Hepatitis C Virus Core Protein: An Update on Its Molecular Biology, Cellular Functions and Clinical Implications

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Key Words

Core protein · Hepatitis C virus · Hepatocellular carcinoma · Apoptosis

Abstract

The present review article is an update on various features of hepatitis C virus (HCV) core protein including its molecular biology, role in HCV replication, involvement in HCV pathogenesis, etiological role in hepatocellular carcinogenesis, significance in diagnosis and vaccination against HCV infection. Core protein is a structural protein of HCV virus and has only recently been characterized. It was found to play a major role in HCV-induced viral hepatitis. Although published information shows a lot about the clinical significance of HCV core protein, several studies are still needed to demonstrate its exact significance in viral biology and underlying HCV pathogenesis.

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Introduction

Hepatitis C virus (HCV) causes a spectrum of liver diseases ranging from an asymptomatic carrier state to hepatocellular carcinoma (HCC). HCV infection has been detected globally and poses a serious public health problem throughout the world. The majority of individuals infected with HCV fail to resolve the infection and

suffer from chronic hepatitis. Infection with HCV has been identified as a leading cause of HCC in many countries around the world [1]. Evidence of an important role for HCV infection includes high seroprevalence of anti-HCV antibodies among patients with HCC and documented progression from chronic hepatitis to cirrhosis to HCC. Most of these patients have HCV RNA present in serum as well as in liver tissue and, in many cases, within the tumor itself.

While intravenous drug use has become the main route of transmission in most industrialized countries, blood transfusion still represents a major contamination risk in developing countries where blood screening is not widely implemented. The prevalence of HCV infection in various locations around the world ranges from 0.5 to 10% [2]. It is believed that more than 70% of HCV-infected patients become chronic carriers [3] with the risk of developing cirrhosis and HCC. While HCV has infected an estimated 175 million people worldwide [4], only a minority of these benefit from antiviral therapies. The development of a vaccine is thus highly desirable.

Molecular biology of HCV has revealed that it has different structural components with diverse functions [5]. The nonstructural component of HCV, HCV core protein has attracted special attention after its characterization and various reports on its important role in HCV pathogenesis. This review is an update on HCV core protein with emphasis on its structure, cellular functions and clinical implications during HCV infection.

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Molecular Structure

HCV Genome

After the genome of HCV was cloned in 1989, this virus was classified as a new member of the Flaviviridae family [6, 7]. HCV is reported to have 6 different genotypes and at least 30 subtypes [8, 9]. HCV has a positive sense, single-stranded RNA genome that has a single long open reading frame flanked by 5' and 3' untranslated regions (UTRs). It codes for polypeptides amounting to a total of 3,000 amino acids. These are subdivided into 3–4 structural proteins at the amino-terminus (core, E1 and E2/P7) and six nonstructural proteins [NS2 (protease) NS3 (protease-helicase), NS4A (co-factor for NS3), NS4B, NS5A (serine phosphoprotein) and NS5B (RNA polymerase)] at the carboxyl terminus [5].

The presence of an internal ribosome entry site for initiation of polyprotein translation has been reported for the highly conserved 5' UTR [10]. The 3' UTR is thought to be critical for RNA replication and packaging and has been found to be essential for infectivity of HCV cDNAs [11]. The polyprotein is processed by both viral and cellular proteases into at least 10 polypeptides including structural and nonstructural proteins [12]. Between structural and nonstructural proteins, an alternative cleavage can lead to the formation of a small protein named p7 [13].

HCV Core Protein Expression

Physical association of the core protein with E1 and E2 glycoproteins, may have a role in virus morphogenesis [14, 15]. In the absence of microsomal membranes, the core protein is synthesized as a polypeptide of ~22 kDa. Simultaneously, additional polypeptides of higher molecular mass may also appear. All these represent complex oligomeric aggregates of the core protein. However, in the presence of microsomal membranes, the polypeptide profile of core is changed. This change is likely derived from a posttranslational proteolytic cleavage of the signal peptide at the carboxy terminus. Most of the core analysis reported here has been made in artificial core expression systems in the absence of a complete HCV replication cycle.

