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Review

Unique features of human T cell responses to HCV-1b

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ABSTRACT

Hepatitis C virus (HCV) is estimated to cause chronic infection in 3% of the population globally. Only limited treatment options are available for these millions of people chronically infected with HCV. In the absence of a complete remission, these patients are at risk of developing liver cirrhosis and hepatocellular carcinoma, both of which carry serious consequences. As yet, there is no preventative vaccine that can control further spread of new HCV infection. HCV is a single positive stranded RNA virus that belongs to the *Flaviviridae* family. It exists as 7 genotypes, which have uneven geographic distributions. It is postulated that human immune responses to different HCV genotypes may be divergent. The experimental evidence for this hypothesis, however, is insufficient. In this paper, we review the existing literature on the adaptive human immune responses to HCV, with specific emphasis on T cell responses to HCV 1b subtype of genotype 1. We also discuss the importance of gaining additional information on human T cell responses to the development of a vaccine for HCV.

KEYWORDS: HCV-1b, T cell, epitope, vaccine

1. INTRODUCTION

Hepatitis C virus (HCV) was first discovered in 1989 and has become one of the major causes of liver disease. Three to four million people are estimated to be newly infected every year. Three percent of the global population, about 170 million people, are chronically infected and at risk of developing severe liver diseases, including cirrhosis and hepatocellular carcinoma (HCC). There are approximately 350,000 deaths as a consequence of HCV infection each year [1, 2]. HCV can be divided into 7 genotypes based on the complete coding region. Genotype 1, namely 1a and 1b, is most prevalent in Europe, North America and East Asia. Genotypes 2 and 3 are more prevalent in Thailand, India and West Africa. Genotype 4 is predominant in Africa, and genotypes 5 and 6 are common in South Africa and South-East Asia [3]. Genotype 7 was identified in Canada in 2011 [4].

HCV is a single positive stranded RNA virus that belongs to the family *Flaviviridae*. Its genome is about 9.6 kb with 5' and 3' untranslated regions (UTRs) and one long open reading frame. The encoded polyprotein can be processed into 3 structural (core, E1 and E2) and 7 nonstructural (NS1, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [5].

HCV infection has different consequences in the population. About 15-25% of acute infected individuals recover spontaneously, while the majority of infected people develop chronic liver infection. Even though chronic hepatitis is nonprogressive in about 15-20% of the infected population, and 10-20% of infections are sensitive to the treatment with pegylated IFN-α and ribavirin, the rest may face an array of severe outcomes, including compensated and decompensated cirrhosis and HCC [6]. Ten to fifteen percent of HCV infected people are estimated to develop cirrhosis within the first 20 years post infection. The rate of disease development is affected by the gender,

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ethnicity, age and host immune responses [7]. The risks tend to increase with older age, co-infection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV), drug and alcohol abuse [8].

Currently, combination therapy with pegylated IFN-α and ribavirin is widely used in the treatment of HCV infection [9]. The response rate to this therapy is about 40-50% for genotype 1 and more than 75% for genotypes 2 and 3 [10]. Two new drugs, simeprevir and sofosbuvir, showed higher response rates in phase III trials when combined with pegylated IFN-α and ribavirin [11, 12]. Due to the high cost of these new drugs, the development of a safe and effective HCV vaccine to help reduce HCV prevalence or therapeutically treat HCV-infected patients is needed.

There are many obstacles to the development of a vaccine, including HCV genotype diversity, unknown mechanisms of spontaneous clearance of the virus, and lack of adequate animal models [13]. Chimpanzee was the only animal model until one genetically humanized mouse model was developed in 2013. The humanized mouse can support the completion of an entire HCV life cycle [14]. This new small animal model may facilitate the testing of new drugs and vaccines. There are a number of vaccine modalities being tested experimentally, including recombinant E1/E2 protein vaccines, DNA vaccines, adenovirus-based vaccines, peptide vaccines and virus-like particle (VLP)-based vaccines [15-19]. However, none of them have been approved for human use yet.

In this paper, we review the existing published literature on the adaptive human immune responses to HCV, with specific emphasis on T cell responses to HCV 1b subtype (HCV-1b) of genotype 1. We identify some knowledge gaps and discuss the importance of gaining additional information on human T cell responses to HCV on vaccine design.

