Therapeutic vaccines against the hepatitis C virus in the age of direct-acting antivirals

REVIEW

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ABSTRACT

Hepatitis C is a significant health problem worldwide, with incidence estimates around 160 million people and 500 000 annual deaths. The limited success of treatments in chronic genotype 1 hepatitis C virus (HCV) patients and the numerous and significant adverse effects of the therapeutic treatment with pegilated interferon and ribavirin have encouraged the development of different direct-acting antivirals (DAAs) with promising results. This was also stimulated by advances of the knowledge on virus cell cycle and the properties of its structural properties. However, DAAs are very expensive and some of those compounds have developed multiple adverse events, all these limiting their use in certain infected populations. Moreover, its use does not prevent from HCV reinfections. Hence, new treatments, such as therapeutic vaccines, have arisen as additional or combined therapies against chronic HCV infection.

Keywords: hepatitis C virus, chronic infection, therapeutic vaccine, antiviral therapy

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RESUMEN

Vacuna terapéutica contra el virus de la hepatitis C en la era de los antivirales de acción directa. La hepatitis C constituye un problema de salud mundial, con estimados de más de 160 millones de personas infectadas. Esta enfermedad es responsable de alrededor de 500 000 muertes anuales. El éxito limitado del tratamiento en pacientes infectados con el virus de la hepatitis C (VHC), genotipo 1, así como los numerosos e importantes efectos adversos de la terapia con interferón pegilado más ribavirina, unido a los avances en el conocimiento del ciclo de vida y de las características de las proteínas estructurales del virus, han estimulado el desarrollo de diferentes antivirales de acción directa (AAD), muy prometedores en sus efectos terapéuticos. Sin embargo, estos nuevos productos son extremadamente costosos y además algunos de ellos han presentado múltiples eventos adversos, lo que limita su empleo en determinadas poblaciones de individuos infectados, y no evitan la reinfección con el VHC. Por lo que se necesitan tratamientos con vacunas terapéuticas como un tratamiento adicional o alternativo para las infecciones crónicas por el VHC.

Palabras clave: virus de la hepatitis C, infección crónica, vacuna terapéutica, terapia antiviral

*I*ntroduction

Hepatitis C is a significant health problem worldwide, with incidence estimates around 160 million people infected with the hepatitis C virus [1] and 3-4 million new cases per year [2]. This diseases causes 500 000 deaths yearly [3] and it is considered as the first cause for the indication of liver transplants in US and Europe [4]. The prevalence of the infection in adults oscillates between 0.5 and 25 % [5], and particularly in Cuba, there is a seroprevalence between 0.7 % and 1.2 % among blood donors during the last 4 years [6].

In HCV patients an immune response is generated against practically all the viral antigens [7]. Such a response is generally ineffective to eliminate the virus. Consequently, 85 % of infections are persistent HCV infections and nearly 25 % of all the chronic carriers of the virus can develop cirrhosis 20 years after the infection, 1.4 % of them developing hepatocellular carcinoma [5, 8, 9].

The studies on individuals who spontaneously eradicate the HCV virus suggest that the early developed immune responses, long-lasting cellular and humoral immune responses targeting several viral antigens, can precondition a favorable prognosis in terms of infection elimination [10].

*M*odifying the immune response induced by HCV

HCV induces liver damage by two mechanisms: the direct viral cytopathic action (a minor contribution to the damage) and the one mediated by cytotoxic T lymphocytes and the inflammatory cytokines produced by the natural immune response against the virus [11].

In fact, viral clearance is only possible during the acute phase if potent CD4+ and CD8+ T cell responses are entangled [12]. Moreover, a correlation between neutralizing antibody levels and virus eradication has been observed [13].

Several alterations have been described in the immune response induced by HCV principally during the chronic phase of infection: a) decreased levels of natural killer cells with activated cytolytic capacity; b) altered antigenic presentation in dendritic cells and

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macrophages which desensitize T cells or leads to the failure in the maintenance of the memory cells; c) alterations in the function and differentiation of T cells; d) viral resistance to interferon, through the action of some viral proteins like NS5A and E2 which interacts with protein kinase R and inhibits the antiviral effects of interferons; e) late development of specific antibodies [14, 15]. All these interferences in the normal functioning of the immune response leads to a misbalanced immune state unable to clear the HCV virus and also contributing to liver damage.

