The Wild-Type Hepatitis C Virus Core Inhibits Initiation of Antigen-Specific T- and B-Cell Immune Responses in BALB/c Mice

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Received 2 December 2009/Returned for modification 9 April 2010/Accepted 19 May 2010

In this study, the effects of wild-type and deletion mutant hepatitis C virus (HCV) core proteins on the induction of immune responses in BALB/c mice were assessed. p2HA-C145-S23, encoding a core protein with the C-terminal 46 amino acids truncated, significantly produced stronger antibody and cellular responses than p2HA-C191-S23. The induction of immune responses by p2HA-C145-S23 was dose dependent. However, increasing the doses or repeated administration did not enhance immune responses by the wild-type core protein. In addition, p2HA-C191-S23 was apparently able to interfere with the priming of specific immune responses by p2HA-C145-S23 when the two were coadministered. These results demonstrated that the wild-type HCV core protein itself could inhibit the priming of immune responses in the course of a DNA vaccination, whereas the truncated HCV core protein could provide potential applications for the development of DNA- and peptide-based HCV vaccines.

Hepatitis C virus (HCV) has been identified as the major cause of posttransfusion and sporadic non-A, non-B hepatitis (4). One of the remarkable features of HCV infection is the high rate of persistent infections that eventually progress to liver cirrhosis and hepatocellular carcinoma (1, 36). The frequent progression of HCV infection to the chronic disease course has been largely attributed to the inability of the host immune system to clear the initial HCV infection (38).

Current data indicate that HCV-specific T-cell responses play a critical role in the control of HCV infection (5, 24). Robust HCV-specific CD4+ and CD8+ T-cell activation is associated with viral clearance in acute infection. However, HCV-specific T-cell clones in chronic HCV-infected persons are directed to many viral determinants, occur with low frequency, and are apparently functionally ineffective. Additional immune response abnormalities in chronic HCV infections include inadequate activation of the innate immune system, which includes excessive proinflammatory cascades in monocytes and altered dendritic cell (DC) functions (47). Therefore, effective new therapies and improved vaccines aimed at preventing HCV infection should induce intense, multispecific, and long-lasting T-cell immune responses that can suppress the replication of HCV in the early stages of infection.

Genetic immunization is a potent vaccine strategy for inducing effective antigen-specific CD4+ and CD8+ T-cell responses. Induction of HCV-specific T-cell responses by plasmid DNA vaccines has been demonstrated in a variety of experimental systems (13, 25). However, compared with DNA vaccines like those coding for hepatitis B virus proteins, HCV DNA vaccines appeared to be less efficient and induced only transient and weak responses (18, 20, 37). The failure of HCV DNA vaccines may be explained by the fact that HCV proteins are able to interfere with host cellular functions and thereby prevent the efficient induction of immune responses (6, 10, 23).

The HCV core gene is highly conserved among the various HCV genotypes and contains several well-characterized B-cell and cytotoxic T-lymphocyte (CTL) epitopes. Antibodies against the core proteins often appear first during natural HCV infections (35). In chronically infected individuals, cellular immune responses against HCV core proteins are always attenuated (11, 39). This implies that HCV core protein-specific immune responses may be important for the control of HCV infections. Therefore, it is worthwhile to test HCV core genes as candidate HCV vaccines for the prevention and therapy of HCV infections. However, the immune response induced by the HCV core in DNA vaccination is always weak and transient. Interaction of the HCV core protein with a wide variety of cellular proteins has been reported to influence host cell functions (26, 34). The HCV core protein is also able to suppress host immunity through several mechanisms, such as impairment of the function of dendritic cells in vitro, promotion of apoptosis of immune cells, or suppression of type I interferon (IFN) signaling, among other mechanisms (7, 14, 22, 28, 31, 32, 52). In the case of DNA vaccination, a high level of expression of the HCV core protein is desired for the priming of specific immune responses. However, the overexpressed viral protein may interfere even more strongly with host functions (2).

