PROSPECTS FOR HEPATITIS C VACCINE

G.M. BHOPALE*, R.K. NANDA

Research and Development Division, Hindustan Antibiotics Ltd., Pimpri, Pune 411018, India

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Summary. – Hepatitis C virus (HCV) is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma worldwide. Unfortunately, neither a vaccine nor any effective therapy is available. Efforts are now directed towards the development of an effective vaccine besides chemotherapy. This review briefly summarizes the properties of an effective vaccine for the control of HCV infection. The mechanisms of protective immune response induced by HCV are not well understood. It is presumed that humoral and cellular immune responses play an important role. Even though there are various obstacles in the development of HCV vaccine, we describe a few promising approaches such as DNA vaccine, recombinant virus vaccine, HCV-like particles (HCV-LPs), peptide-based vaccine and plant-derived recombinant subunit vaccine.

Key words: Hepatitis C virus; vaccine; protective immune response

Contents:

1. Introduction

HCV infection poses a major threat to public health as it is frequently becomes chronic and in this way it may lead to cirrhosis and hepatocellular carcinoma (Boyer and Marcellin, 2000). Approximately 170 million people are infected with HCV worldwide (Cohen, 1999). Currently, the most effective therapy utilizes a combination of interferon alfa with ribavirin. However, only about 50% of treated patients have sustained benefit from such a therapy (Manns *et al.*, 2001; Fried *et al.*, 2002). Under the present scenario, development of either a new drug or vaccine is an attractive alternative. This article highlights the progress made towards the development of a vaccine against HCV.

2. Hepatitis C virus

HCV is a spherical enveloped virus of approximately 50 nm in diameter. Its genome consists of single-stranded positive-sense RNA molecule of approximately 9.5 kb (Clarke, 1997). The genome consists of highly conserved 5'- and 3'-noncoding regions and a single large ORF that encodes a polyprotein of 3008–3037 amino acids. The polyprotein is processed co- and post-translationally by both host and viral proteases into at least 10 structural (C, E1, E2 and P7) and nonstructural proteins (NS2, NS3, NS4a, NS4b, NS5a and NS5b) (Fig. 1). The core C protein appears to play multiple roles in various cellular processes.

The viral envelope proteins E1 and E2 are heavily glycosylated transmembrane proteins that form stable noncovalent heterodimers. P7 is a small hydrophobic peptide. NS2 and NS3 are responsible for the cleavage of all nonstructural proteins. Furthermore, NS3 has a helicase activity, which plays a role in viral RNA replication. NS4

*E-mail: girishbhopale@rediffmail.com; fax: +9120-27425327.

Abbreviations: CTL = cytotoxic T = lymphocyte; GM-CSF = granulocyte macrophage colony-stimulating factor; HCV = Hepatitis C virus; HCV-LPs = HCV-like particles; IL = interleukin; NS = nonstructural

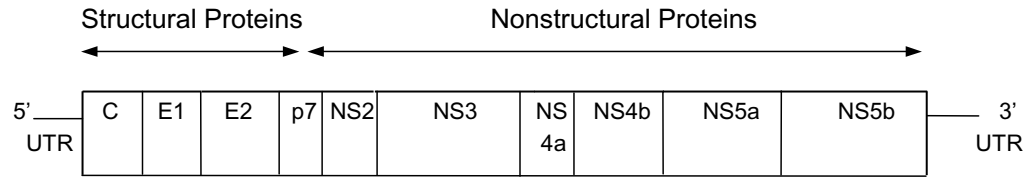

Fig. 1
Genomic organization of Hepatits C Virus

comprises two proteins, namely NS4a and NS4b. The former appears to have diverse functions such as anchorage of replication complexes and cofactor for the NS3 protease. NS4b is likely to play an integral role within replication complexes. NS5 is composed of two major proteins, NS5a and NS5b, which are released as mature products by the action of the NS3 protease in conjunction with NS4a. NS5a appears to be involved in interferon resistance.

There are at least six genotypes and more than 50 subtypes of HCV (Bukh *et al.*, 1995). HCV enters a susceptible host mainly either directly, through needle (injection or transfusion of contaminated blood products) or sexually. Acute hepatitis C is marked by appearance of HCV RNA in the serum within 1 to 2 weeks of exposure followed by elevation of serum alanine aminotrasferase and then jaundice. The latter occurs in less than 20% of the infected cases and is often preceded and accompanied by fatigue, lethargy, myalgia, low grade fever, nausea, vomiting and right upper quadrant pain or discomfort. The chronicity appears to be more frequent in young individuals and in African blacks in comparison to Caucasians and Hispanic whites (Howell *et al.*, 2000; Bellentani and Tiribelli, 2001). The quasi-species of HCV may also contribute to the development of chronicity. Hepatitis steatosis is a characteristic feature of hepatitis C and contributes to the progression of liver disease. Major longterm complications of chronic hepatitis C are cirrhosis and hepatocellular carcinoma, which develop after many years. Approximately 20% of those infected chronically develop cirrhosis and 1% to 5% of the patients with cirrhosis progress to hepatocellular carcinoma (Cohen, 1999).