*H*epatitis C treatment

During the last decade, chronic hepatitis C has been treated by the combination of pegylated interferon (alpha-2a and alpha-2b; pIFN) and ribavirin, the dosage adjusted to the body weight and applied for 24 weeks in patients infected by HCV genotypes 2 or 3, or 48 weeks in patients carrying genotypes 1, 4, 5 or 6 [16]. Treatment is intended to induce a sustained viral response (SVR), defined as the lack of detection of HCV RNA during six months after concluding the treatment. SVR is associated to a reduction in inflammation and the severity of fibrosis, and therefore, it is considered as an indirect marker of hepatitis C viral resolution [17].

The application of these treatment regimes in individuals having no previous treatment (so-called 'naïve') successfully attained SVR in 40-50 % of the patients carrying genotype 1; in 65-85 % of those carrying genotypes 4, 5 or 6; and in 75-85 % of those infected by genotypes 2 or 3 [18].

Its limited success in chronically infected HCV patients carrying genotype 1 together with the numerous and significant adverse effects of the pIFN plus ribavirin therapy and recent advances on the knowledge of the viral life cycle and the properties of the viral structural proteins have fostered the development of different direct-acting antivirals (DAAs) with very promising therapeutic results. Nevertheless, these new products are extremely expensive and some of them has provoked multiple adverse events [19], limiting their use in certain populations of infected individuals and also, they have shown unable to protect from HCV reinfection. For instance, in 2011, the protease inhibitors boceprevir and telaprevir were approved with the single indication to patients infected with HCV genotype 1 [20]. Both inhibitors required to be combined with pIFN plus ribavirin, since monotherapy was reported to fastly induce drug resistance.

Concerning the prices, in Spain, the price of the triple therapy based in pIFN plus ribavirin and a protease inhibitor administered for 48 weeks was about 35 000 € when including telaprevir (Incivo®) or 42 000 € for boceprevir (Victrelis®)[21]. This triple therapy achieved SVR levels of 75 % in genotype 1 naïve carriers and nearly 50 % in non-responders to previous treatments [22, 23]. The adverse effects (anemia and cutaneous manifestations) caused by the treatment and the drug interactions were so significant that American Association for the Study of Liver Diseases (AASLD) decided to do not recommend these drugs in 2014 [24].

The second generation of DAAs (simeprevir and sofosbuvir) has caused less adverse events and minimal drug interactions in patients co-infected with HCV and the human immunodeficiency virus (HIV). Moreover, treatments are shorter but with a similar efficacy for all the viral genotypes, with reports of 90 % SVR in treated patients. Nevertheless, the prices remain high, around 90 000 USD the treatment, which are unaffordable by most patients [25]. Additionally, there is no clear view on how efficacious the new treatments may be in certain immunocompromised infected populations such as those comprising cancer or kidney failure patients. There are also no evidences on the efficacy of these new treatments to prevent reinfection, a very relevant task in risk groups such as drug addicts and patients under hemodialysis. Hence, the abovementioned aspects and the emerging evidences of resistance against the antiviral therapy [26], make necessary to develop new immunological therapies that could include vaccines as additional and alternative treatment for the chronic HCV infections [27].

*V*accination: a feasible alternative for HCV treatments

In order to solve the demand for new and efficacious treatments, with less adverse effects and at lower costs, several studies has been carried out at preclinical and clinical development stages of therapeutic vaccine against chronic HCV [19]. Nevertheless, there is no vaccine available in the market in spite of great efforts and the substantial resources spent in research. Hence, the need for a preventive, therapeutic or both types of agents is still unmet [28].

Major scientific challenges include: a) the genetic diversity of the virus; b) the lack of a reliable immunological correlate for protection; and c) the absence of a small animal model of the HCV infection to test the passive and active immunization strategies, the impact of the genetic background of the individual on the course of infection and the development of the immune response [29-31].