Biophysical Characterization of the Core Protein

Biophysical characterization of the core protein indicates that the C-terminal residues 125–179 are critical for the folding and oligomerization of the core protein. The mature core protein contains significant secondary structure and associates as a large multimer of ~24 monomers,

while the C-terminally truncated core protein is unstructured and monomeric [16]. Additionally, the tryptophan-rich region, previously shown to facilitate self-association *in vivo*, was not responsible for this interaction *in vitro*.

The biophysical profile of the C-terminally truncated core is different from that of the mature core protein. It implies that this truncated core may interact with viral and host-cell factors differently from the mature core protein. The lack of structure in C-terminally truncated core suggests that it is likely to recognize host macromolecules through sequence-specific interactions, while structured noncontiguous epitopes may regulate mature core protein interactions with host proteins.

It is also possible that the C-terminal deletion core proteins used to investigate host-cell interactions became structured through their interaction with nucleic acid [17, 18]. Since the core protein nonspecifically binds nucleic acid *in vitro* [19, 20], it is likely that the core protein is associated with nucleic acid in cell culture. Possibly the binding of nucleic acid to the core protein influenced the reported interactions between the C-terminal deletion core proteins and host-cell proteins or the multimerization of the core protein in cell-based systems [21, 22].

The role of the multimeric mature core protein is unknown. The formation of the 11S species of the core protein may be an intermediate in the assembly pathway. The accumulation of the core protein at the site of assembly may accelerate the formation of the capsid shell and decrease the number of additional interactions that the core protein must make upon assembly. However, results from self-assembly of the 11S core protein into nucleocapsid-like particles imply that additional interactions or steps are required for the 11S core proteins to form particles consistent with the morphology and size of nucleocapsids from infectious viruses [17].

Role of the Core Protein in Cell Regulation and HCV Replication

Many studies have focused on the biological effects of HCV core in cells, its activities on transcription regulation, signal transduction and cell-cycle regulation. The core protein is the viral nucleocapsid protein that binds and packages the viral RNA genome. Core has been shown to form multimers [21, 23] and can efficiently self-assemble *in vitro* into nucleocapsid-like particles. In addition, core is able to interact with the envelope protein (E1) [23]. Taken together, these data suggest that core, along with the envelope proteins, forms the capsid of

a

10 20 30 40 50 60 70 80 90

MSTNPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATRKTSERSQPRGRRQPIFKARRPEGRMTAQPGYPMPLYGNEGCGNAGM

→ Domain 1

100 110 120 130 140 150 160 170 180 190

LLSPRGSRPSWGPDPRRRSRNLGRVIDTLTCGFADLMGYIPLVGAPLGGAARALAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASA

Domain 2

Domain 3

Fig. 1. Schematic representation of HCV core protein. **a** Amino acid sequence of HCV core protein. HCV core protein constructs expressed in *Escherichia coli* (**b**) and *Pichia pastoris* (**c**).

HCV. Core is localized in the cytoplasm, but deletion of the C-terminal hydrophobic region causes core to translocate to the nucleus [24–26]. Core interacts with several cellular proteins including tumor necrosis factor, lym-

photoxin- β receptor [24, 27–29] and RNA helicases [30–33]. Since full-length core is found in the cytoplasm, its role in transcriptional regulation could be through its interaction with cytoplasmic proteins and signal transduc-

tion pathways [33–36] so as to cause the transcription or repression of nuclear genes. Simultaneously, core has been reported to regulate apoptosis [37–38], steatosis [39], tumorigenesis [40] and abnormal lipid metabolism [41].

A novel interaction between core and NS5A using the yeast two-hybrid method has been reported [42]. The minimal region of NS5A required for binding with core contains the ISDR, PKR-binding and proline-rich regions. However, it is possible that the two proteins formed a complex through other proteins. Core has been shown to be associated with lipid droplets [43, 44], while NS5A is localized to the perinuclear region.

The function of the core-NS5A interaction for viral assembly or replication is not clear. Core binds specifically to the 5' NCR of the HCV genome and possibly suppresses its translation [19]. Alternatively, core-NS5A may couple viral replication to viral assembly, since NS5A is likely to be involved in replication and core is the nucleocapsid protein that binds the viral RNA.

