Interferon-based treatment as adjuvant therapy for hepatitis C virus vaccine candidates against chronic infection

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ABSTRACT

Therapeutic vaccine candidates against hepatitis C virus infection have demonstrated immunogenicity, but none of them have been able to induce complete viral elimination. Reviewed evidence shows hepatitis C virus-specific T cell exhaustion as a major obstacle for the immune containment of the virus. The possibility that hepatitis C virus-specific T cells may be rescued from exhaustion by interferon-based treatment makes this therapy a promising candidate to combine with hepatitis C virus-specific therapeutic vaccine interventions. This review summarizes the main effects of interferon-based therapy on hepatitis C virus-specific cellular immune response in chronic patients and, focuses on its potential to favorably impact the performance of therapeutic vaccine approaches for sustained viral clarification.

Keywords: Interferon, hepatitis C virus, vaccine candidates, therapy

RESUMEN

El tratamiento basado en interferón como terapia adyuvante para los candidatos vacunales contra la infección crónica por el virus de la hepatitis C. Los candidatos vacunales terapéuticos contra el virus de la hepatitis C han demostrado ser inmunogénicos, pero no han sido capaces de inducir la eliminación viral completa. Las evidencias experimentales acumuladas indican que el agotamiento de las células T específicas contra este virus es un obstáculo fundamental, que debe ser salvado si se persigue el control de la infección por parte del sistema inmune. El potencial mostrado por los tratamientos basados en interferón para rescatar la funcionalidad de dichas células, convierte a esta terapia en un candidato promisorio para ser combinado con las estrategias vacunales contra el virus de la hepatitis C. La presente revisión se centra en los principales efectos que tienen las terapias basadas en interferón sobre la respuesta celular específica contra el virus de la hepatitis C, en los pacientes crónicos. Particularmente, se hace énfasis en su potencial para modificar favorablemente la eficacia de los enfoques vacunales terapéuticos para alcanzar una eliminación viral sostenida.

Palabras clave: Interferón, virus de la hepatitis C, candidatos vacunales, terapia

Introduction

Hepatitis C virus (HCV) infects about 170 million people worldwide [1]. No preventive or therapeutic vaccines are available against this virus, which establishes chronic infection in most cases and becomes the leading cause of liver transplant in Western countries [2].

Correlates of immune protection have not been entirely established for HCV infection. Evidence of a significant role of antibody responses in viral clearance during the chronic phase of the infection, seems conflicting [3, 4]. In contrast, the importance of sustained multispecific CD4+, as well as CD8+ T cell responses, targeting numerous epitopes, has been highlighted [5, 6].

In persistently infected patients CD4+ and CD8+ T cells are typically found at low frequencies in peripheral blood, but they seem to be enriched in the liver [7]. Impaired production of interferon (IFN)-gamma and interleukin- (IL) 2, as well as the inability to proliferate *in vitro*, have been demonstrated for CD4+ T cells of chronically infected patients [8, 9]. Similarly, CD8+ T cells of persistently infected subjects fail to produce IFN-gamma and tumor necrosis factor (TNF)-alpha in functional assays [10, 11].

The inability to clearly define correlates of protection against HCV has hampered the development of efficient vaccine strategies. So far, vaccine candidates reaching clinical stages have demonstrated immunogenicity [12, 13], but none of them have been able to induce significant reductions or complete elimination of viral load. There is much evidence showing HCV-specific T cell exhaustion as a major obstacle for immune containment or elimination of the virus. Unfortunately, the causes of T cell failure to control HCV infection are not completely established. In this respect, studies have found significant correlations between high viral load and HCV-specific T cell hampered functionality [14]. Nevertheless, there is no generally accepted consensus of whether the demonstrated T cell impairment is a cause or a consequence of the high viral load. In any case, immune restoration seems more achievable with a moderate, instead of a high viral load. In fact, there is evidence that HCV-specific T cell dysfunction can be reversed by viral clearance [15].

