficial effect against carcinogenesis and persistent viral infection.

In HCV infection, viral proteins such as the core protein and, possibly, NS5A protein induce oxidative stress, intracellular signaling, and transcription factors, which are not reflected in blood ALT level, contributing to the progression of carcinogenesis. Carcinogenesis, however, is slow as is observed in humans and core gene transgenic mice, the latter of which developed HCC in the latter half of their life. Recently, Okanou et al. reported a long-term follow-up study of subjects with persistent HCV infection who had persistently normal ALT levels (PNAL) [93]. In their study, serum thioredoxin levels were not elevated in those with PNAL compared to those with chronic hepatitis. This may apparently seem contradictory to the results of our above-mentioned animal model studies. However, we should realize that anti-oxidant system is also instrumental in the liver. In these relatively younger people with PNAL than those with CH [93], active anti-oxidant system may erase the apparent elevation of ROS. Such a phenomenon was described in a mouse model by Moriya et al. [2], in which ROS was apparently normal in young core gene transgenic mice with the activation of catalase and reduction of GSH. Clinically, the presence of inflammation is thought to facilitate the process of hepatocarcinogenesis.

10. Conclusions

A very close pathological relationship between oxidative stress and HCV infection is observed, as shown by the above overview of relevant publications and discussion. The causes of oxidative stress in HCV infection are considered to include various factors such as mitochondrial damage, endoplasmic reticulum stress, iron accumulation, and lipid accumulation in the liver. Various study results demonstrated that even only viral proteins, mainly the HCV core protein, cause oxidative stress. When inflammation via immunoreactions to viral infection is added to oxidative stress, ROS production is expected to further increase, leading to a state in which the anti-oxidation system cannot cope with. In this sense, inflammation in chronic hepatitis C is considered to be qualitatively different from inflammation observed in other types of hepatitis such as autoimmune hepatitis or hepatitis B [94] (Fig. 5). As a treatment of chronic hepatitis C, the eradication of the virus is ideal. If it is not possible, however, the control of factors that exacerbate oxidative stress, such as inflammation via immune reaction and alcohol, and the relief of oxidative stress by the iron removal therapy and the administration of an anti-oxidation agent are considered to delay the progression of chronic hepatitis.

The development of such new anti-oxidation agents is being awaited. In further studies on the development of new therapies for hepatitis C and control methods for hepatocarcinogenesis in the future, the importance of those focusing on oxidative stress is expected to markedly increase.

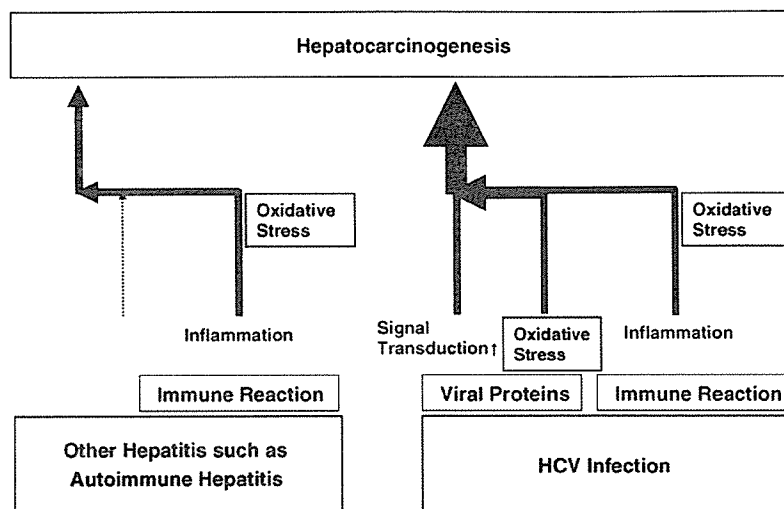


Fig. 5. Oxidative stress and hepatocarcinogenesis in various types of hepatitis (hypothesis). Oxidative stress is generated in all types of hepatitis via inflammation accompanied by continual cell death and regeneration. In HCV infection, HCV itself causes the production of oxidative stress in a synergy with inflammation. In this sense, the quality of “inflammation” in HCV infection may be different from that in other types of hepatitis. Additional impact of HCV proteins on the intracellular signal transduction would provoke the development of HCC. These may explain the conspicuous properties of HCC development.