In this study, we began by investigating whether HCV core-
based DNA vaccines could be improved by truncating the HCV core protein. The removal of the carboxy-terminal region significantly enhanced specific T- and B-cell responses. Through codigestion with other DNA vaccines, it was shown that the full-length HCV core protein was able to inhibit the initiation of antigen-specific T- and B-cell responses. Our findings imply that the HCV core protein could interfere with host immune responses and therefore contribute to the development of chronic infection.

**MATERIALS AND METHODS**

**Mice and cell lines.** Female BALB/c mice (6 to 8 weeks of age) were kept under specific-pathogen-free conditions in the Central Animal Laboratory of the Wuhan Institute of Virology, Chinese Academy of Sciences (license number SYXK2003-0034), and were handled by following the guidance of animal ethical standards.

The cell lines HepG2, Huh7, and HeLa, kept in our laboratory, were used in this study. HepG2 cells were cultured with RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco). Huh7 and HeLa cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) (Gibco) supplemented with 10% FBS.

**Construction of expression vectors.** The full-length and truncated forms of the HCV core gene, each with a hemagglutinin (HA) tag sequence (YPYDVPDYA), was constructed. The identities of these plasmids were confirmed by DNA sequencing.

**Western blot analysis.** HepG2 cells were seeded into six-well plates at a density of 3 × 10^5 cells/well and transfected with 3 μg of plasmid DNA using Lipofectamine 2000 (Invitrogen). Transfected cells were harvested at 48 h posttransfection and resuspended in lysis buffer (0.15 M NaCl, 1% NP-40, 50 mM Tris, 0.5% deoxycholate, and 1% sodium dodecyl sulfate [SDS]). Proteins were separated by 15% SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto nitrocellulose membranes. Fat-free milk powder solution (3%, wt/vol) was used to block the membranes. Proteins with HA tags were detected by Western blotting using a primary mouse anti-HA monoclonal antibody (1:500 dilution; Shanghai Genomics, China) and a secondary HRP-conjugated anti-mouse IgG.

A schematic map of full-length and truncated mutant fusion HCV core proteins expressed by DNA constructs. p2HA-C191-S23 is a construct containing the full-length HCV core sequence (aa 1 to 191), p2HA-C191-S23 (aa 1 to 177) and p2HA-C191-S23 (aa 1 to 145) are C-terminally truncated fusion HCV core constructs. p2HA-N20-S23 (aa 21 to 191) and p2HA-N49-S23 (aa 50 to 191) are N-terminally truncated fusion HCV core constructs. Solid bars indicate the HCV (wt) epitope. Hollow bars indicate the wt or mutant HCV core.

**TABLE 1. Primers used in this study**

| DNA target fragment (aa) | Primer | Primer sequence | Primer position (aa) |
|--------------------------|--------|-----------------|---------------------|
| HA-Core (1–191)          | F1     | 5′ CCAGAGCTTACATTGACATCACTGATTGCATGCAAGATCGCTAAACC 3′ | 345–361 |
|                          | R1     | 5′ CGGATCCGAAGATCCGATGCAAGATCGCTAAACC 3′ | 345–361 |
| HA-Core (1–177)          | F1     | 5′ CCAGAGCTTACATTGACATCACTGATTGCATGCAAGATCGCTAAACC 3′ | 345–361 |
|                          | R2     | 5′ CGGATCCGAAGATCCGATGCAAGATCGCTAAACC 3′ | 345–361 |
| HA-Core (1–145)          | F1     | 5′ CGGATCCGAAGATCCGATGCAAGATCGCTAAACC 3′ | 345–361 |
|                          | R3     | 5′ CGGATCCGAAGATCCGATGCAAGATCGCTAAACC 3′ | 345–361 |
| HA-Core (20–191)         | F2     | 5′ CCAGAGCTTACATTGACATCACTGATTGCATGCAAGATCGCTAAACC 3′ | 757–776 |
|                          | R1     | 5′ CGGATCCGAAGATCCGATGCAAGATCGCTAAACC 3′ | 757–776 |
| HA-Core (50–191)         | F3     | 5′ CCAGAGCTTACATTGACATCACTGATTGCATGCAAGATCGCTAAACC 3′ | 402–418 |
|                          | R1     | 5′ CGGATCCGAAGATCCGATGCAAGATCGCTAAACC 3′ | 402–418 |
| HBsAg (23–44)            | SF     | 5′ CCAGATCTCTTATATCCGGAATACATAC 3′ | 221–239 |
|                          | SR     | 5′ CCAGATCTCTTATATCCGGAATACATAC 3′ | 271–286 |
| 2HA-C191-S23             | F4     | 5′ CCAGAGCTTACATTGACATCACTGATTGCATGCAAGATCGCTAAACC 3′ | 271–286 |
| 2HA-C177-S23             | SR     | 5′ CCAGATCTCTTATATCCGGAATACATAC 3′ | 271–286 |
| 2HA-C145-S23             | SR     | 5′ CCAGATCTCTTATATCCGGAATACATAC 3′ | 271–286 |
| 2HA-N20-S23              | R1     | 5′ CCAGATCTCTTATATCCGGAATACATAC 3′ | 271–286 |
| 2HA-N49-S23              | SR     | 5′ CCAGATCTCTTATATCCGGAATACATAC 3′ | 271–286 |