Thus, if the idea that high viral load is a pivotal determinant of T cell exhaustion is correct, IFN-based treatment could be taken into consideration as an appealing adjuvant therapy for HCV vaccines, given its capacity to reduce viral load, as well as its immune modulatory properties [16, 17]. The present review summarizes the main effects of IFN-based therapy on HCV-specific cellular immune response in chronic patients and, thus, focuses on its potential to favorably impact the performance of therapeutic vaccine approaches to achieve sustained viral clarification.

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Cellular immune response is impaired in chronically infected HCV patients

Early studies performed in acutely HCV-infected patients showed that vigorous, multi-specific and sustained CD4+ T cell responses are associated to a self-limited course of the infection [18]. Similarly, the multi-functional capacity of these cells, in terms of their ability to proliferate and secrete IFN-gamma, has been recognized as an important factor for viral control [19, 20].

Likewise, CD8+ T cell responses are vigorous and directed against several epitopes in acute, self-limited infections [21, 22] and these responses, when long-lasting, contribute to viral elimination [23].

Thus, it has been demonstrated that in most HCV-infected subjects a detectable cell mediated immune response is generated at the onset of acute infection, but this response progressively disappears in those evolving toward persistent viral infection [9].

Several mechanisms have been proposed to explain T cell response failure. Primary failure to induce a T cell response and the exhaustion of an initially vigorous response are identified as important factors predicting viral persistence [24]. In fact, various studies demonstrate that patients developing a chronic infection typically show weak and oligospecific CD4+ and CD8+ T cell responses during both acute and chronic phases of the infection [24]. Additionally, direct loss, by exhaustion of CD4+ and CD8+ T cell responses during acute hepatitis, in patients transiently controlling the infection and subsequently progressing to viral persistence, has been documented [25]. Mechanisms responsible for the primary failure or T cell exhaustion are not yet clear. It has been suggested that antigen presentation by dendritic cells and macrophages may be affected in HCV infection [26], resulting in ineffective T cell priming or memory maintenance. Another explanation for T cell exhaustion is the elimination of virus specific cells, when there is high viral load, according to that found for lymphocytic choriomeningitis virus infection in adult mice [27] or by rapid activation-induced death in the liver [28].

A functional anergy of virus-specific T cells against viral antigens is also frequently described in HCV-infected patients. Various studies have demonstrated that the dysfunction of CD8+ T cells occurs in the acute and chronic phases [25, 29]. According to this, a CD8+ T cell phenotype known as 'stunned', characterized by impaired proliferative, cytotoxic and TNF-alpha and IFN-gamma secreting capacity, has been described [30].

In many cases, regardless of viral outcome, a T cell dysfunction has been described in the early course of infection. However, in patients with a self-limited infection, recuperation of CD8+ T cell functionality is associated with viral load reduction and resolution of disease [25, 29]. In contrast, the functionality of these cells remains suppressed in patients progressing toward chronic infection [31]. CD8+ T cell dysfunction is also associated to an immature phenotype, mainly CD28+ and/or CD27+, indicating an early differentiation state [32]. In fact, it has also been documented that in chronic HCV patients most of the intrahepatic HCV-specific T lymphocytes, are not able to behave as fully differentiated cytotoxic lymphocytes, despite a high expression of perforine [33].

On the other hand, it is speculated that the different mechanisms of CD8+ T cell failure are a direct result of CD4+ T cell dysfunction, which is common in HCV persistent infection [8, 34]. The development of IL-10-secreting CD8+ T cells, that are specific against HCV antigens, has also been described [35]. Data suggest that these cells are induced as a compensatory mechanism that limits the inflammation and the immunopathology, resulting in suppression of CD4+ cells, an increase in viremia, and the evolution to chronicity. Despite being antigen specific in their induction phase, in the effector phase the suppression they exert is antigen non-specific, mediated by IL-10 production [33].

Additionally, the existence of CD4+CD25+ T cell subpopulations, which are specifically activated via the T cell receptor has been described in chronic patients with normal alanine aminotransferase (ALT) levels and minimum hepatic damage [36]. It is postulated that in chronic patients these regulatory cells may be preferentially distributed to the liver, where they exert a low level and sustained inflammation, which is critical for the survival of the patient and the pathogen [36]. More evidence shows that CD4+CD25+ T cells play a pivotal role in the suppression of HCV-specific CD8+ T cells. They were found to be enriched in chronic individuals compared to patients clarifying the infection and healthy controls, and are able to suppress IFN-gamma production in response to peptide stimulation, as well as CD8+ T cell proliferation [37, 38].