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Novel Dengue Virus Type 1 from Travelers to Yap State, Micronesia

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Dengue virus type 1 (DENV-1), which was responsible for the dengue fever outbreak in Yap State, Micronesia, in 2004, was isolated from serum samples of 4 dengue patients in Japan. Genome sequencing demonstrated that this virus belonged to genotype IV and had a 29-nucleotide deletion in the 3' noncoding region.

Dengue virus (DENV) is a mosquito-borne flavivirus; there are 4 serotypes, DENV-1, -2, -3 and -4. DENV has been found in ≥ 100 countries and 2.5 billion people live in areas where dengue is endemic. Fifty to one hundred million cases of dengue infection are estimated to occur annually (1). In Japan, outbreaks of dengue fever occurred in Nagasaki, Hiroshima, Kobe, and Osaka from 1942 to 1945, but none thereafter (2). However, ≈ 50 imported dengue cases occur annually in Japan.

The DENV genome is a single-stranded positive-sense RNA of $\approx 11,000$ nucleotides (nt) that encodes 3 structural proteins (capsid, membrane, and envelope) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (3). Surrounding the open reading frame (ORF) are 5' and 3' noncoding regions (NCRs) that form RNA secondary structures (4). These regions are ≈ 100 and ≈ 400 nt, respectively (5).

Dengue fever developed in 9 Japanese patients in 2004 after they returned from Yap state. We report the genetic characterization of RNA from DENV-1 isolates from these patients.

The Study

Yap is the westernmost state of the Federated States of Micronesia and composed of 4 major islands. Yap has a total area of 102 km² and a population of 11,241 (2000 census). The climate is moderate and fairly constant. The mean annual temperature is 27°C. Relative humidity ranges from 65% to 100% (annual mean 83%). Rainfall averages 120 inches a year and is seasonal.

In 1995, a dengue epidemic caused by DENV-4 occurred in Yap state (6), but no dengue outbreaks have since been reported. However, the Yap EpiNet Team reported a dengue outbreak caused by DENV-1 in Yap state that began in the last week of May 2004. A total of 658 reported dengue fever cases (defined by the World Health Organization) occurred as of December 29, 2004. No deaths or dengue hemorrhagic fever/dengue shock syndrome cases were reported (7).

Fever, headache, and diarrhea developed in 7 Japanese adults who visited Yap after their return to Japan in August 2004. DENV infection was serologically confirmed in 5 patients (patients 1–5) by an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA) (Focus Diagnostics Inc., Herndon, VA, USA) and an IgG ELISA (PANBIO Ltd., Brisbane, Queensland, Australia) at the National Institute of Infectious Diseases in Tokyo, Japan. Of these 5 patients, 4 had a primary DENV infection and 1 had a primary dengue infection and a secondary flavivirus infection. DENV infection was serologically confirmed in the sixth patient at another institute. The seventh patient did not visit a medical facility but had symptoms of dengue fever. In addition, 2 other Japanese patients who traveled to Yap in September 2004 were diagnosed with dengue (data not shown).

Four virus isolates (NIID04-27, -31, -41, and -47) were obtained from serum samples from patients 1–4, respectively. Two hundred microliters of serum samples diluted 1:40 was injected onto C6/36 cells in minimal essential medium supplemented with 2% fetal calf serum. The cells were incubated at 28°C for 7 days and culture supernatant fluids were collected. DENV isolates were used for analysis without any further passage.

Complete nucleotide sequencing of RNA of NIID04-27, -31, and -47 and partial sequencing of NIID04-41 were performed. Viral RNA was extracted by using a High Pure RNA extraction kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions, transcribed to cDNA, and amplified by polymerase chain reaction, as described previously (8). The cDNA was purified and sequenced by using the ABI PRISM 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Seventeen pairs of primers were designed based on the DENV-1 NIID02-20 sequence (GenBank accession no. AB178040) and used in the analyses (9).