*a* The underlined bases are restriction endonuclease sites.

*b* NCBI Reference Sequence accession no. NC_004102.1.

*c* GenBank accession no. AP011094.1 [hepatitis B virus DNA; complete genome; strain B0503102(D1L/F)].

FIG. 1. Schematic map of full-length and truncated mutant fusion HCV core proteins expressed by DNA constructs. p2HA-C191-S23 is a construct containing the full-length HCV core sequence (aa 1 to 191). p2HA-C177-S23 (aa 1 to 177) and p2HA-C145-S23 (aa 1 to 145) are C-terminally truncated fusion HCV core constructs. p2HA-N20-S23 (aa 21 to 191) and p2HA-N49-S23 (aa 50 to 191) are N-terminally truncated fusion HCV core constructs. Solid bars indicate the HCV (wt) epitope. Hollow bars indicate the wt or mutant HCV core.
antibody (1:2,000 dilution; Novagen). Chemiluminescent signals were detected using an ECL kit (Pierce).

Confocal immunofluorescence microscopy. Two days after transfection, cells on slides were washed twice with phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde in PBS for 20 min, permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature (RT), and blocked with 3% nonfat milk. Subsequently, 30 μl of anti-HA monoclonal antibody (1:50 dilution) was added to each slide, and the slides were incubated in humidified chambers at RT for 2 h. The slides were then washed three times with PBS. A fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG secondary antibody (1:100 dilution; Novagen) was added, and the slides were incubated in humidified chambers for 1 h. After three washes with PBS, cell nuclei were stained with Hoechst 33258 (Beyotime, China) for 5 min. All specimens were examined with a Leica SP2 laser scanning confocal microscope (Leica, Germany). Twenty to thirty positive-stained cells were observed in at least five different fields. All of the cell fluorescence images shown in this paper were taken from a single confocal slice.

Plasmid DNA immunization. DNA immunization of mice was performed in vivo electroporation, according to a protocol described previously (49). Mice were randomly divided into groups, with six mice in each group. Mice were immunized with defined amounts of plasmid DNA dissolved in 50 μl of Tris-EDTA (TE) buffer. The mice were inoculated by electroporation at multiple sites in the quadriceps muscles (ElectroSquarePorator T830 M; BTX, San Diego, CA). Two boost immunizations were carried out at time intervals of 3 weeks. Ten days after the last immunization, the mice were sacrificed. Spleenocytes from the vaccinated mice were prepared for enzyme-linked immunospot (ELISPOT) analysis. Sera were collected and stored at −20°C.