In patients evolving to chronicity, the *in vivo* selection for epitope variants, which are less efficiently recognized by CTL than wild type sequences, is also regarded as an important element contributing to T cell failure to control viremia [22, 39]. It is also known that human leukocyte antigen (HLA) alleles affect the capacity of spontaneous and therapy-induced viral control [40-42].

The cells of the innate arm of the immune system are also considered important effectors in viral clearance. It has not been defined which one of the functions of the natural killer (NK) cells, namely cytotoxicity or cytokine secretion, is more relevant for virus control [43]. In this sense, it has been observed that while the *ex vivo* cytotoxicity of NK cells does not seem to be compromised in chronic patients; a reduction in the number of these cells has been detected in their peripheral blood [44]. It has also been demonstrated that the ability of NK cells to activate dendritic cells is hampered in HCV infected individuals, due to the enhanced expression of CD94-NK2A receptor and the production of cytokines such as IL-10 and tumor growth factor (TGF)-beta [45]. Additionally, it has been detected that HCV E2 protein is able to inhibit NK cells' cytotoxicity and cytokine secretion, by cross linking of CD81 [43].

Particularly, impaired antigen presentation and reduced cytokine secretion by antigen presenting cells [46-48] may negatively affect T cell functions. Some authors report normal functional capacity of both myeloid and plasmacytoid dendritic cells in chronic HCV infection [49]. However, certain studies have demonstrated that in persistent infection both cell subsets are numerically and functionally impaired

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[50, 51]. Furthermore, attenuation of toll-like receptor (TLR) innate sensing, dependent on the HCV RNA density associated to myeloid dendritic cells, has been demonstrated [52].

Achievements and drawbacks of therapeutic vaccination against HCV in clinical practice

Until now, there is no preventive or therapeutic vaccine available against HCV. There are many approaches, focusing on vaccines composed of one or several antigens or representative epitopes, either as recombinant proteins, synthetic peptides or vectors. Basically, these approaches aim at the induction of B-cell and/or T-cell mediated immunity, in addition to the modulation of the immune response already established in infected individuals. The rationale behind these approaches is based on evidence provided by studies showing significant associations between neutralizing antibodies and T cell responses and the resolution of infection [20, 22, 53].

Various HCV therapeutic vaccine candidates have been tested in healthy individuals as proof of concept, demonstrating their immunogenic properties [54-56]. Nevertheless, the analysis of their immunogenicity in healthy volunteers is out of the scope of this review, which focuses on the capacity of vaccine interventions to impact the already established HCV-specific immune response in infected individuals.

A vaccine candidate based on a recombinant truncated variant of HCV E1 protein, formulated in aluminum hydroxide [57] demonstrated its ability to induce humoral and proliferative responses, including IFN-gamma secretion in chronic patients [57]. In immunized individuals a decrease in ALT levels was observed, and at the end of the study, the total Ishak score indicated improved or stabilized hepatic histology in most of them. Nevertheless, further results evidenced its inability to sustainably reduce liver damage and to decrease circulating viral RNA levels [57].

The first trial to prove the synthetic peptide immunization concept against HCV in humans was carried out with IC41 vaccine candidate, comprising five HCV-derived cytotoxic T lymphocyte epitopes, restricted by HLA-A2, and three highly promiscuous CD4+ T cell epitopes formulated in poly-L-arginine [56]. This candidate demonstrated its ability to induce CD4+ T cell proliferation and IFN-gamma secretion by CD4+, as well as CD8+ T cells in chronically infected patients, who are non-responders to the antiviral therapy [12]. Nevertheless, a transient viral load reduction greater than 1 log10 was observed in only three out of twenty four individuals, and this was positively associated with vigorous IFN-gamma secretion [12].