2. Immune correlates of protection against HCV infection

2.1. T cell responses to HCV

T cell responses are important in the control of HCV infection. In patients with acute HCV infection, viremia is detectable within the first 2 weeks and remains at high titers during the first few months. The vigor of $CD4^+$ T cell responses at the early stage of HCV infection may be a critical determinant of the later outcomes [20]. Antiviral treatment-induced viral clearance is also associated with the magnitude of IFN-γ and interleukin-2 production by HCV specific T helper 1 (Th1) cells [21]. HCV-specific $CD8^+$ T cell responses are significantly stronger in acute-resolving patients who had decreased viremia than in chronically infected patients [22, 23]. The appearance of strong cytotoxic T lymphocyte (CTL) responses is temporally associated with a sharp decline in viral load [24].

Similarly, in experimentally infected chimpanzees, viral load control was accompanied by early, vigorous, multi-specific $CD4^+$ and $CD8^+$ T cell responses [25]. Different subsets of T cells have different roles. When $CD4^+$ T cells were depleted by antibodies, memory $CD8^+$ T cell responses alone were insufficient to curtail the viral escape mutations and unable to exert complete control of viral replication [26]. On the other hand, when $CD8^+$ T cells were depleted by antibodies in chimpanzees, prolonged virus replication was observed in the absence of memory $CD4^+$ T cells [27]. These data suggest that $CD4^+$ and $CD8^+$ T cell responses are both essential and their combined actions may play a key role for viral clearance in HCV acute infection.

T cell responses in HCV infected patients with viral persistence have different characteristics. During the early stage of infection, broadly HCV-specific $CD4^+$ T cell responses are detectable irrespective of divergent clinical outcomes. However, persistence of viremia is often accompanied by early proliferation defects, rapid physical deletion or loss of function of $CD4^+$ T cells [28-30]. Although there is massive recruitment of memory effector $CD8^+$ T cells to the liver of chronic HCV infected patients, these cells are deficient in IFN-γ production due in part to an inhibition by IL-10 produced by $CD8^+$ regulatory T cells that can be expanded by HCV antigens [31]. T cell responses can also be rendered ineffective as a result of viral escape mutations within human leukocyte antigen (HLA) restricted T cell epitopes [32]. Most viral mutations appeared during the early acute infection, but remained fixed in the viral quasispecies for many years [33].

2.2. Other immune responses to HCV

Besides T cell responses, neutralizing antibody and innate immune responses may also contribute to

protection against HCV infection. Virus neutralizing antibodies are often the most direct and efficient tools to impede HCV invasion. Clear evidence came from a single-source outbreak of HCV, where viral clearance was strongly associated with a rapid production of neutralizing antibodies in the early stage of infection. In contrast, chronic HCV infected patients displayed absence or low titer of neutralizing antibodies in the early phase of infection [34]. Most known B cell epitopes are located within the envelop glycoproteins E1 and E2, which are the key elements for virus entry. It is worth noting, however, that spontaneous recovery from an acute HCV infection can happen in the absence of detectable antibody responses [35]. Thus, antibody response is not the only crucial factor in HCV clearance.

Recent studies have revealed that innate immune responses in hepatocytes have significant functions in viral defense during HCV infection. When HCV invades hepatocytes and initiates viral replication, its RNA is sensed by pattern recognition receptors (PRRs) and activates innate immune response through signaling pathways [36-40]. To counter this, HCV has evolved several strategies to escape from the innate immunity. NS3-NS4A protease, core, NS5A and E2 proteins – all have the capability to block the innate signaling pathway or inhibit functions of specific proteins to some extent [41-46]. The balance between innate immune defense and the evasion strategies of HCV may be a crucial determinant for HCV control.

Therefore, both antibody responses and innate responses may play important roles in the control of HCV, but neither of them alone can determine the outcomes of HCV infection. More antiviral immune responses are needed to be identified.

3. Human T cell responses to HCV 1b subtype of genotype 1

As mentioned above, T cell responses are essential for viral load control in infection by all 7 HCV genotypes. HCV-1b is one subtype of HCV genotype 1. T cell responses to this particular subtype show some unique features. Among the 7 HCV genotypes, only genotypes 1, 2 and 3 have a worldwide distribution, and genotype 1b is the most common genotype with a high prevalence worldwide. In China and Japan where most of the global HCV infected population resides, more than 60% of HCV infections are caused by HCV-1b. In the United States and Europe, HCV-1b is also responsible for a large proportion of HCV infections [47-51].