3. Protective immune response

The immune response to HCV is multispecific both in terms of humoral and cellular immune responses. These responses remain poorly defined despite an increasing evidence suggests that both humoral and cellular immune responses are likely to contribute to protection and/or neutralization of the virus.

HCV infection generates antibodies against structural and nonstructural viral proteins. The hypervariable region of the E2 protein is a major site of antienvelope antibody response and contains a principal neutralization epitope. Antibody responses to envelope proteins develop slowly and achieve only modest titers during primary infection (Chen *et al.*, 1999). Therefore, neutralizing antibodies may emerge too late to prevent chronic infection. The cellular immune response plays an important role in the clearance of HCV infection based on strong association of a sustained vigorous and multispecific antiviral CD4 and CD8 cell response (Chen, 2003). A strong T cell proliferative response against structural and nonstructural proteins was found to be associated with self limited infection (Ferrari *et al.*, 1994; Missale *et al.*, 1996). Following acute infection the vigorous CD4 T cell response to HCV is maintained for many years, while the memory CD8 T cell response may be maintained with variable efficacy. Several studies (Ferarri *et al.,* 1994; Missale *et al.*, 1996; Bassett *et al*., 2001) have suggested that strong HCV-specific cytotoxic T Cell (CTL) responses against structural and nonstructural proteins are likely to be important in viral clearance and possible protection. Thus an ideal HCV vaccine should induce a strong humoral response and prime strong HCV-specific T helper and CTL responses.

4. Vaccine candidates

The identification of potential vaccine candidates is not easy due to difficulties in culturing the virus *in vitro*. However, most of the work related to the HCV vaccine development was focused on structural proteins (C, E1 and E2) and nonstructural proteins (NS3, NS4 and NS5). These proteins play crucial functions in viral host recognition, virus neutralization and virus life cycle. Among them, NS3 seems to play a key role in virus clearance.

5. Approaches for vaccine development

Several approaches have been used to develop the vaccine (Table 1) (for reviews see Lechmann and Liang, 2000; Brinster and Inchauspe, 2001; Forns *et al.*, 2002). In this article we summarize a few recent promising studies on HCV vaccine development.

Table 1. Current approaches to HCV vaccine development

Vaccine approach	Vaccine candidate	Animal models	Remarks
DNA vaccine	Structural and nonstructural protein genes	Mice, rats, rabbits monkeys, chimpanzees	Induce moderate humoral immune response. Induce strong cellular immune response. Immunogenicity can be increased by adjuvants. Results obtained in one animal species cannot be directly applied to other species.
Recombinant virus vaccine	Adenovirus, Vesicular stomatis virus, Herpes simplex virus, Semiliki forest virus, Rabies virus, Canarypox virus containing structural and nonstructural HCV protein genes	Mice	Induce moderate humoral immune response. Induce strong cellular immune response (Th1 type). Immunogenicity can be increased by administration of a recombinant virus expressing adjuvants such as IL-2.
Recombinant bacterium vaccine	Attenuated bacteria (*Salmonella* and BCG) containing nonstructural protein genes	Mice	Induce strong cellular immune response. Safety and regulatory issues connected with implementation of the approach may be of concern.
HCV-LPs	Recombinant baculovirus containing structural proteins	Mice	Induce strong humoral and cellular immune response. Immunogenicity can be increased by addition of adjuvants. Difficulty in the generation of sufficient amount of HCV-LPs.
Peptide-based vaccine	Peptide-containing epitopes from structural and nonstructural proteins	Mice	Induce strong humoral and cellular immune response. Synthesis of correct sequence of desired peptide in a large amount is a constraint.
Plant-derived recombinant subunit vaccine	Plant extract containing recombinant HVR1/CTB	Mice	Induce humoral immune response.

5.1 DNA vaccines

A DNA vaccine consisting of a recombinant plasmid encoding the virus antigen of choice is a novel approach to induction of immunity against target protein. The ability of the encoded antigen to induce the immune response offers the possibility of generating effective prophylactic immunity against the HCV.