A further phenotypic characterization of vaccine-induced CD8+ T cells in chronic patients, who are non responders to therapy, revealed the ability of IC41 to induce a clear shift in the phenotype of memory cells in single individuals [58]. T central and effector memory cells increased, while T effector memory RA+ cells declined, during vaccination in patients in which CD8+ T cell frequency increased [58]. This was an encouraging result since it demonstrated that the phenotype of HCV-specific T cell may be changed by

vaccination in chronic HCV patients. Nevertheless, no significant changes in IFN-gamma production were observed, nor was there an obvious correlation of HCV-RNA levels and HCV phenotype with IFN-gamma/tetramer+ ratios. Moreover, during follow-up, HCV-specific cells showed a backshift in memory phenotype, with the loss of T effector memory and the increase of T effector memory RA+ [58]. The impossibility to enhance CD8+ T cell functionality, as well as being unable to induce a sustained phenotypic change would explain the lack of a significant anti-viral effect in these patients.

Other peptide-based vaccine candidates tested in HCV genotype 1b chronically infected patients, who are non responders to IFN-based therapy, have been able to induce humoral and cellular immune responses in a number of patients, and have a tendency to a positive correlation between cellular responses, while a favorable clinical course has been observed [13, 59]. None of these studies offered results on histological evaluation and none of the patients cleared circulating viral RNA [13, 59].

A vaccine candidate, CIGB-230, based on the combination of a plasmid DNA encoding the structural region of HCV genotype 1 and a truncated variant of HCV core protein [60], was the first to test the DNA vaccination concept in HCV chronically infected individuals. The immune response elicited by CIGB-230 was characterized in a Phase I clinical trial in IFN-alpha and ribavirin non responders [61]. Remarkably, individuals immunized with CIGB-230 significantly modified their neutralizing antibody response during the treatment, with de novo generation of these immunoglobulins in 40% of immunized patients. In addition, after the treatment with CIGB-230, a significant number of individuals developed de novo proliferative responses, particularly against HCV core, and the stabilization or improvement (reduction) in liver fibrosis correlated with cellular immune response directed against more than one HCV antigen at the end of treatment, even when viral clarification was not achieved [61].

In general, all vaccine candidates tested against HCV in humans, thus far, have demonstrated that it is possible to stimulate HCV specific immune response in chronically infected patients; nevertheless, the ultimate objective, complete viral eradication, has not been accomplished. Effects on viral load have generally been null or only transient reductions have been observed. A main obstacle in this sense is the ignorance of the optimal quality and magnitude of the immune response required to clear the infection. On the other hand, most studies have not focused on a detailed evaluation of the impact of these interventions on key issues such as the phenotype and function of vaccine-induced effector T cells. Additionally, the influence of these candidates on specific antigen presenting cells, and other arms of the innate immune system, remains mostly unexplored and must be optimized to improve vaccine performance. An important objective would be to enhance anti-viral functionality of existing T cells with the generation of long lived memory T cells. It seems reasonable to consider that any therapeutic intervention should be conceived on the basis of repeated inoculations, incorporating novel

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adjuvants and combining various strategies, concomitantly or sequentially.

IFN-based treatment as a modifier of HCV-specific T cell response in chronic patients

The best antiviral treatment available today against HCV infection is based on the combination of pegylated interferon (PEG-IFN) and ribavirin. Diverse mechanisms have been proposed to explain how IFN exerts its antiviral action against HCV. It seems that this activity is exerted through the direct interaction with IFN-stimulated response elements on DNA, leading to the translation of proteins that interfere with HCV replication (not directly related to the virus or replication complex), while having immune modulation actions on innate and adaptive immune systems [62]. Pegylation has improved IFN pharmacokinetics, making it possible to reduce the administration frequency to only once a week. Two types of PEG-IFN are available in the market: PEG-IFNalpha-2b (PEG-Intron; Schering-Plough, Kenilworth, EEUU) and PEG-IFNalpha-2a (PEGASYS; Roche). Both PEG-IFN have different pharmacokinetics; however, differences have not been found in sustained virological response (SVR) rates when combined with ribavirin [63].

In vitro studies suggest possible mechanisms of action for ribavirin. As a nucleoside analogue, its incorporation into the viral genome may increase mutagenesis rate [63-65]. It could also directly inhibit HCV non-structural 5B polymerase, as well as the activity of the inosine monophosphate dehydrogenase cell enzyme, affecting GTP availability, thus inhibiting viral replication [63, 65]. Immune modulatory properties have also been described for ribavirin [64]. Remarkably, ribavirin alone has been shown to have very little antiviral activity *in vivo*, but when combined with PEG-IFN it improves SVR rates [65].