Expression of anti-HCV core antibodies. Anti-HCV core antibodies were measured using specific enzyme-linked immunosorbent assays (ELISA). Microwell plates were coated with recombinant HCV core protein (obtained from the Academy of Military Medical Science, China) at a concentration of 3 μg/ml and incubated overnight at 4°C. The plates were washed with PBS containing 0.5 mg/ml Tween 20 (PBS-T) and blocked with blocking buffer (50 mg/liter fat-free PBS containing 0.5% BSA and 0.05% Tween 20 (PBS-T) and blocked with blocking buffer (50 mg/liter fat-free PBS-T). Subsequently, mouse serum samples diluted in blocking buffer were added and incubated for 1 h at 37°C. After three washes with PBS-T, HRP-conjugated goat-anti-mouse IgG (Novagen), HRP-conjugated goat-anti-mouse IgG1 (Southern Biotech), or HRP-conjugated goat-anti-mouse IgG2a (Southern Biotech) antibodies were added, and incubation proceeded for 1 h at 37°C. The plates were then developed with substrate buffer. After 30 min of incubation at room temperature, the reaction was stopped with 50 μl of 2.5 M H2SO4, and absorbance was measured at 450 nm using a microplate reader.

Statistical analysis. One-way analysis of variance (ANOVA) and Student's t tests were used for comparison of results among the different groups. All tests were performed using SPSS software. P values of <0.05 were considered to be statistically significant.

RESULTS

Expression of wild-type and truncated core proteins in Huh7 cells. Western blot analysis was performed to detect the expression of wild-type and truncated HCV core proteins in transiently transfected Huh7 cells using an anti-HA monoclonal antibody (Fig. 2A). Expression of p2HA-C191-S23 led to the synthesis of a major protein product of approximately 21 kDa and a minor protein product of 26 kDa. The minor protein band may represent the unprocessed wild-type fusion HCV core protein; the major protein band represents the processed core protein fused with HA at the N terminus but truncated around aa 173 at the C terminus. Consistently, protein bands of 24 and 20 kDa were detected in cells transfected with p2HA-C177-S23 and p2HA-I45-S23, respectively (19, 41). They were apparently not processed further, as these proteins did not have the carboxy-terminal hydrophobic sequences (Fig. 2A). The expression of p2HA-N20-S23 also led to two forms of fused HCV core proteins, with molecular masses of approximately 21 kDa and 16 kDa, consistent with incomplete processing. Transfection with p2HA-N49-S23 resulted in the expression of a protein with a molecular mass of 20 kDa, which represented an unprocessed protein. Using β-actin as an internal control, the relative expression levels of the recombinant proteins were analyzed (Fig. 2B). The truncated fusion HCV core proteins expressed by p2HA-C145-S23 and p2HA-N20-S23 groups versus p2HA-C191-S23 groups: *, P < 0.05; **, P < 0.01.

Subcellular localization of HCV core proteins. The subcellular localization of the HCV core protein may influence its function (50). The extensive modifications of the HCV core protein in our study may lead to an altered distribution of recombinant proteins in cellular compartments. To assess the subcellular localizations of full-length and truncated HCV core proteins, HeLa cells were transfected with p2HA-C191-S23, p2HA-C177-S23, p2HA-I45-S23, p2HA-N20-S23, or p2HA-C191-S23.
Immunofluorescence (IF) staining with anti-HA MAb indicated that 2HA-191-S23 and 2HA-N49-S23 were distributed mostly in the cytoplasm. However, the fused mutant core proteins 2HA-C145-S23 and 2HA-N20-S23 were located in both the nucleus and cytoplasm, with 2HA-N20-S23 apparently enriched in the nucleoli (Fig. 3). Our results were consistent with previous studies (30, 45, 46), which showed that the two C-terminal hydrophobic regions (approximately 70 aa) of the core protein are related to endoplasmic reticulum (ER) targeting, while the N-terminal region of the core protein is responsible for nuclear targeting.