The HCV core protein is highly conserved among various genotypes and therefore an attractive target for DNA-based vaccine. Immunization with the core protein DNA results only in a weak humoral immune response in mice. To enhance the immune response Geissler *et al.* (1997) have coinjected recombinant plasmids expressing the granulocyte macrophage-stimulating factor (GM-CSF), interleukin-2 (IL-2) or interleukin-4 (IL-4) with the core protein DNA. The results showed increased humoral and cellular immune responses as compared with the core protein DNA alone.

The E1 protein appears to be less immunogenic. However, Fournillier *et al.* (2001) have demonstrated that mutations of the N-glycosylation sites in the E1 protein enhanced the humoral immune response in mice. The results suggest that the deglycosylation of the E1 protein made some epitopes more accessible to the immune system.

Another study has explored the immune responses elicited in chimpanzees by DNA immunization with a plasmid encoding the E2 protein (Forns *et al.*, 2000). This DNA vaccine candidate was also shown to induce immune responses in mice and macaques (Forns *et al.*, 1999). The results suggest that the DNA-based immunization with the E2 protein may modified the course of infection and prevent progression to chronicity.

The DNA immunization using recombinant plasmids encoding NS3, NS4 and NS5 proteins individually or together has demonstrated immunogenicity for mice and rats. In mice, these nonstructural proteins produced strong cellular immune responses and specific antibody responses (Encke *et al.*, 1998). In rats, the immune response to all three nonstructural proteins increased provided GM-CSF was used (Cho *et al.*, 1999).

Recently, Duenas-Carrera *et al.* (2004) have evaluated the capacity of a plasmid encoding three HCV structural proteins (core protein, E1 and E2) to induce immune response in rabbits and macaques. Their results indicate that such an immunization is able to elicit both humoral and cellular immunity against HCV structural antigens in animal models different from mice.

The DNA-based vaccination may prove useful to generate virus-specific CTL responses. Nishimura *et al.* (2000) have used a plasmid expressing HCV structural proteins (core protein, E1 and E2) under the control of the human elongation factor 1 alpha (EL1-α) promoter. A single injection of the plasmid was shown to induce a specific CTL response in mice. This study indicates the potential utility of EF-1α promoter in development of HCV vaccine.

A vaccine strategy directed to increase the Th1 cellular immune response has considerable potential. Jiao *et al.* (2003) have demonstrated that cationic liposome-mediated DNA immunization induces strong HCV NS3-specific immune responses and also triggers high level of nonspecific

IL-12 production in mice. Further detailed studies on other animal models are required.

It is well known that the immunogenicity of DNA vaccines can be increased by changing the route of immunization or by addition of an adjuvant (Inchauspe, 1999; Krieg and Davis, 2001; Ma *et al.*, 2002; Encke, 2003). IL-23 has been shown to possess IL-12-like biological activity. IL-23 induced long lasting Th1 and CTL immune responses to the HCV E2 protein, which were much stronger than the IL-12-mediated immune response (Ha *et al.*, 2004). These data suggest that IL-23 could be an effective adjuvant of DNA vaccine for the induction of durable antigen-specific T cell immunity. However, the DNA-based immunization has some limitations, mainly the fact that the results obtained in one animal species cannot be simply applied to other species relevant for HCV.

5.2 Recombinant viral or bacterial vaccines

Recombinant viruses are an efficient way to deliver heterologous DNA that can mediate high levels of protein expression in host cells. Studies in mice have shown that a recombinant adenovirus containing genes of structural and/or nonstructural proteins of HCV induce both humoral and cellular immune responses (Makimura, 1996; Brunna-Romero *et al.*, 1997; Arribillaga *et al.*, 2002). Administration of a recombinant adenovirus expressing IL-12 led to a marked increase in cellular immune response (Lasarte *et al.*, 1999). Also vesicular stomatis virus expressing high levels of HCV E1 and E2 proteins generated strong immune responses (Buonocore *et al.*, 2002). A recombinant Herpes simplex virus expressing the HCV E2 glycoprotein induced high levels of E2 antibodies (Lucas *et al.*, 2003) in mice. There are some other promising recombinant viruses based on Semiliki forest virus (Brinster *et al.*, 2002), Rabies virus (Siler *et al.*, 2002) and Canarypox virus (Pancholi *et al.*, 2000; Pancholi *et al.*, 2003), which encode either structural or nonstructural proteins and induce strong immune responses.

As for nonviral vaccine vehicles the attenuated *Salmonella typhimurium* should be mentioned (Shata *et al.*, 2000; Wedemeyer *et al.*, 2001). Oral or nasal immunization of mice with *S. typhimurium* induced both mucosal and systemic immune responses against the encoded HCV NS3 antigens. This approach deserves further investigation in other animal models. Uno-Furuta *et al.* (2003) have assessed the capacity of an attenuated tuberculosis bacillus, Calmette-Guerin bacillus (BCG) as a vaccine vehicle to elicit HCV-specific CTLs. The results showed a substantial reduction of vaccinia virus titers in mice. These findings suggest BCG as a vaccine vehicle and the necessity of its further investigation.