An early study showed that the addition of ribavirin to IFN-alpha in a combined treatment did not alter CD4+ T cell proliferation to HCV antigens, compared with IFN monotherapy, but it modulated cytokine balance in favor of a T helper 1 (Th1) response, with the suppression of IL-10 production [66]. Unfortunately, the combination of IFN-alpha and ribavirin produced many adverse effects, requiring dose adjustments, treatment interruption or contraindications in certain patients [63]. The mechanisms of action of both compounds are not completely understood. Thus, it is hard to define the causes for therapy failure, whether it is due to suboptimal potency, or because the effects are not sustained.

Early viral RNA kinetics may predict treatment success. Specifically, it is considered that genotype-1 infected patients, not showing a reduction of more than 2 log10 in circulating RNA, or having an RNA concentration of 30 000 IU/mL after a twelve week treatment are not likely to attain a SVR [67]. For these patients a halt of the treatment is recommended. It has been observed that patients infected by genotype 2 and 3 may be cured in over 75% of the cases, while only 40-50% of genotype-1 infected patients achieve a SVR [63, 68, 69]. Additionally, host genetic factors have been shown to influence response to therapy [70].

Investigations performed in patients under IFN-alpha and ribavirin treatment suggest that a favorable clinical course may be associated to the stimulation of CD8+ T cell activity and the secretion of Th1 cytokines [71]. In transplant settings a successful antiviral treatment is also related to significant enhancement of multi-specific and sustained CD8+ T cell responses, while an unsuccessful treatment is not [23]. In addition, enhancement of memory T-cell proliferation and prevention of T-cell apoptosis by Type 1 IFNs have been reported [72].

Studies show that before therapy, either alone or combined with ribavirin, HCV-specific T cell reactivity was hardly detected in the majority of chronic patients [66, 73]. Nevertheless, IFN-based treatment increased the number of patients with T cell reactivity to one or more HCV antigens [66]. Moreover, the induction of persistent HCV-specific CD4+ T cell reactivity was found to be associated with treatment response, and the magnitude of this response against the core was directly associated to SVR [66]. During treatment the functional response was induced and maintained in SVR patients, while a patient not responding to IFN-alpha monotherapy lost the response after therapy withdrawal [73]. These results suggest that HCV-specific IFN-gamma production persists after therapy in patients achieving an SVR, while it disappears in non responders.

At the same time, seemingly contradictory evidence has also been found regarding the enhancing effect of IFN-based therapy on HCV-specific cellular immune response. A study involving persons with acute infection, who did not rapidly control viremia indicated that virus-specific CD4+ and CD8+ T-cell responses uniformly decrease with successful treatment and increase transiently, with recrudescence of viremia in those failing to achieve an SVR [74]. Hence these data show that when treatment is successful, it is not associated with persistent augmentation, but with a sustained decline of the immune response [74]. Other reports have found no significant effect of this treatment on T cell functionality or frequency, either in SVR or non-SVR [14].

Many factors may contribute to the discrepancy between studies: the heterogeneity of the cohorts of patients and of the treatment regimes applied make comparisons impossible; most of the studies are biased due to the reduced number of epitopes tested, which lead to underestimation of responses. In addition, different time points and diverse immune correlates, either involving frequencies or functionality, are evaluated in different trials. Such heterogeneity makes it impossible to find consensus regarding the net effects of IFN-based therapies on HCV-specific T cell response.

Nevertheless, Badr et al. showed that even when HCV-specific T cells do decline in frequency over time, they remain detectable and polyfunctional up to 1 year following discontinuation of therapy in all SVR [20]. In contrast, while the polyfunctionality of HCV-specific T cells, in terms of production of IFN-gamma, IL-2, and CD107a, could be enhanced by IFN monotherapy in a relapsing patient, their proportion declined and were undetectable after viral rebound, consistent with their inability to up-regulate

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CD127 and Bcl-2 expression, common characteristics of long-lived memory T cells [20].