The rationale for vaccination against HCV infection resides on the fact that just 15 % of infected individuals effectively and spontaneously clear the virus, by means of potent humoral and cellular immune responses [18]. Moreover, there is a significant modification of the natural immune response against HCV in those patients achieving a sustained immune response during the antiviral treatment, their ineffective immune responses contributing to liver damage [32].

Consequently, a vaccine against HCV has to be intended to induce a strong immune response, either cell-mediated or through the induction of neutralizing antibodies, of high cross-reactivity, long-lasting and targeting various viral antigens. Its main therapeutic effects should be: a) the elimination of the viral infection through complete clearance of viremia; b) the modification of the pattern of ineffective immune response, ameliorating its mediated liver damage; and c) the induction of anamnestic immune responses which could limit viral rebound and reinfection after its combination with other antiviral treatments.

A myriad of vaccine strategies have been evaluated with relevant HCV antigens: protein subunit vaccines, virus-like particles, synthetic peptides, live recombinant viruses and plasmid DNA vaccines [33].

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Table. Vaccine candidates against the hepatitis C virus other than the Cuban CIGB-230 candidate

Vaccine candidate	Clinical phase	Antigen	HCV genotype	Vaccine technology or vector	Immune response	Company	Country
Inno-101 (discontinued)	II	E1 C-terminal (135 aa.)	1	Recombinant protein	Cell-mediated response	Innogenetics/ GenImmune	Belgium
IC41	II	8 peptides from Core, NS3, NS4	1	Synthetic peptide vaccine	Cell-mediated responses	Intercell AG	Austria
GI-5005	II	NS3-core fusion protein	1	Heat-killed yeast cells	Dendritic cell activation, innate immunity and specific cell-mediated response	GlobeImmune	United States
TG4040	I-II	NS3, NS4 NS5B	1b	Modified Vaccinia Ankara vector	Increased CD8+ T cell response	Transgene Inc.	France Canada
CHRON VAC-C®	I-IIa	NS3, NS4A	1a	DNA vaccine	Cell-mediated response	ChronTech Pharma	Sweden
HCV core ISCOMATRIX®	I	Core	1a	Recombinant protein	Cell-mediated and antibody responses	Commonwealth Serum Laboratories Ltd.	Australia
PEV2A PEV2B	I	NS3	ND	Virosomos-formulated synthetic peptides	Cell-mediated and antibody responses	Pevion Biotech Ltd	Switzerland
Autologous dendritic cell immunotherapy	I	Core, NS3, NS4B CTL peptides	1	Autologous dendritic cells pulsed with lipopeptides	Cell-mediated response	Burnet Institute	Australia
Adenoviral vector vaccine (Ad6 and AdCh3)	IIb	NS3, NS4, NS5	1a	Adenovirus vectors	Cell-mediated response	GSK plc. (Okairos)	United Kingdom
Emulsified core peptide	II	Core 35-44 peptide	1b, 2a	Peptide vaccine	Cell-mediated and antibody responses	Kurume University (leading institution)	Japan

* All the candidates shown were demonstrated to be safe, with predominantly local adverse reactions, and viral loads were modified transiently. ND: Not declared.

Either the case, the vaccine has to be able to redirect the anomalous immune response in infected individuals, steadily diversifying and enhancing it, for the concerted and effective functioning of the immune system components [34].

Recent evaluations support the view of therapeutic vaccination against HCV as a promising alternative: Particularly, recombinant protein vaccines are very attractive even for those patients unresponsive to conventional therapies. They induce potent humoral immune responses and also cell-mediated responses to a lesser extent, this last of specific T cells developed by the direct presentation of the antigen to the T cell receptor through human leukocyte antigen (HLA) molecules. Nevertheless, they are limited by the high antigen variability among the population, the strategy been effective only in some patients. Vector-based vaccines (e.g., adenovirus vectors) present some alternatives to those shortcomings of protein vaccines. DNA vaccines also provide some technical advantages, such as the preferential induction of cell-mediated responses and adverse effects milder than those generated by viral vectors, in spite of their limited effectiveness. Noteworthy, some delivery strategies are being explored to improve their effectiveness by enhancing their uptake and antigen expression, such as electroporation [35].