Increasing doses or repeated administration of plasmids encoding the full-length HCV core protein did not enhance specific T- and B-cell immune responses. Previous studies indicated that DNA vaccination with plasmids encoding the full-length HCV core protein led to low levels of B- and Th-cell responses (18, 27). Enhancement of the expression of the HCV core gene does not enhance core-specific immune responses in DNA immunization (2). Thus, we tested three different doses of plasmids, 30, 50, and 100 µg per injection, for DNA immunization. The effects of the DNA immunizations were assessed by measuring anti-HCV core antibodies with ELISA and HCV core-specific T-cell responses with ELISPOT assay. The test results showed that all three doses induced low levels of anti-HCV core antibodies and T-cell responses (Fig. 4A and C). Kinetically, the anti-HCV core antibody levels induced by the DNA immunizations with p2HA-C191-S23 were increased by boosting with the DNA vaccine 3 weeks after the primary immunizations (Fig. 4B). However, a third boost with p2HA-C191-S23 did not result in a significantly stronger anti-HCV core antibody response (Fig. 4B). A similar tendency for the cellular immune responses measured by ELISPOT assay was also observed (Fig. 4D). A primary immunization induced T-cell responses to the CTL epitopes at aa 39 to 48 of the HCV core protein and aa 29 to 38 of HBsAg. The boost immunizations did not sig-
significantly increase T-cell responses to the specific CTL epitopes of the HCV core protein and HBsAg (Fig. 4D).

**Antibody and T-cell responses to the truncated HCV core proteins.** Current data demonstrated that HCV core proteins that are truncated at the carboxy termini are able to evoke stronger immune responses than the full-length HCV core protein (9, 18, 27). Therefore, we systematically investigated whether amino- and carboxy-terminal truncations could enhance the ability of the HCV core protein to induce specific immune responses. Plasmids encoding the truncated HCV core proteins with HA tags and a fusion to the HBsAg epitope at aa 23 to 44 were used to immunize mice. Antibody and T-cell responses to the HCV core protein were measured.

While p2HA-C191-S23, p2HA-C177-S23, p2HA-N20-S23, and p2HA-N49-S23 induced both low seroconversion rates and low levels of anti-HCV core antibody responses, four mice immunized with p2HA-C145-S23 developed significantly higher levels of anti-HCV core IgG than other groups ($P < 0.05$) (Fig. 5). IgG2a antibody was the only subclass detected following immunization in all tested samples (data not shown).

The cell-mediated immune responses induced by full-length or truncated HCV core proteins were assessed by ELISPOT assays, using peptides corresponding to the HCV core-specific epitope at aa 39 to 48 or the HBsAg-specific epitope at aa 29 to 38. All plasmids were able to induce T-cell responses to the fused HBsAg epitope (Fig. 6B). The plasmids p2HA-C145-S23

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**FIG. 4.** Effect of dosing or repeated boosting of DNA vaccine of the full-length HCV core gene. (A) Analysis of humoral responses in mice immunized with the full-length HCV core gene in different doses by ELISA. Sera obtained from 6 mice in each group on the 10th day after the last inoculations were tested, and a positive result was defined as a value greater than the mean value $\pm 2$ SD (cutoff) of the results from the mock DNA-immunized control group. (B) Analysis of humoral responses in mice immunized with the full-length HCV core gene with different boosting times by ELISA. Sera were collected and tested on the 10th day after the last inoculations with different boosting times in each group. Filled triangles and rectangles represent the means of the OD values in each group. (C) Analysis of cellular responses in mice immunized with the full-length HCV core gene in different doses by ELISPOT assay. The number of HCV core-specific or HBsAg CTL peptide (aa 29 to 38)-specific IFN-$\gamma$-producing cells was determined. Results represent the mean numbers of spot-forming cells per $2 \times 10^5$ splenocytes. (D) Analysis of cellular responses in mice immunized with the full-length HCV core gene with different boosting times by ELISPOT assay. The number of HCV core-specific or HBsAg-specific CTL peptide (aa 29 to 38)-specific IFN-$\gamma$-producing cells was determined. Results represent the mean numbers of spot-forming cells per $2 \times 10^5$ splenocytes.
and p2HA-N20-S23 induced the highest levels of IFN-γ-producing cells, which were significantly different from the low level of T-cell response triggered by p2HA-C191-S23. These results were also confirmed the use of the HCV core CTL epitope at aa 39 to 48 as a stimulator (Fig. 6A). The plasmid p2HA-N49-S23 was not able to induce CTLs to the HCV core epitope, due to the deletion of the sequence. Thus, truncated HCV core proteins expressed by p2HA-C145-S23 and p2HA-N20-S23 were able to more potently trigger specific T- and B-cell responses. It also implies that the full-length HCV core protein may possess the ability to generally interfere with the development of antibody and cell-mediated immune responses.