In fact, comprehensive studies on the impact of IFN-based therapy on HCV-specific T cells have shed light on important possible correlates of treatment induced viral clarification. In treatment-naïve and early chronic individuals, T cells predominantly express a CD127low-Bcl-2low phenotype, which can be rapidly reverted by successful IFN monotherapy upon virus elimination [20]. CD127+ Bcl-2+ HCV-specific populations, which are selected for during therapy in SVR, were shown to be long-lived after discontinuation of therapy [20]. In agreement with other studies [74], T cell proliferation was not found to be a predictor of the outcome of HCV infection [20]. In contrast, a marked increase in IFN-gamma secretion, restoration in all functions, and generation of polyfunctional T cells were observed in patients following the initiation of therapy and coincided with virus elimination [20]. Badr and colleagues further demonstrated that CD127 expression distinguished a unique subset of HCV-specific memory T cells bearing the phenotypic signature of T effector memory cells (CCR7- CD45RA-) and yet bearing the functional features of both T central memory (rapid proliferation and high IL-2 production) and T effector memory (high IFN-gamma production and cytotoxic potential) [20]. Another study linking HCV-specific T cell phenotype and response to PEG-IFN-alpha and ribavirin demonstrated that treatment was able to induce a predominance of early and late differentiation phenotypes (based on CD28 and CCR7 expression) in SVR, while non-SVR showed a predominance of pre-terminally differentiated, not fully functional T cells, that could not be reversed by therapy [75]. This could be interpreted in the sense that a predominance of early and late differentiation phenotypes is advantageous because the former is a source of effectors, while fully differentiated cells are ready-to-act effectors that can control viral replication. However, the sustained predominance of pre-terminally differentiated cells, that do not reach a fully mature state, results in the inability to clear infection.

The importance of persistent HCV T cell responses to reach a SVR has been highlighted, not only in the context of HCV monoinfection [66, 73], but in HCV-HIV co-infection as well [76].

Combined IFN-alpha and ribavirin therapy not only impacts the adaptive arm, but also the innate arm of the immune system (Table). While a significant reduction in NK cell frequency and a quantitative imbalance of NK cell subsets may be observed in HCV-infected patients, it has been demonstrated that antiviral treatment is able to reverse this situation [77, 78]. Additionally, the expression of NK cell receptors, and function of NK cells (CD107a and IFN-gamma expression) returned to normal levels in SVR, in contrast to relapsing patients [78]. Also an enhancer effect of dendritic cell maturation has been described for IFN-alpha [72].

The reasons for the decline in HCV-specific T cells during therapy are not completely understood. Some authors consider that the decline of HCV-specific CD8+ T-cell responses during therapy is possibly due to the withdrawal of the antigen, and argue against treatment-induced immunological containment of

Table. Immune correlates of viral clarification in IFN-alpha-based treatment with a possible beneficial impact on the efficacy of HCV vaccine candidates

Innate immunity
Enhancement of dendritic cell maturation
Induction of a quantitative balance of NK cell subsets
Increased frequency of NK cells and recuperation of their functionality (CD107a and IFN-gamma expression)

T cell immunity
Modulation of cytokine balance in favor of a T helper 1 (Th1) response, with suppression of IL-10
Generation of persistent HCV-specific CD4+ T cell reactivity
Enhancement of long-lasting poly-functionality of HCV-specific T cells (production of IFN-gamma, IL-2, CD107a)
Reversion of CD127lowBcl-2low phenotype, towards a CD127+Bcl-2+ long-lived memory T cells phenotype
Induction of a predominance of CD28+CCR7+ and CD28-CCR7- CD8+ T cells