Although all these approaches using viral as well as nonviral vehicles are very promising, safety and regulatory issues connected with their implementation may be of concern.

5.3 HCV-like particles-based vaccines

HCV-like particles (HCV-LPs) are attractive as a recombinant protein vaccine, because they might mimic more closely the properties of native viruses (Baumert *et al.*, 1999). The HCV-LPs synthesized through a recombinant baculovirus contain the complementary DNA (cDNA) encoding structural HCV proteins. These HCV-LPs have biophysical, ultrastructural and antigenic properties similar to those of the putative virion (Baumert *et al.*, 1999). The mice immunized with HCV-LPs generated a strong humoral and cellular immune response against the HCV core and E2 proteins (Baumert *et al.*, 1999; Lechmann *et al.*, 2001). Moreover, adaptive transfer of lymphocytes from HCV-LPs-immunized mice to naive mice provided protection against a recombinant HCV-vaccinia challenge in mice and this transferred immunity could be abrogated by either CD4 or CD8 depletion (Murata *et al.*, 2003).

The effects of the adjuvants ASO1B (contains monophosphoryl lipid A and a naturally occurring saponin QS21) and CpG10105 were evaluated in mice (Qiao *et al.*, 2003). The results showed that the immunogenicity of HCV-LPs was enhanced by the adjuvants at both humoral and cellular levels. A potential obstacle for the use of this approach could be the difficulty in the generation of sufficient amount of HCV-LPs.

5.4 Peptide-based vaccines

Induction of multispecific cellular immune response directed simultaneously against multiple HCV epitopes appears to be important for the development of effective HCV vaccine. Peptides containing epitopes from the core, NS4 and NS5 regions have been shown to induce strong CTL responses in mice (Shirai *et al.*, 1996; Hiranuma *et al.*, 1999). A covalent attachment of CTL peptide to T helper peptide seems to be crucial for generating a strong CTL response. A sequence encompassing aa 121–135 of the E1 protein was capable to induce both CD4 cells as well as CTLs (Lopez-Diaz de Cario *et al.*, 1999). However, this approach has attracted only a limited interest .

5.5 Plant-derived recombinant subunit vaccines

Recently plant derived subunit vaccine against HCV has been described (Nemchinov *et al.*, 2000; Natilla *et al.*, 2004). Intranasal immunization of mice with crude plant extract containing a recombinant tobamovirus (HVRI/CTB), which encoded a consensus sequence from the HCV hypervariable region 1 fused to the C-terminus of the B subunit of cholera

toxin, elicited an antiserum containing antibodies to both CTB and HVRI (Nemchinov *et al.,* 2000). Recently, Natilla *et al.* (2004) have reported Cucumber mosaic virus as carrier of a HCV-derived epitope. A plant derived recombinant HCV vaccine can potentially reduce expenses normally associated with production and delivery of conventional vaccine.

6. Future research strategy

During the last decade, many efforts have been dedicated to develop the HCV vaccine. However, considerable obstacles in practical fulfilment of this task have appeared. The most important guidelines for the future research are as follows.

1. The development of a reliable, reproducible and efficient culture system for propagating HCV is considered to be of highest priority. Such a system would provide further insights into the structure of HCV and function of its polypeptides.
2. To date, the chimpanzee model is the only animal model that can be used to test the efficacy of HCV vaccine candidates. Chimpanzees are not readily available, require specific facilities and are very expensive. Therefore, a small animal and less expensive model should be found for studying the correlation of protective immunity and viral clearance.
3. The mechanisms by which HCV escapes the host immune responses and establishes a chronic infection are not well defined. Detailed studies are needed to define viral escape mechanisms and role of cytokines in the establishment of chronic HCV infection.
4. To improve the immunogenicity of HCV vaccine candidates new adjuvants, cytokines and chemokines that would favor a strong cellular response should be evaluated.
5. Several genotypes of HCV have been identified worldwide. Their characterization is likely to facilitate the development of an effective vaccine against HCV infection.
6. HCV exists in numerous quasi species in the infected population. It is therefore desirable to develop a vaccine that would induce strong immune responses to several both variable and conserved regions of the virus at the same time.

7. Conclusions

The development of a vaccine against HCV is obviously faced with multiple challenges. At present no commercial hepatitis C vaccine for human use is available but the results of various experimental studies using novel approaches have provided optimism that an effective HCV vaccine for human use is feasible.

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