In addition, HCV-1b is more likely to escape from host T cell immune responses. Mutation rate of $CD8^+$ T cell epitopes, especially those within NS3/4A and NS5B regions, are significantly higher in HCV-1b than in other genotypes. The viral evolutionary pressure may come from complex interactions of many factors, but the immune pressure within individuals and at population level plays an important role [52, 53]. In a study of HCV-1b NS3 $CD8^+$ T cell epitopes, rapid appearance of mutations produced high variability in epitopes to escape host T cell responses, which occurred during acute infection [54]. One possible explanation is that HCV-1b is the prototype of all HCV genotypes, which emerges earlier in HCV evolution history, and it is the most epidemic genotype worldwide. Therefore, it has a higher probability to evolve with variable immune escape strategies [51].

In contrast to other genotypes, another feature of HCV-1b is that it is the least likely to have a response to combination therapy with pegylated IFN-α and ribavirin [48, 55]. The therapy leads to a sustained virologic response (SVR) in only 50% of patients with HCV genotype 1 infection, along with substantial side-effects [56]. This may be related to the specific HCV-1 sequence which has up to 20% of amino acid diversity from various HCV genotypes [57]. Interferon sensitivity-determining region (ISDR) within the NS5A of HCV-1b is related closely to SVR during the antiviral therapy, and the treatment outcome of patients with HCV-1b infection was influenced by mutations within ISDR [58, 59, 60]. Additionally, there are significantly more T helper 2 (Th2) cells in patients with more variable ISDR sequences. Significant Th2 expansion during the IFN-α plus ribavirin combination therapy correlated with the therapeutic responsiveness, which could be a predictor of SVR. In contrast, Th1 cells showed no correlation with treatment outcome [61-63]. The general function of Th2 is to facilitate humoral immunity by secreting cytokines like IL-4 and IL-5. This evidence implies that the level of Th2 in human peripheral blood may be an important factor that influences the efficacy of antiviral therapy and viral clearance. Hence, strengthening the HCV specific

T cell responses during antiviral therapy may improve the efficacy of therapy, and therapeutic T cell epitope-based vaccines could also make a contribution towards the treatment response when combined with interferon and ribavirin.

Core protein of HCV-1b was also found to be related to antiviral response during pegylated interferon-α and ribavirin therapy. Point mutations at positions 70 and 75 of HCV-1b were significantly associated with treatment outcome and this was not found in HCV-1a infection [64]. Furthermore, core protein can block interferon signaling pathway *in vitro*. Thus, the therapeutic action of IFN-α might be weakened by core protein [43, 44, 65]. Since core protein also contains many T cell epitopes, inducing stronger T cell responses before or during antiviral therapy with interferon and ribavirin may increase the magnitude of responses.

These unique characteristics of HCV-1b including their wide geographic distribution, various immune escape strategies, insensitivity to interferon therapy and interactions with host factors, should be better understood in order to overcome the challenges in dealing with HCV-1b infection.

4. Identification of T cell epitopes

Within the Immune Epitope Database (IEDB), a total of 1683 possible T cell epitopes on HCV genotype 1b have been reported. Among these epitopes, only 658 peptides have been identified experimentally. These epitopes are distributed within several host species, including *Homo sapiens*, *Mus musculus*, *Macaca mulatta* and *Pan troglodytes*. Since the entire polyprotein is composed of only 3010 amino acids, redundancy and overlap must exit in these 658 epitopes. In addition, the sequences of these epitopes vary among different HCV-1b isolates.

To comprehensively analyze the T cell epitopes of HCV-1b identified so far, we selected all defined HLA-I and HLA-II restricted epitopes in human infection and discarded all the redundancy (100% sequence identity). This analysis has yielded 82 $CD4^+$ T cell epitopes and 114 $CD8^+$ T cell epitopes (Fig. 1A). Epitopes were then classified and counted. All the $CD4^+$ T cell epitope sequences are distributed in regions of core, E1, E2 and NS3 proteins (Fig. 1B). Over 90% of epitope sequences are from core and E1 protein regions, suggesting that these two proteins are more critical to $CD4^+$ T cell responses in HCV-1b infection. Furthermore, among the identified major histocompatibility complex (MHC)-II alleles, HLA-DR is more commonly associated with $CD4^+$ T cell responses (Fig. 1C). The sequence distribution of $CD8^+$ T cell epitopes is different from that of $CD4^+$ T cell epitopes. Core, E2 and NS3 proteins contain more than 70% of $CD8^+$ T cell epitopes (Fig. 1D). This indicates these three proteins are more important in triggering $CD8^+$ T cell responses. Among MHC-I alleles, HLA-A2 and HLA-A24 are the more frequently identified restriction elements for $CD8^+$ T cell responses in HCV-1b infection (Fig. 1E).