ongoing viral replication; an alternative explanation proposed is that T cells redistribute to the liver during therapy, and therefore decline in frequency in peripheral blood mononuclear cells [79]. Nevertheless, an important distinction between those who control the virus and those who do not is that responses tend to be stronger and broader in the long term, in the absence of viremia. This fact strongly highlights that virus-specific immune response may actually contribute to viral containment and that the withdrawal of the antigen does not necessarily imply that long-lived functional cells may not be generated and maintained in the absence of antigenic stimulation. In fact, there is evidence in favor of an important role for HCV-specific T cells in viral control during therapy. The findings that the maximal induction of virus-specific T-cell reactivity occurs at about weeks 4-8 of the treatment, with clear differences in cytokine profiles between treatment responders and non responders, suggest that this may be an important effector mechanism of viral elimination [66]. However, whether the enhancement in HCV-specific Th reactivity seen with antiviral treatments results from the induction of new T-cell clones or if it is the result of restoration of pre-existing T-cell reactivity, is unknown. It has also been proposed that the restoration of virus-specific T-cell reactivity during IFN-alpha and ribavirin treatment could result from the observed inhibition of IL-10 production, which would reduce the immunosuppressive effects of this cytokine [66, 80]. Others favor the hypothesis that the reconstitution of a polyfunctional immune response is a consequence of virus elimination and prevention of continued T-cell exhaustion, similar to acute resolving HCV [20, 81]. In this sense, when more and more evidence highlight the pervasive effect of high viral load on HCV-specific cellular immune responses [19, 29, 72], IFN-alpha and ribavirin therapies could be considered effective adjuvant therapies to be used with HCV-specific vaccines. Thus, several hypotheses support the idea of IFN-based treatment as a modifier of HCV-specific immune response in chronic patients, of which the favorable impact responds to its ability to improve quality, instead of quantity, of the immune response.

An important finding from a recent study by Abdel-Hakeem and colleagues [81] demonstrated that functionality of HCV-specific T cell, both of CD4+ and CD8+, is more effectively rescued when treatment is started early after HCV infection. However, studies evaluating the efficacy to attain viral clarification of

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the combination of IFN-based treatments with HCV-specific vaccine candidates are still scarce.

The first study published in this sense was that of Wedemeyer and colleagues [82]. In that study, peptide vaccine candidate IC41 was tested as a late add-on to standard PEG-IFN and ribavirin treatment, in chronic treatment-naïve patients. The main objective was to determine if IC41 could reduce relapse rate when administered from 28 to 48 weeks after the start of the standard treatment, but the observed relapse rate was inconclusive, so that proof of therapeutic benefit could not be demonstrated [80]. IC41 did not delay viral relapse either; nevertheless, vaccine-specific T cell responses were exclusively detected in SVR and not in relapse patients, offering more evidence that HCV-specific T cell responses can contribute to the long-term control of the virus [80]. One important limitation of this study was that it was uncontrolled, a fact that made a true comparison with unvaccinated, but treated patients, impossible.

Preliminary results of the inclusion of GI-5005, a yeast vector vaccine expressing an NS3-core fusion protein, in a phase II trial evaluating a triple therapy combined with PEG-IFN-alpha and ribavirin regime, compared with the antiviral regime alone, showed improved early virological responses in all treatment-naïve patients [83]. Nevertheless, end-of-trial results, including data of SVR, have not yet been published.

HCV elimination. Nevertheless, the probability that a threshold may be reached cannot be ruled out. The generation of multifunctional long-lived T effector cells is regarded as an advantage of paramount importance that would lead to the attainment of a protective state, in which the probability of viral relapse or reinfection is reduced. HCV therapeutic vaccine candidates, based on an array of important epitopes may contribute to the diversification of these responses. On the other hand, the possibility that HCV-specific T cells may be rescued from exhaustion by IFN-based treatment make this therapy a promising candidate that is to be combined with HCV-specific therapeutic vaccine interventions. So far, IFN-alpha based therapy has shown a potential to induce such changes, through its capacity to reduce viral load, which is a pivotal impact. However, the optimal treatment schedule, for the combination with novel T cell stimulating vaccine candidates, must be defined. In fact, results indicate that the effects are actually modest and still insufficient. Future studies should consider longer IFN-alpha courses, the inclusion of more potent vaccine adjuvants and novel antiviral compounds, as part of multi-therapy approaches. Hence, this type of therapy would help reduce viral load and lead to the recovery of functional immune responses that would contribute to a sustained viral clarification in chronic HCV patients.

Conclusions

Data reviewed seems to point to the quality of the immune response, rather than its quantity (number of effectors) as the key factor associated to effective

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