Inhibitory effect of the full-length HCV core protein on the induction of immune responses. Truncated HCV core proteins were able to trigger stronger immune responses in DNA vaccination than the full-length HCV core protein. Some researchers have attributed this observation to that fact that carboxy-terminally truncated HCV core proteins can enter the nucleus (27). However, other explanations were possible. Therefore, we asked whether the full-length HCV core protein would interfere with the induction of specific immune responses by truncated HCV core proteins. We performed additional immunization schemes, with coadministration of plasmids expressing full-length and truncated HCV core proteins. BALB/c mice were immunized twice, with the constructs in different doses or combinations, by electroporation in vivo (Fig. 7). On the 10th day after the last immunization, antisera and splenocytes were collected for detection of antibody and cell-mediated immune responses. First, immunizations with p2HA-C145-S23 at doses of 30, 50, or 100 μg per injection showed a clear dose-dependent anti-HCV core antibody response (Fig. 7). At the highest dose of 100 μg per injection, 5 of 6 mice showed seroconversion and high reactivity in an ELISA for detection of anti-HCV core antibodies. In contrast, only 2 of 6 mice seroconverted after immunization with p2HA-C191-S23, and reactivity to the HCV core protein was very low, consistent with the previous results. Coadministration of p2HA-C191-S23 (50 μg) and pHA-C145-S23 (50 μg) resulted in a low level of anti-HCV core antibody response, similar to immunization with 100 μg of p2HA-C191-S23. Thus, the ability of p2HA-C145-S23 to induce specific immune responses was apparently inhibited by p2HA-C191-S23. ELISPOT analysis confirmed that p2HA-C145-S23 induced specific T-cell responses to the HCV core protein and HBsAg at aa 29 to 38 in a dose-dependent manner (Fig. 8). Coadministration of p2HA-C191-S23 and p2HA-C145-S23 triggered a low T-cell response, similar to that triggered by immunization with p2HA-C191-S23 alone. It appeared that p2HA-C191-S23 inhibited the priming of immune responses by p2HA-C145-S23.

**DISCUSSION**

The HCV core protein may represent a valuable component in the development of a vaccine, as it is the most conserved viral antigen. However, earlier studies using a full-length core sequence have revealed a rather limited immunogenicity of the HCV core when administered to a host in the form of naked
DNA (18, 20, 25). In this study, we found that genetic vaccination with plasmids expressing the full-length HCV core protein was ineffective for inducing specific antibody and T-cell responses. Truncating the HCV core protein could improve its immunogenicity and evoke stronger immune responses in DNA-vaccinated mice. These findings are largely consistent with those of previous studies (27). We demonstrated that DNA immunization using the truncated HCV core protein induced specific T- and B-cell responses in a dose-dependent manner, while no significant improvement of immune responses could be reached by administration of increased doses of plasmids expressing the full-length HCV core protein. Strikingly, our study again demonstrated that the full-length HCV core protein was apparently able to actively interfere with the priming of specific immune responses. Plasmid DNA expressing the full-length HCV core protein inhibited the priming of T- and B-cell immune responses by a truncated HCV core protein.

It will be of great importance to understand the molecular mechanisms leading to the inhibitory action of the full-length HCV core protein on the priming of specific immune responses. It has been recently proposed that HCV core proteins may be the “core” of the immune deception (48). The innate immune pathway, through engagement of pattern recognition receptors such as Toll-like receptors (TLR) and helicases, expressed in cells of the classical immune system and in hepatocytes, is affected by the HCV core protein (47). The immunomodulatory role of the HCV core protein in the adaptive immune system has also been characterized. The HCV core protein is able to inhibit T-cell activation through its interaction with gC1qR (51). Upon stimulation of human peripheral blood mononuclear cells (PBMC) with either ConA or anti-CD3/CD28, it was found that the HCV core protein inhibited the proliferation of T lymphocytes in a dose-dependent manner (23). In addition, the production of interleukin-2 (IL-2) and IFN-γ in cells treated with the HCV core protein was markedly diminished compared to that in control cells (12, 44). Finally, several studies demonstrated that the HCV core protein may cause dysfunction in human DCs and mouse myeloid DCs in vitro (3, 8, 22).