Among all the human $CD4^+$ and $CD8^+$ T cell epitopes of HCV-1b in IEDB, many epitopes had more than one record. Epitopes which had more than 4 records were analyzed (Fig. 2). Most of them were identified by different assays, and hence, these epitopes are more likely to be definitive epitopes for T cell responses.

The analyses showed an overview of the characteristics of T cell epitopes of HCV-1b identified thus far. Many specific HLA allele restricted epitopes are indicated to be associated with T cell responses in HCV-1b infected humans. Their identification should help to evaluate T cell mediated immunoprotection and construction of new vaccines.

5. Associations between T cell epitopes and disease status

Since many HCV T cell epitopes, including both $CD8^+$ and $CD4^+$ T cell epitopes have been identified, the association between these epitopes and viral load control need to be analyzed.

Many previous studies have investigated HCV specific CTL or $CD4^+$ T cell responses based on T cell epitopes. Three HCV-2a-derived epitopes, which are located within the regions of HCV E2 and NS3 proteins, were demonstrated to induce peptide-specific CTLs in peripheral blood mononuclear cells (PBMC) of HCV-infected patients [66]. A novel epitope derived from core protein region of HCV-1b was reported to induce peptide-specific CTLs in PBMC of HCV-1b infected patients [67]. Four long peptides from NS3, NS4 and NS5B of HCV nonstructural proteins, which contain both $CD4^+$