Binding between the two proteins is apparently not necessary for NS5A cleavage, as other apoptotic signals can induce NS5A cleavage [45] in the absence of core. However, cells infected with HCV will express core, which could provide an intrinsic apoptotic signal in cells infected with HCV; the level of core may not be high enough to induce programmed cell death, but other factors such as cytokine stimulation [26] and the activation of certain signal transduction pathways involving Fas signaling [46] could enhance the proapoptotic signal of core. NS5A modulates cell-cycle regulatory genes and promotes cell growth. Coexpression of NS5A with core does not reduce apoptosis. It is not evident from these observations that NS5A modulates the apoptotic effect of core.

To activate transcription, both core and the serine phosphoprotein (NS5A) need to be translocated into the nucleus. This may be made possible by the deletion of regions of NS5A and core that contain the cytoplasmic retention signal [24, 25, 47]. For NS5A, the cleavage by caspases produces N-terminally deleted forms that can enter the nucleus and act as transcription activators. Core, when expressed in mammalian cells, is truncated at the C-terminal [48], which should remove the hydrophobic tail that localizes core to the cytoplasm. Nuclear-localized core could not be detected in immunofluorescence experiments, possibly because full-length core complexes with the truncated core and prevents the truncated core from being transported to the nucleus [48]. Fractionation methods to separate nuclear proteins from cytoplasmic

proteins cannot definitively determine the presence of nuclear-localized NS5A protein as nuclear fractions are inadvertently contaminated by cytoplasmic or membrane-associated proteins. Failure to detect nuclear-localized forms of NS5A and core by immunofluorescence does not exclude the possibility that small amounts of these proteins are sufficient to affect transcription of certain genes that may lead to pathogenesis in the long term. The identification of genes that are regulated by NS5A and core individually or in combination would be of interest for the understanding of HCV-related pathology [49].

Role of the Core Protein in HCV Pathogenesis

Effect of Core on Apoptosis

Although diverse effects of the core protein on apoptosis have been reported, the underlying mechanisms are not fully understood [39]. The HCV core protein exhibits both proapoptotic or antiapoptotic actions. Modulation of apoptosis may involve binding of HCV core protein to the intracellular signal-transducing portion of death receptors and displacement of signaling molecules [50]. Previous studies showed that hepatocytes resemble type II cell lines, in which Fas-induced death is dependent on mitochondria [51], and that bcl-2 members act as a selective link between upstream signals and downstream death effectors [52–53]. In a study using an RNase protection assay and Southern blotting, it was demonstrated that not only Bcl-x mRNA, but also Bcl-x_L protein was increased in the core-producing HepG2 cells. As Bcl-x_L is known to block the cleavage-mediated activation of procaspase-3, a critical downstream effector caspase in the apoptotic pathway [54–56], the core-producing HepG2 cells were considered to inhibit procaspase-3 activation through enhanced expression of Bcl-x_L protein.

Although the core protein activates the NF-κB pathway [39, 57–59], the enhanced expression of Bcl-x_L by the core protein was not dependent on the NF-κB site in the Bcl-x_L. The regulation of Bcl-x_L expression appears to be quite complex, and the contribution of NF-κB to this process may depend on cell type or activating stimuli. In fact, other studies failed to reveal alterations in Bcl-x_L transcript levels by NF-κB activation [58–62].

The Fas system is known to play an important role in liver cell injury by HCV and in the clearance of viruses [63]. The ability of the core protein to inhibit the Fas-mediated apoptotic pathway by upregulation of Bcl-x_L expression may help in evading host antiviral defense mech-

anisms. It was reported that the expression of Bcl-x_L is high in HCC in transgenic mice [64]. Therefore, Bcl-x_L-expressing cells by their capacity to inhibit apoptosis could account for the development of HCC in hepatitis C patients.

Immunomodulatory Role of HCV Core

The immunomodulatory role of HCV core protein in the inhibition of T cell responsiveness has characterized the effect of HCV core on T cell activation through its interaction with gClqR. Upon stimulation of human PBMC with either Con A or anti-CD3/CD28, it was found that HCV core inhibited the proliferation of T-lymphocytes in a dose-dependent manner. In addition, the production of IL-2 and IFN- γ [65–67] in core-treated cells was markedly diminished, as compared with control cells. The addition of high doses of exogenous rIL-2 (50–100 U/ml) to core-treated T-cell cultures partially restored the core-induced inhibition of T cell proliferation, suggesting that HCV core might interfere with the expression of IL-2R or its downstream signaling events. Indeed, HCV core affected the expression of high-affinity IL-2R by downregulating IL-2R α chain expression. Subsequent analysis of the effect of HCV core on the ERK/MEK MAP kinase showed that HCV core inhibited the activation of ERK/MEK MAP kinase, which led to the inhibition of IL-2 and IL-2R α chain gene transcription. These results suggest that the impaired activation of ERK/MEK MAP kinase due to the core/gClqR interaction inhibits the transcription of early genes involved in T cell activation and leads to the suppression of T cell responsiveness.