The nucleotide sequences of the viral isolates were compared with published complete sequences of DENV-1 (Table 1). Sequence alignment and analysis were performed by using ATGC analysis programs (version 4.02; Genetyx Corp., Tokyo, Japan). Phylogenetic analyses of nucleotide sequences were conducted with ClustalX software version 1.83 (<ftp://ftp-igbmc.u-strasbg.fr/pub/ClusterW/>). A phylogenetic tree was reconstructed for

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Table 1. Dengue virus (DENV) strains used in the study

Virus	Strain	Origin	Year isolated	GenBank accession no.
DENV-1	NIID04-27	Yap Island	2004	AB204803
DENV-1	NIID03-41	Republic of Seychelles	2003	AB195673
DENV-1	FGA/89	French Guiana	1989	AF226687
DENV-1	BR/90	Brazil	1990	AF226685
DENV-1	BR/97-111	Brazil	1997	AF311956
DENV-1	BR/01-MR	Brazil	2001	AF513110
DENV-1	Abidjan	Côte d'Ivoire	1998	AF298807
DENV-1	Mochizuki	Japan	1943	AB074760
DENV-1	S275/90	Singapore	1990	M87512
DENV-1	16007	Thailand	1964	AF180817
DENV-1	GZ/80	China	1980	AF350498
DENV-1	A88	Indonesia	1988	AB074761
DENV-1	Cambodia	Cambodia	1998	AF309641
DENV-1	Djibouti	Ethiopia	1998	AF298808
DENV-1	West Pac 74	Nauru	1974	U88535
DENV-1	98901530	Indonesia	1998	AB189121
DENV-1	98901518	Indonesia	1998	AB189120
DENV-1	259par00	Paraguay	2000	AF514883
DENV-1	295arg00	Argentina	2000	AF514885
DENV-1	ARG9920	Argentina	1999	AY277664
DENV-1	NIID02-20	Thailand	2002	AB178040
DENV-1	99-36-1HuNIID	Paraguay	1999	AB111065
DENV-1	01-27-1HuNIID	The Philippines	2001	—
DENV-1	01-32-1HuNIID	The Philippines	2001	—
DENV-1	01-36-1HuNIID	Singapore, Malaysia	2001	AB111067
DENV-1	01-42-1HuNIID	Thailand, Cambodia	2001	AB111069
DENV-1	01-44-1HuNIID	Tahiti	2001	AB111070
DENV-1	01-54-1HuNIID	India	2001	—
DENV-1	01-54b-1HuNIID	India	2001	—
DENV-1	01-61-1HuNIID	Cambodia	2001	AB111071
DENV-1	01-65-1HuNIID	Thailand	2001	AB111072
DENV-1	01-66-1HuNIID	Thailand	2001	—
DENV-2	DENtype2-TB16i	Indonesia	2004	AY858036
DENV-3	DENtype3-TB55i	Indonesia	2004	AY858048
DENV-4	DENtype4-8976/95	Indonesia	2004	AY762085

aligned nucleotide sequences by using the neighbor-joining method. Bootstrap reassembling analysis of 1,000 replicates was used to assess confidence values for virus groupings. The phylogenetic tree was constructed by using Treeview software version 1.6.6 (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.htm>).

The full-length RNA genomes of NIID04-27, -31, and -47 were 10,706 nt. A previous study reported that the full-length RNA genome of DENV-1 was 10,735 nt (8). The differences in the genome sequence between NIID04-27 and the other 2 isolates (NIID04-31 and -47) were subtle; identities with NIID04-31 and -47 were 99.94% and 99.92%, respectively. The results suggest that these 3 isolates belong to the same strain. Therefore, we used NIID04-27 as a representative isolate for further analysis.