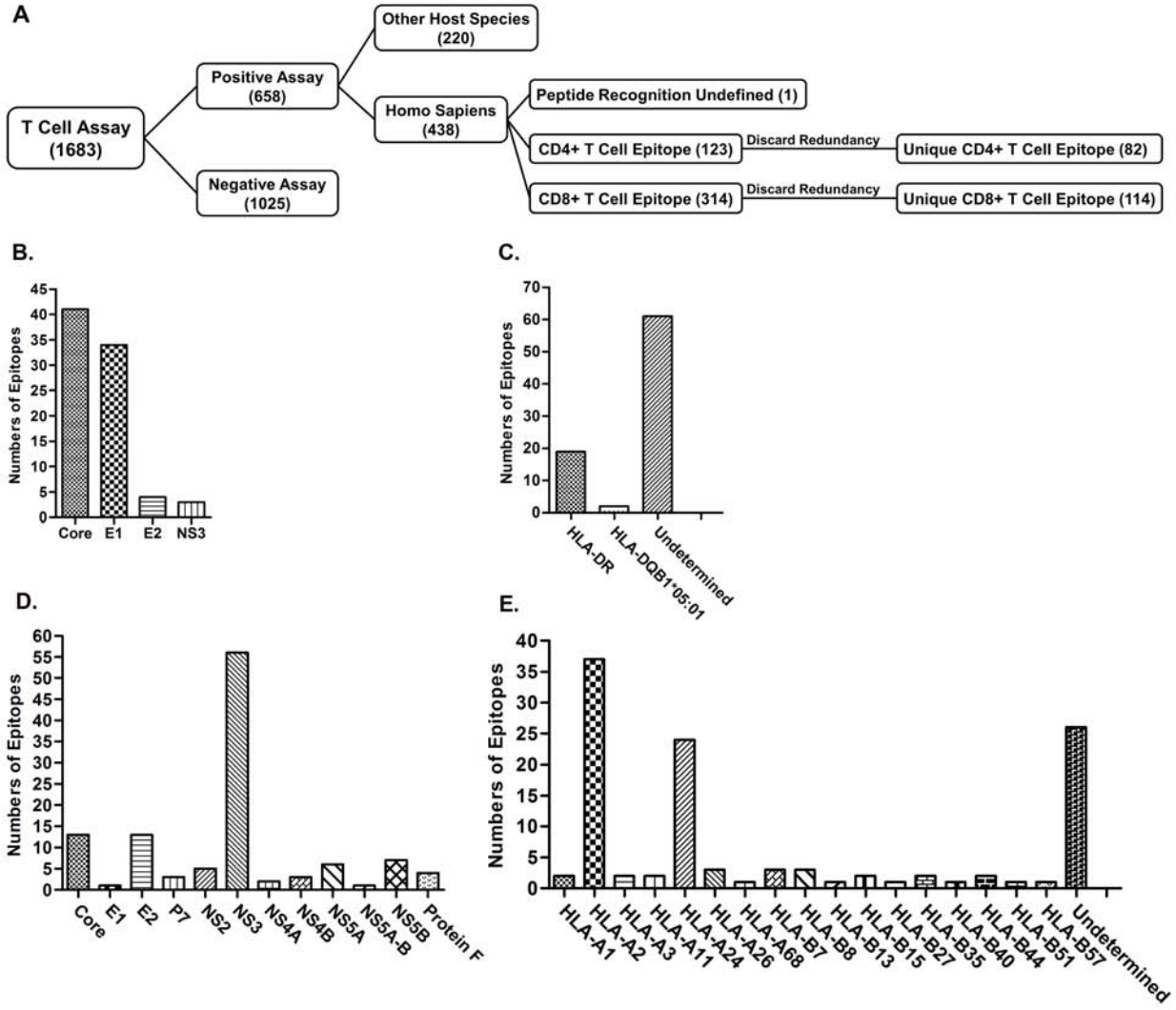

Fig. 1. Protein distribution and HLA allele distribution of HLA-1b T cell epitopes. Dataset was retrieved from IEDB (updated till Nov. 6th, 2013) by specifying "Hepatitis C virus subtype 1b" as source organism and selecting "T cell assay" choice to generate 1683 results. These assays include ^{51}Cr release assay, ELISA, ELISPOT, MHC tetramer/multimer staining, intracellular cytokine staining, ^{3}H-thymidine assay, cytokine bioassay and cytometric bead array. After discarding all epitopes from negative assays, epitopes of nonhuman species, peptides recognition undefined and redundancy (100% sequence identity), 82 unique $CD4^+$ T cell epitopes and 114 unique $CD8^+$ T cell epitopes were left and analyzed (**A**). All the epitopes were classified and counted. All $CD4^+$ T cell epitopes were distributed in four proteins of HCV (**B**). Only 21 epitopes were clearly identified with two restricting HLA-II alleles (**C**). $CD8^+$ T cell epitopes were distributed in all HCV proteins including protein F (**D**). Many specific HLA-I alleles were identified to be restriction elements for HCV-1b $CD8^+$ T cell epitopes (**E**).

and $CD8^+$ T cell epitopes, were shown to stimulate IFN-γ-producing $CD4^+$ and $CD8^+$ T cells in a mouse model and in PBMC of HCV-infected patients [68]. Additionally, other studies have reported that $CD4^+$ T cell epitopes from HCV NS3 and core proteins could prime $CD4^+$ T cells that augment CTL responses [69, 70].

What is the impact of these epitope-specific responses in determining clinical outcomes of HCV infection? One study found that a HLA-B27 restricted HCV-specific $CD8^+$ T cell epitope could be recognized in the majority of HLA-B27 positive recovered patients but not in chronic infected patients because of the emergence of escape mutations [71]. Another study