The observed interference of HCV core proteins with the priming of immune responses in the course of DNA vaccination may be explained by the dysfunction of dendritic cells in vivo (42, 43). The interference of the HCV core protein was not limited to HCV core protein-specific effector cells but extended to other antigens. This mechanism may be important for the establishment of chronic HCV infection, since the action of the HCV core protein would at least attenuate HCV-specific immune responses.

The ability of the HCV core protein to interfere with the priming of immune responses explained the failed attempts to develop HCV core protein-based vaccines. A high level of expression of the HCV core protein would not induce a better immune response but, rather, would more strongly inhibit the priming of immune responses. Previous studies of murine models consistently showed that immunization of mice with plasmids expressing the HCV core gene could reduce the number of CD4+ T cells; additionally, expression of the HCV core protein in transgenic mice can interfere with the host immune response (21, 44). However, an adenovirus vector expressing...
the HCV core gene did not induce obvious immunosuppression in the immunized mice (29). The mechanisms involved have not yet been clarified.

Manipulation of the HCV core protein may influence its expression and subcellular localization. There is considerable evidence that HCV core protein maturation involves cleavage by signal peptidase (at position 191) and a second cellular protease at the signal peptide to give a product of about 173 aa in length (19, 41). Other in vitro studies reported that the HCV core protein could also be truncated at aa 151 or 121, but a detailed processing mechanism was not clear (16, 33, 45). The processing of the HCV core protein after expression is related to many factors, including the presence of the downstream sequence, expression conditions, or methods to detect HCV core protein. In our experiment using Huh7 hepatoma cells, three cleavage sites (at positions 191, 173, and 151) were apparently used in the processing of the HCV core protein, consistent with results from previous reports using different in vitro expression systems (16, 41, 45). We also showed that the HCV core protein, truncated at aa positions 145 or 151, displayed low expression levels, which was likely due to the lower stability levels of these truncated proteins. It has been reported that an HCV core mutant bearing a deletion of aa 125 to 166 is rapidly degraded by the proteasome (17). The subcellular localization of the HCV core protein is related to the ER targeting and nuclear localization signaling (NLS) sequences of its N terminus. The effect of ER anchoring can obstruct the functions of NLS sequences. Our data were in accordance with those of previous studies (30, 45, 46). The proteins 2HA-191-S23/2HA-177-S23 and 2HA-N49-S23 were largely located in the cytoplasm, which is due mainly to the effect of ER anchoring. In contrast, 2HA-C145-S23 and 2HA-N20-S23 displayed the tendency of shifting to the nucleus, which is due to the functional NLS sequences after the deletion of the ER anchor.

Truncated HCV core proteins may lead to a rational vaccine design. DNA-based immunization with a C-terminally truncated HCV core construct induced enhanced host immune responses. A C-terminal 15-aa truncation of the HCV core protein allowed the generation of a slow but potent immune response (9), while induction of immune responses by an HCV core protein with the C-terminal 37 amino acids removed was more immediate and vigorous (27). A detailed mechanism of the relationship between immune induction and the length of C-terminal truncation is worthy of further investigation. In addition, a recombinant HCV core protein (aa 1 to 164) was also a good immunogen (18). Furthermore, the induction of immune responses by truncated HCV core proteins may be enhanced by increasing their doses.

ACKNOWLEDGMENTS

We are grateful to Ze Chen for his technical assistance with DNA immunization. We are indebted to Xuefang An and Youlin Zhu for their assistance with animal breeding and experiments. We also thank Juxin Liu for technical support with confocal microscopy and Yuan Zhou for her excellent work in cell culture.

This work was supported by the National Basic Research Program of China (973 Program projects 2005CB522901, 2006CB933100, and 2007CB512901) and a grant from Deutsche Forschungsgemeinschaft, Transregio TRR60.

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