To characterize the molecular structure of the genome, the complete NIID04-27 nucleotide sequence was compared with those of other DENV-1 strains available in GenBank (Table 1). NIID04-27 shared sequence identity ranging from 90.9% to 96.9% (Table 2) with 12 other

DENV-1 strains. With respect to the alignment of full-length genomes, some alterations were found in the 3' NCR. These alterations included a deletion of 29 nt starting at the 13th position from the ORF termination codon (Figure 1). The same deletion in the 3' NCR was found in the viral genome amplified directly from the serum sample from patient 1 and was also observed in NIID04-31, -41, and -47.

To further analyze the genetic variation in the 3' NCR of DENV-1, we analyzed the sequence of 24 other DENV-1 strains. Only the NIID03-41 strain, which was isolated in our laboratory from a patient returning from the Republic of Seychelles, had a 17-nt deletion in the 3' NCR (Figure 1). The complete genomes of the 25 DENV-1 strains analyzed showed high levels of nucleotide sequence identity in the 3' NCR, except for a small region of 50 nt immediately after the ORF, which is the hypervariable region. The nucleotide sequence identities in the 3' NCR between NIID04-27 and 12 other DENV-1 strains ranged from 89.3% to 92.5% (Table 2).

Table 2. Pairwise comparisons of full-length genome and 3' noncoding region sequences of dengue virus type 1 (DENV-1) strains*

Virus strain	% identify of nucleotide												
	NIID 04-27	FGA/89	BR/90	BR/97 -111	Abidjan	Mochizuki	S275/90	16007	GZ/80	A88	Cambodia	Djibouti	West Pac74
Full-length genome													
NIID04-27	91.5	91.5	91.3	90.9	93.4	91.9	93.1	92.3	96.9	91.7	91.7	95.6	
FGA/89	89.5		98.3	97.9	94.5	93.7	93.4	93.5	92.5	92.3	92.0	92.1	93.3
BR/90	89.5	99.4		98.8	94.5	93.9	93.7	93.7	92.7	92.3	92.2	92.0	92.9
BR/97-111	89.3	98.7	99.4		94.4	93.7	93.5	93.5	92.5	92.2	92.1	91.8	92.8
Abidjan	89.7	94.0	93.8	94.2		92.9	92.8	92.9	92.1	92.0	91.7	91.6	92.3
Mochizuki	91.2	94.8	94.7	94.9	93.4		95.0	95.4	96.1	94.6	95.3	95.2	95.1
S275/90	91.2	91.7	91.5	91.9	96.6	94.4		93.8	96.1	93.0	96.4	95.2	93.7
16007	90.8	96.2	95.9	95.7	93.2	97.4	93.4		94.2	94.2	93.7	93.5	94.7
GZ/80	90.2	94.1	93.8	94.4	93.6	97.4	94.4	95.7		93.3	96.7	98.0	93.9
A88	92.5	93.8	93.6	93.8	92.3	96.8	92.9	96.8	95.5		92.7	92.7	97.2
Cambodia	89.9	92.1	92.1	92.1	96.4	94.4	96.9	93.6	95.1	92.8		95.9	93.4
Djibouti	89.5	92.1	91.9	91.9	95.3	95.3	96.9	94.0	95.5	93.2	97.6		93.3
WestPac74	91.4	95.1	94.9	95.1	93.8	93.8	93.2	97.2	95.1	97.2	93.0	93.4	
3' noncoding region													

*The percentage nucleotide sequence identities of the complete genomes are shown in the upper right half of the table. The percentage nucleotide sequence identities of the 3' noncoding region of the genomes are shown in the lower left half of the table. The percentage sequence homologies between NIID04-27 and each of 12 other DENV-1 strains are shown in boldface.

To understand the genetic relationships and evolution of DENV-1 strains, we also performed phylogenetic analysis of the fully sequenced DENV-1 strains that included NIID04-27 (Figure 2). NIID 04-27 belonged to genotype IV along with A88, 98901518, 98901530, NIID03-41 and West Pac74. This cluster was called the Pacific group in a previous report (10). NIID04-27 and NIID03-41 are the first DENV-1 strains to have deletions in 3' NCR.