It is important to point out that patients chronically infected with HCV exhibit immune dysfunction with a Th2-dominant cytokine profile, while Th1 cytokines are prominent in those with self-immune HCV infection [68–71]. The decreased levels of Th1 cytokines in the periphery of chronic HCV patients can be recovered by treatment with IFN- α and ribavirin [72]. The strength and quality of both Th1 cell and CTL responses have been reported to play a crucial role in recovery from HCV infection [73, 74]. The immunomodulatory function of HCV core through its interaction with the gClqR, as described in this report, may play a critical role in the establishment of HCV persistent infection during the early viral infection by suppressing T cell responses including the IL-2 and IFN- γ production. A critical issue relating to the role of core/gClqR-induced immune suppression in chronic HCV infection is the presence of circulating core protein in the blood of HCV-infected patients that could

potentially interact with peripheral T-lymphocytes. Strikingly, it has been reported that HCV core protein was secreted from tissue culture cell lines [75], and that the circulation of free HCV core protein has been detected in the plasma of HCV-infected patients [76, 77]. The potential impact for circulating HCV core protein to suppress host immune responses of HCV is further supported by several reports of high levels of the core protein detected during the early stage of infection before the production of anti-core Ab [78, 79]. These studies support the views of core-induced immune suppression in HCV persistence. During the early acute phase of HCV infection, circulating core protein (i.e. core protein free of anticore Ab binding) could inhibit T cell responses by binding to the gClqR on peripheral T cells. Studies on underlying mechanisms of core/gClqR-induced immunosuppression will provide a rational basis for developing therapeutics and immunization strategies.

Experiments carried out in cell lines expressing various HCV proteins alone or in different combinations revealed that the expression of the structural region (core-E1-E2-p7) clearly impaired IFN- α -mediated antiviral activity, indicating that the expression of the structural proteins alone may interfere with the IFN system. In cells expressing the core protein in very high levels, there was also some impairment of the antiviral response. Thus, HCV core protein has significant effects at the cellular level. On one hand it can stimulate various cell signaling pathways such as NF- κ B and AP-1 [34], while at the same time it can inhibit TNF- α -mediated signaling and regulate cell growth and apoptosis [80]. In mice, HCV core expression impaired their antiviral resistance [81], suggesting that the core protein alone may have immunosuppressive or anti-IFN activities.

Role of Core in Oxidative Stress

HCV core protein directly produces oxidative stress and changes in mitochondria. Several possible mechanisms could explain core-induced changes in mitochondrial function. One explanation is that the core protein alters signal transduction pathways that promote the mitochondrial permeability transition. The core protein is known to bind to the cytoplasmic domains of the lymphotoxin- β receptor [82] and tumor necrosis factor receptor-1 [26]. Oligomerization of each of these receptors initiates a signaling cascade that involves activation of caspase-8, proteolytic activation of Bid, and translocation of activated Bid from cytosol to mitochondria. This results in mitochondrial permeability transition, release of cytochrome-C, and ultimately cellular apoptosis [83].

Other evidence also suggests that changes in signaling to the mitochondria are not the primary effect of the core protein. In a recent study in transgenic mice, Machida et al. [84] observed no change in caspase-8 or Bid activation resulting from expression of HCV proteins in the liver. In preliminary studies, they also did not observe any effect of the core protein on the activation state or subcellular localization of Bid. This evidence suggests that core-induced ROS production is not a consequence of caspase-mediated signaling to the mitochondria [43].

An alternative hypothesis is that the core protein interacts directly with mitochondria, impairing electron transport, and thereby increasing ROS production. The core protein has been previously demonstrated to be associated with the ER⁵ and intracellular lipid droplets [44, 75]. In the Huh-7/191-20 cells, the core protein colocalized with perinuclear mitochondria, was present in purified mitochondria and ER fractions, and was expressed in structures adjacent to the mitochondria. Immunoelectron microscopy in transgenic mice has also shown an association of the core protein with mitochondria [85]. Close interactions of ER and mitochondria have also been observed in other cells [86].