At present, several candidates are under investigation with satisfactory results in terms of immunogenicity and good safety profiles in animal models and also clinical trials [36, 37]. The table shows the properties of the main vaccine candidates being tested in phase I or II clinical trials against different antigens, which have proven to be immunogenic, safe and with predominantly local adverse reactions [38, 39].

Therapeutic vaccination in Cuba

Since 2006, a new therapeutic vaccine candidate against HCV has been developed in Cuba, named CIGB-230 (formerly Terap C), which is a mix of the recombinant HCV capsid protein with a DNA plasmid vector coding for the structural HCV antigens. The mechanism of action resides on the simultaneous presentation of the capsid antigen, which is highly conserved among viral isolates, through both the T-helper 1 (Th1)-prone antigen presentation pathway (the plasmid DNA-encoded antigen) and the Th2 pathway (the recombinant protein antigen). The immune activation is also reinforced by the synergic interaction of the plasmid DNA and the capsid protein vaccine components, which effectively protect the plasmid DNA from degradation, and, conversely, provides an effective activation of the innate immune response against the protein antigen by the CpG motifs present in plasmidic DNA. This vaccine has proven to be save, well tolerated and induces positive changes in the immune response and liver histology [40].

Preclinical results with CIGB-230

Various studies in mice, rabbits and macaques animal models provided relevant evidences on the potential effectiveness of CIGB-230. All the animals were provided by the Cuban National Center for the Production of Laboratory Animals (Cenpalab) and maintained in the Bioterium at the Center for Genetic Engineering and Biotechnology of Havana (CIGB), under good laboratory practices. All the animals immunized with CIGB-230 developed detectable antibody levels against antigens of the structural region of HCV (E1, E2 and the capsid). Moreover, preclinical studies helped to determine the adequate proportion of recombinant protein and plasmid DNA (pIDKE2 construct) able to induce protection in a model of challenge with a viral surrogate, as evidence of a functional immune response *in vivo* [38, 41].

The results obtained through the immunization with the pIDKE2 plasmid and the evaluation of adequate

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plasmid DNA amounts in different animal models [42-44], provided the experimental support for the equivalent dose to be administered in humans, together with previous DNA immunization studies in humans [45, 46]. Remarkably, the amount of plasmid DNA to be administered and the intervals between doses are key aspects of further optimization, since there are relevant differences in the structure of muscle tissues from mice to man which considerably influence on the immune response obtained.

Clinical evaluation of CIGB-230

Two clinical trials have been conducted with CIGB-230: 1) a phase I study to evaluate the safety and preliminary efficacy of the vaccine candidate in 15 genotype 1b HCV-infected patients who were vaccinated with CIGB-230 alone, and 2) a phase II clinical trial to evaluate the efficacy and safety of the concomitant administration of CIGB-230 with interferon plus ribavirin in 92 genotype 1b HCV-infected patients who were naïve to antiviral treatment [40].

CIGB-230 showed to be immunogenic and safe in both trials. Significantly, it induced cross-reactive, neutralizing antibody responses and *de novo* cell-mediated responses against the target viral antigens [40, 47]. Additionally, the phase II clinical trial demonstrated the relevance of the administration schedule for the modification of the virological response when the vaccine candidate was administered in combination with antiviral therapy. This last widens the therapeutic perspective for CIGB-230.

Conclusions

In general, all the therapeutic vaccine candidates against HCV tested so far in humans have demonstrated to be safe, generating local adverse events predominantly and have stimulated the specific immune response in chronically infected HCV patients. But the major goal of viral eradication remains to be attained, with discrete results in the reduction of viral load.

The conventional antiviral treatments have proven to be improved with the use of therapeutic vaccines, followed by increased antiviral responses and lower adverse events. This may lead to combination treatments of increased therapeutic outcomes, minimal adverse effects and affordable by all the patients. The optimization of vaccination schedule and the administration routes, together with formulation development including more potent and suitable adjuvants, are envisaged as immediate fields of research.

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