Conclusions

We have genetically characterized DENV-1 isolate NIID04-27 by determining its complete nucleotide sequence and comparing the sequence with most of the available DENV-1 full-length sequences. Sequence heterogeneity in the 3' NCR of the genus *Flavivirus* has been reported for tickborne encephalitis virus, Japanese encephalitis virus, DENV-2, and DENV-4 (11-13). For example, DENV-2 isolated in Texas, Peru, Venezuela, Mexico, and Puerto Rico had a 10-nt deletion starting at

the 19th nucleotide position from the ORF termination codon (13).

The terminus of the 3' NCR has a conserved sequence and secondary structure. The functions of the 3' NCR of flaviviruses have not been fully determined. The 3' NCR in flaviviruses affects RNA replication but does not affect viral translation (14,15). Introduction of a 30-nt deletion starting at the 212th position from the ORF termination codon in the 3' NCR of DENV-4 reduced the ability of the virus to propagate in vivo and in vitro (16).

We have identified a 29-nt deletion in the 3' NCR of DENV-1 isolated from a dengue patient returning to Japan from Yap. Isolates from 3 other patients infected in the same outbreak also had the same deletion. The DENV-1 strain with a 29-nt deletion in the 3' NCR was responsible for the dengue epidemic in Yap in 2004. The biologic characteristics induced by this deletion should be further analyzed.

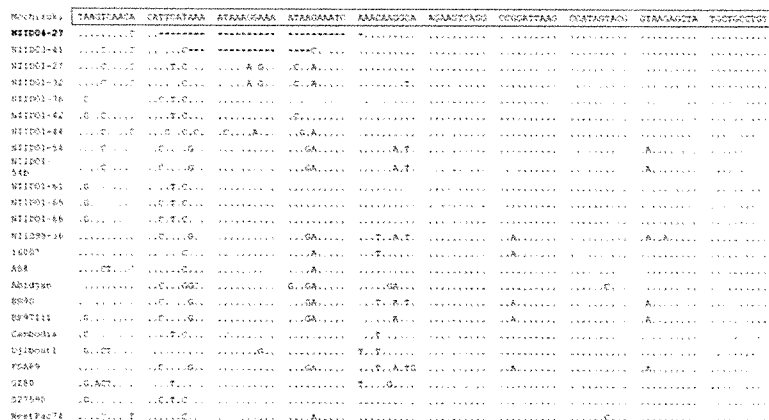


Figure 1. Nucleotide sequence alignment of the variable region in the 3' noncoding region of dengue virus type 1 strains, including NIID04-27 sequenced in the present study. The Mochizuki strain was used as the consensus sequence, and the sequence of 100 nucleotides immediately downstream of the open reading frame termination codon is shown at the top. Solid dots indicate nucleotides identical to the consensus sequence and hyphens indicate deletions.