Evidence obtained from studies by Okuda et al. [87] does not directly show a functional effect of the core protein on mitochondria, but it suggests that mitochondria are the major spheres of ROS because DPI, an inhibitor of electron transport in flavoenzymes, completely abolished the core-induced increment in ROS content. This inhibitor, which was not toxic to their cells under these conditions, blocks mitochondrial complex I at a proximal site and has been shown to prevent almost all mitochondrial ROS production [88]. DPI can also inhibit ROS production from microsomal NADPH oxidase. Two other mitochondrial electron transport inhibitors, rotenone, a distal complex I inhibitor, and antimycin A, a complex III inhibitor, increase mitochondrial ROS production by themselves [89] and thus could not provide information on core-induced ROS.

The observations of Okuda et al. [87] suggest a new model for the pathogenesis of chronic hepatitis C. An increased abundance of ROS occurs as a direct result of core protein expression. This is likely to further impair mitochondrial electron transport, amplifying the effect of core on the mitochondria and sensitizing cells to other oxidative insults. Such a positive feedback effect of ROS on mitochondrial ROS generation is documented [90]. Most T cells of the body use a number of antioxidant mechanisms to respond to these circumstances, including induction of antioxidant proteins that contribute to

cell survival. Antioxidant gene expression could explain some of the divergent effects of the core protein on apoptosis seen in other experiments [37, 46, 84]. Different T cells are likely to respond to the core-induced ROS increase in different ways. In the Huh-7/191-20 line, there is a dramatic induction of antioxidant gene expression. The core protein has been shown to activate antiapoptotic factors such as NF- κ B [34]. This could have a net effect that suppresses apoptosis. In cells that fail to activate antioxidant genes or NF- κ B, core protein-induced ROS production may sensitize cells to apoptosis. What is consistent is that core protein expression increases cellular ROS. This interpretation is further supported by the recent report of Moriya et al. [91], who have demonstrated that HCV core protein causes a state of oxidative stress in transgenic mice.

As a consequence of chronic oxidative stress, there is a reduction in mitochondrial metabolic processes, which might contribute to the development of steatosis by inhibition of β -oxidation and oxidative damage to both mitochondrial and chromosomal DNA. The combination of oxidative DNA damage and suppression of apoptosis favors the development of HCC. Recent observations in transforming growth factor α /c-myc mice show that chronic oxidative stress promotes hepatic tumor formation and that this can be prevented by antioxidant therapy [92]. The consequences of impaired mitochondrial function and abnormal ROS generation would be exacerbated by the immune-mediated inflammatory process present in patients with chronic hepatitis C, and the additional oxidant load it would present to the HCV-infected liver.

The hypothesis that HCV core protein stimulates prooxidant production has also been supported by the recent studies of Okuda et al. [87], who demonstrated increased ROS in Huh-7- or HeLa cyclin-inducible expression system. Acute core expression was accompanied by increased lipid peroxidation and induction of some antioxidant enzymes [93]. Although the data were collected from cultured cells which probably overexpress HCV core as compared to infected human liver, the amount of viral replication and viral proteins produced during HCV infection in vivo is currently unclear [94, 95]. On the other hand, available evidence suggests that the core protein promotes oxidative stress in vivo and the consequences of this stress cause hepatic injury. Transgenic mice that constitutively express HCV core protein showed increased hepatic lipid peroxide species, steatosis, and HCC [90]. Hepatic steatosis and development of HCC are highly associated with HCV infection in humans [96]. Lerat et al.

[97] reported that transgenic mice that express either HCV structural genes or full-length HCV gene sequences also develop steatosis and HCC. The transgenic mice studies suggest that individual HCV proteins, such as core, manifest extraviral activities *in vivo*. Collectively, the *in vitro* and *in vivo* data imply that the prooxidant actions of core may have a mechanistic role in the pathology of the virus and should be studied further.

Role of HCV Core in Hepatocellular Carcinogenesis

The core protein of HCV has been shown to induce HCC in transgenic mice and has been suggested to play a central role in the development of HCC in chronic hepatitis C [85]. However, it still remains unclear how the core protein operates in the development of HCC: modulation of certain cellular gene products such as helicase lymphotoxin B receptor or dead box protein [59] as shown in cell culture system, may contribute to hepatocarcinogenesis. Induction of oxidative stress is also a possibility. Few studies found an age-dependent increase in oxidative stress in the livers of transgenic mice that develop HCC in the absence of inflammation as a consequence of the core protein [90].

Hepatic steatosis is a risk factor for HCC in patients with chronic HCV infection [98]. Prevalence of hepatic steatosis ranges from 31 to 72%. The pathogenesis of hepatic steatosis in patients with chronic HCV infection has been postulated recently. Both *in vitro* and *in vivo* studies have shown that HCV core protein expression either in cell culture or in transgenic mice led to the development of hepatic steatosis, contributing to carcinogenesis [43, 44]. Steatosis induced by HCV core protein, predisposes to lipid peroxidation and excess free radical activity with the potential risk of genomic mutations. Marreto et al. [99] reported that nonalcoholic fatty liver disease may be a common underlying liver disease in patients with HCC in the US. All these reports support the view that hepatic steatosis plays a role in hepatocarcinogenesis in patients with chronic HCV infection. The possible mechanism is based on the fact that the core protein interacts directly with mitochondria impairing electron transport and thus increasing ROS production. Core is also reported to be associated with ER [43] and lipid droplets [44]. The consequence of oxidative stress is the reduction in mitochondrial metabolic processes, which might contribute to the development of steatosis by inhibition of β -oxidation and oxidative damage to both mitochondria and chromosomal DNA.

HCV core also modulates a number of cellular regulatory functions. In fact, HCV core protein has been found to modulate the expression of the cyclin-dependent inhibitor p21^{WAF1} and to promote both apoptosis and cell proliferation through its physical interaction with p53 [99–102].

The core protein is produced as an innate form (amino acids 1–191) and following processing produces a mature form (amino acids 1–173). The innate form regulates subcellular localization of the mature expression, and the mature form in the nucleus suppresses the p21^{WAF1} expression. Thus, the core protein in the cytoplasm increases the amount of p21^{WAF1} via activating p53, and the core protein in the nucleus decreases the amount of p21^{WAF1} by a p53-independent pathway. The regulation of p21^{WAF1} expression by the core protein via subcellular localization might decide the fate of infected cells either towards proliferation or apoptosis. Thus, the innate form is not only a precursor of the mature form but also a regulator of the localization and functions of the core protein [102, 103].

In addition, HCV core protein has also been seen to regulate p73, a member of the family of p53. p73 is involved in neurogenesis and natural immune response and seems strongly involved in malignancy acquisition and maintenance process [104]. p73/core interaction results in the nuclear translocation of HCV core protein either in the presence of the p73- α or p73- β tumor suppressor proteins. In addition, the interaction with HCV core protein prevents p73- α -, but not p73- β -dependent cell growth arrest in a p53-dependent manner. HCV core protein may directly influence various p73 functions, thus playing a role in HCV pathogenesis [105].

Role of Core in Vaccination against HCV

HCV nucleocapsid (core) may represent a valuable component in the development of a vaccine as it is the most conserved viral antigen. Results of nucleotide and deduced amino acid sequence analysis reveals 93% nucleotide and 96% amino acid core sequence homology [106].

The core protein has been shown to induce both a humoral and a cellular-mediated response in the natural infection: (a) anticore antibodies are among the first antibodies to appear during the acute phase of infection. An increase in IgG1 isotype concomitant with a decrease in IgG3 isotype has been observed during the development of chronicity [107]; (b) core-specific peripheral blood CD4+ T cell proliferative responses are often correlated

with viral remission in IFN- α -treated chronic carriers [107–108], and (c) intrahepatic core-specific CTL cells were found associated with lower levels of viremia and more active liver disease in chronic patients [109].