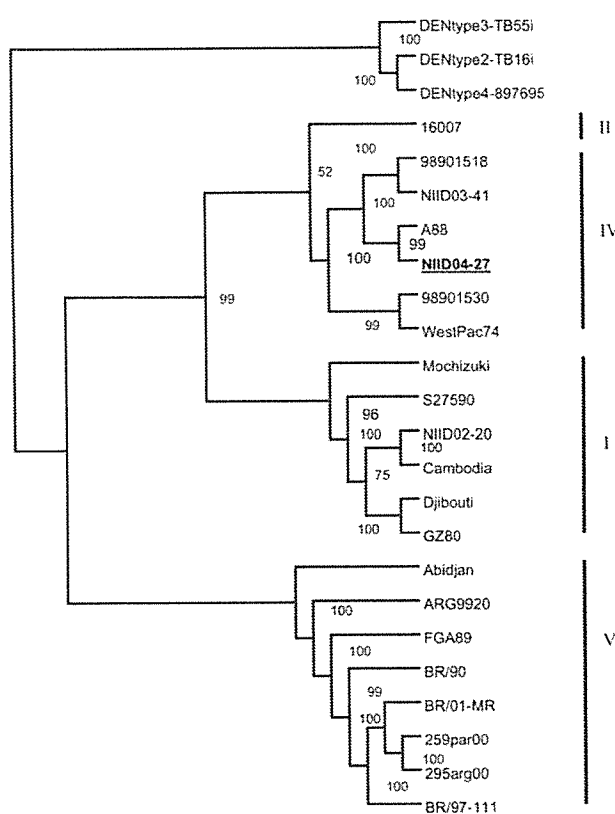


Figure 2. Phylogenetic tree based on the full-length genome sequence of 21 available dengue virus (DENV) type 1 strains and DENV-2, -3, and -4. The multiple sequence alignments were obtained with ClustalX, and the tree was constructed by the neighbor-joining method. The percentage of successful bootstrap replicates is indicated at the nodes. The NIID04-27 strain is indicated in boldface. Genotypes I, II, IV, and V correspond to DENV-1 genotypes as defined by Goncalvez et al. (10).

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Editorial

Oxidative stress and apoptosis in hepatitis C: the core issue

Article on page 257

Hepatitis C virus core protein inhibits deoxycholic acid-mediated apoptosis despite generating mitochondrial reactive oxygen species

HARA Y, HINO K, OKUDA M, et al.

Extra- as well as intracellular stimuli elicit a wide range of responses, such as cell proliferation, differentiation, survival and apoptosis, via the regulation of intracellular signaling. Recent studies have revealed that stress-responsive signal transduction pathways are stringently regulated by the intracellular redox state.¹ The redox state of the cell is determined by the delicate balance between the levels of oxidizing and reducing equivalents, including reactive oxygen species (ROS) and endogenous antioxidants. The production of ROS, a representative of oxidative stress, fluctuates in response to alterations in both external and internal environments and, in turn, triggers specific signaling cascades, such as mitogen-activated protein kinases, which determine cell survival or death. Thus, ROS are profoundly involved in cell death or apoptosis.

In the liver, ROS are also key cytotoxic and signaling mediators in the pathophysiology of liver diseases, including viral hepatitis, in which hepatocytes and resident and infiltrating phagocytes can generate ROS. While ROS are able to cause cell death through massive lipid peroxidation, they also act to modulate signal transduction pathways by affecting redox-sensitive enzymes, transcription factors, and organelles, including mitochondria and endoplasmic reticulum. ROS, thus, directly regulate apoptotic and necrotic cell death.² In addition, ROS have indirect effects on the pathophysiology of cell death by supporting protease activity via inactivation of antiproteases.

In hepatitis C virus (HCV) infection, both ROS and apoptosis are closely involved in the process of progressive liver diseases from chronic hepatitis to cirrhosis and hepatocellular carcinoma (HCC).^{3–7} ROS are assumed to play a major role in the pathogenesis of chronic hepatitis, which is characterized by continual

cell death followed by regeneration. In this condition, the viral proteins of HCV, the core and nonstructural (NS) 5A proteins, have been shown to play a role in inducing ROS as well as in modulating apoptosis of hepatocytes.

The HCV genome comprises the genes of four structural proteins and six nonstructural proteins, and at least two of these viral proteins have been reported to cause oxidative stress in cells. The core protein, a structural protein, has been found to have various actions, including the induction of oxidative stress and the accumulation of lipids, in experimental studies using cultured cells and transgenic mice.^{7,8} Experiments using mice transgenic for the core gene showed increased ROS production, increased intrahepatic catalase activity, a decreased intrahepatic glutathione (GSH) level, and a decreased GSH/GSH-GSSG (dimeric oxidized glutathione) ratio, indicating inhibition of antioxidation effects, although there was neither an increase in the serum alanine aminotransferase (ALT) level nor a histological finding of hepatitis.⁷ Increased levels of intrahepatic peroxide lipids in the core gene transgenic mice with aging, compared with levels in control mice, also indicate an increase in oxidative stress. One possible mechanism underlying oxidative stress induction by the core protein is mitochondrial damage. Morphological abnormalities of the mitochondria have been observed in core gene transgenic mouse liver,⁷ and increased ROS production caused by damage to the mitochondrial electron transport system has been noted in core protein-expressing cells.⁸ Mitochondrial DNA, which has no protective proteins such as histone, is susceptible to damage by ROS. Mitochondrial DNA damage in the core gene transgenic mice appeared when they were as young as 3 months old. This mitochondrial damage disrupts the synthesis of proteins constituting the electron transport system complex and might also increase oxidative stress caused by damage to the electron transport system.