Earlier studies using a full-length core sequence under the control of a cytomegalovirus promoter have revealed a rather limited immunogenicity of HCV core when introduced into a host in the form of naked DNA. The antibody seroconversion rate and the vigor of induced CTL responses in mice reached optimal values of 40 and 30% (for an E:T ratio of 100:1), respectively [110, 111]. Different strategies have subsequently been followed to try and optimize such responses. As previously described for the envelope glycoproteins, the use of plasmids containing truncated sequences of core expressed in fusion with the HbsAg has been tested [112, 113]. HBV-HCV chimeras have been constructed, either by insertion of HCV sequences within the coding sequence of the preS2 gene [114, 115] or downstream from the S gene [112]. Major et al. [115] demonstrated expression of both HCV and HBV antigens in the cytoplasm and supernatants of transiently transfected cells, but Geissler et al. [113] could not document viral antigen secretion in supernatants. In both studies, seroconversion rates and antibody titers were systematically found to be lower for the nonchimeric core-expression plasmid compared with chimeric ones, while up to 60–100% rates and at least one log increase in antibody titers could be observed in mice injected with HBV-HCV chimeras.

The intracellular targeting of HCV core protein to specific cellular compartments was recently evaluated as a way to optimize vaccine responses [115]. A wild-type core expressing plasmid was compared with plasmids expressing either the core sequence in fusion with the ubiquitin gene or with the signal sequence and transmembrane domain of the murine lysosome-associated membrane protein (mLAMP-1). The principal aim of the ubiquitin fusion was to improve degradation of core in a proteasome-dependent fashion in order to optimize core-derived peptide transport to the MHC class I pathway and, as a consequence, the resulting CTL activity. This strategy was very efficient when used for the nucleoprotein of the lymphocytic choriomeningitis virus model [116]. Similarly, the mLAMP-1 fusion was realized to target the core protein to the intracellular endosomal/lysosomal pathway, particularly the MHC-class-II-enriched compartments. These organelles are mainly present in antigen presenting cells, and it may be indeed interesting to try and target the expression of a vaccine antigen to these compartments. LAMP type fusions have recently been

shown to efficiently enhance T cell proliferative response, CTL activity and antibody production in the case of antigens such as papillomavirus HPV-16 E7 [117] or HIV gp 160 [118]. Nevertheless, in contrast to these previously reported studies, Vidalin et al. [116] did not observe any improvement in the induction of either proliferative CTL or antibody-based immune responses specific to HCV core. The reasons for this failure are not clear.

Hu et al. [112] compared BALB/c mice immunized twice with a core-encoding plasmid or primed with the plasmid and boosted with a recombinant purified core protein (amino acids 1–164). Although a protein boost following priming with DNA was shown to induce earlier seroconversion rates as well as higher in vitro CD4+ T cell proliferative responses, CTL activity remained comparable to that observed following DNA vaccination alone.

Role of Core in HCV Diagnosis

There are three common assay procedures used to diagnose HCV infection.

These include anti-HCV antibody assay, HCV-RNA detection and recently introduced HCV core antigen assay. Reports from various studies indicate the presence of HCV core protein in nearly 80–92% of patients positive with anti-HCV antibody [77, 119–121]. At the same time, the concordance between HCV core and HCV-RNA was noted up to 93–95% [77, 121]. In few studies, HCV core assay has been reported to be less sensitive than anti-HCV or HCV-RNA assay, though all those reports found it to be more specific as compared to these techniques [121]. Total HCV core antigen quantification is an accurate and precise indirect marker of HCV replication in HCV-infected patients. However, HCV core assay cannot detect HCV replication for HCV-RNA value below 20,000 IU/ml [122]. Despite several merits of HCV core assay over HCV-RNA detection by PCR, core assay has been reported to be less sensitive in different studies [123]. Whereas the sensitivity of HCV-RNA assay was found to be 99% that of HCV core assay was noted as 98% [124]. Tanaka et al. [125] also reported a sensitivity of 98% for the core assay. It was comparable to that of HCV-RNA. However, Zanetti et al. [123] found only 82% sensitivity of core as compared to that of HCV-RNA assay. In fact, more studies are needed to further authenticate the beneficial effect of HCV core estimation over HCV-RNA detection for the diagnosis of HCV infection.

Future Aspects of HCV Core

It is very interesting to study more aspects of HCV core protein now, particularly when complete cell culture systems are available [126–129]. The role of the core protein can be investigated more extensively for the process re-

lated to HCV entry, replication and virion production. The culture system may also help in developing antiviral agents targeting different stages of virus cycle, and these may prove to be potential therapeutic agents. How HCV core protein acts or plays a role in all these phenomena may now be possible to study and understand.

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