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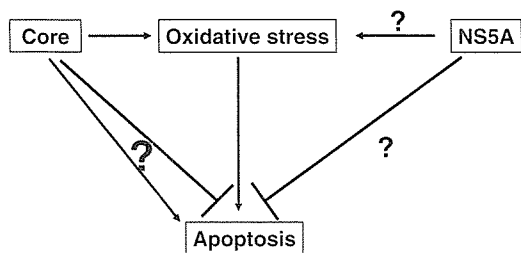


Fig. 1. Relationships among oxidative stress, apoptosis, and HCV proteins

A study using a cell culture system demonstrated that the NS5A protein also causes oxidative stress. NS5A induces the endoplasmic reticulum to release calcium by causing stress to the endoplasmic reticulum, and this release leads to increased ROS production in the mitochondria.⁹ Although the effect of NS5A has not yet been confirmed by other study groups, HCV has the direct action of increasing intracellular ROS production via its proteins, separate from the induction of oxidative stress as a result of inflammation caused by viral infection (Fig. 1). A report that oxidative stress is also observed in HCV carriers with a normal ALT level¹⁰ indicates that it is induced directly, without any mediating inflammatory reactions being necessary.

In contrast to the production of ROS by the core protein, which is now quite evident, the role of HCV core protein in apoptosis is rather controversial. Regarding the HCV-induced apoptotic mechanism, the HCV core protein may have a regulatory function in modulating apoptosis, either by enhancing or inhibiting it. In particular, the core protein exhibits both proapoptotic and antiapoptotic actions, depending on experimental conditions and the type of cells used,^{11–14} whereas both the NS3 and the NS5A proteins have antiapoptotic effects (Fig. 1).¹⁵ Modulation of apoptosis may involve binding of the core protein to the intracellular signal transducing portion of death receptors such as TNF- α , Fas, or lymphotoxin- $\alpha\beta$. Thus, HCV proteins may modulate hepatocyte apoptosis by indirect rather than by direct mechanisms. The real role of the core protein in the apoptotic process is, thus, not defined yet.

In the current issue of *Journal of Gastroenterology*, Hara et al.¹⁶ tried to elucidate this core issue of HCV pathogenesis by separating the two properties of the HCV core protein with cultured cells, Huh-7 and HeLa. They confirmed that the core protein induced ROS, which was followed by activation of the scavenging system and insults to the cellular DNA, as shown previously.⁶ In the study by Hara et al.,¹⁶ the core protein inhibited the proapoptotic action of deoxycholic acid (DCA), which is known to cause both ROS production and apoptosis. Thus, the core protein seems to act to

oppose the proapoptotic function of ROS, which ROS are also induced by the core protein itself. Such apparently opposing actions of the core protein, the production of ROS and the inhibition of apoptosis, might well explain the mode of hepatocarcinogenesis in HCV infection: hepatocytes with ROS-induced DNA damage may evade apoptosis by another effect of the core protein that inhibits apoptosis. Such a mechanism, similar to one previously postulated by other researchers,³ in which both ROS production and mitogen-activated protein kinase activation are ascribed to the core protein, may clarify how cells with DNA damage can survive and develop into buds of HCC. Regrettably, the current study was done using DCA as an agent to induce both apoptosis and ROS, making it difficult to interpret the authentic role of the core protein in the execution of such biological functions. As noted above, the effect of the core protein in apoptosis varies depending on the system used. Therefore, further studies using different systems may be necessary to bring a conclusion to the core issue of HCV-induced pathogenesis associated with the multipotential HCV core protein.

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