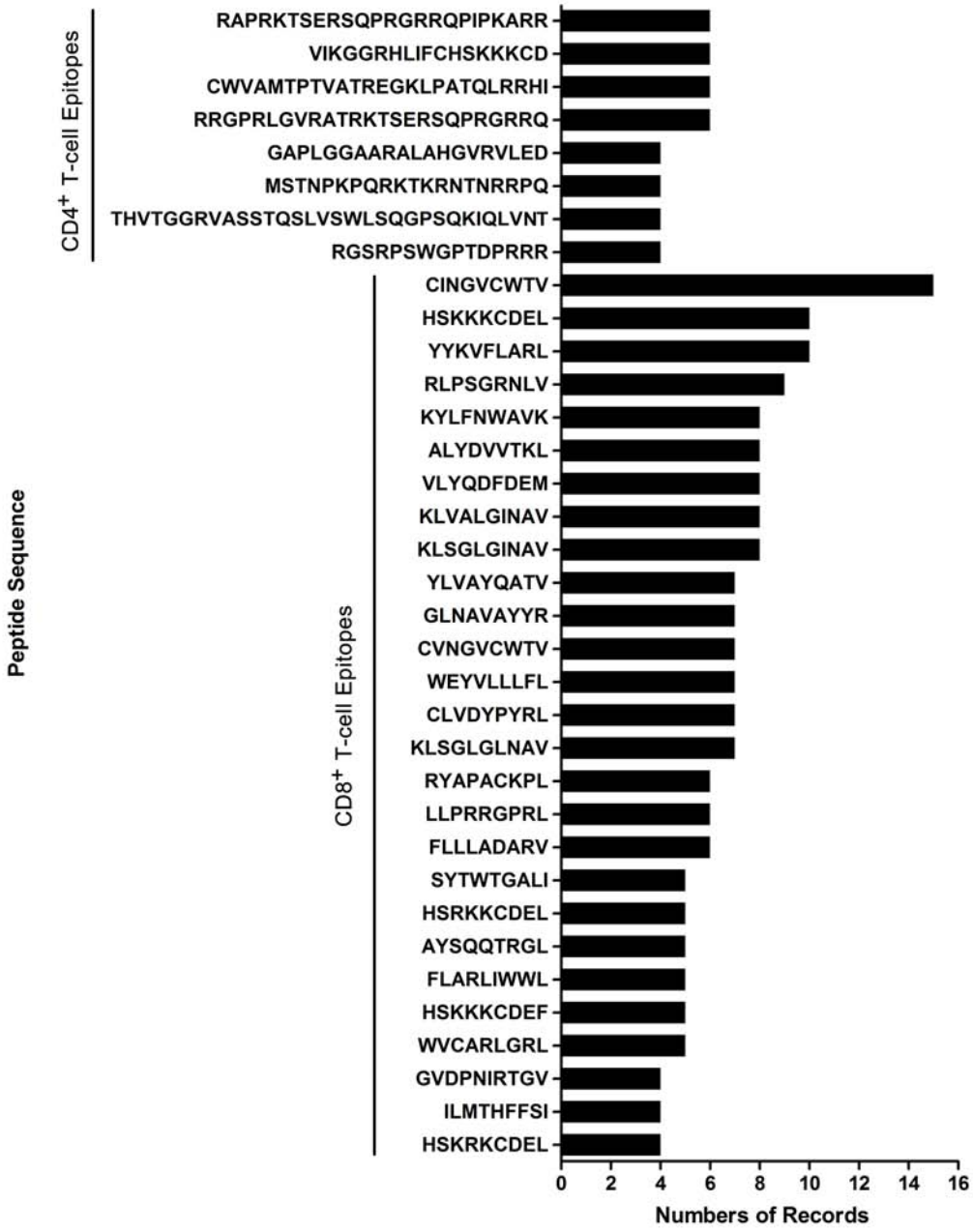

Fig. 2. Record repetition for specific epitopes of HCV-1b in IEDB. All human $CD4^+$ and $CD8^+$ T cell epitopes of HCV-1b (437) from the IEDB were analyzed. Epitopes which had more than 4 records in the database were displayed.

which used peptides from NS3 protein of HCV to stimulate PBMC found that spontaneously recovered HCV-infected patients mounted significantly broader CTL responses of higher functional avidity and wider variant cross-recognition capacity than the chronic infected patients [72]. As peptide-induced T cell responses are different between acute-resolving patients and chronic patients, using these HCV T cell epitopes to induce strong T cell responses may be a strategy of vaccine development or a therapeutic option in chronic infected patients.

To enhance the immunogenicity of peptide antigens, peptides were coupled with liposome. They had the capability to induce long-lived memory $CD8^+$

T cells in a murine model [73]. Using mature dendritic cells loaded with peptides could significantly increase epitope-specific CTL responses [74]. The HLA allotype of patients is another factor that should be taken into consideration. For instance, HLA-B27 had been reported to contribute to viral clearance through rapid antigen processing, which might be the key immunological feature of HLA-B27 associated immune protection.

6. Potential application of T cell epitopes to vaccine design

The low response rate of the current combination therapy of IFN-α and ribavirin to HCV infection has prompted the search for more effective antiviral drugs as well as effective vaccines. Developing peptide-based vaccines is one of the attractive strategies for both prophylactic and therapeutic uses. Since T cell responses are believed to be essential for viral clearance and many T cell epitopes of structural and nonstructural proteins have been identified, a synthetic peptide vaccine could be a viable approach to elicit T cell immune responses against HCV infection [75].

Many trials have been carried out to design vaccines with HCV T cell epitopes including vaccine candidate IC41, which is comprised of seven synthetic peptides representing four HCV specific HLA-A2 restricted CTL epitopes and three $CD4^+$ T cell epitopes from both structural and nonstructural proteins [18]. In a phase I clinical study in 128 HLA-A2 positive healthy volunteers, IC41 was proved to be safe and well tolerated. It also induced T cell responses in a dose-dependent manner. Furthermore, adjuvant poly-L-arginine was shown to be required for the production of functional IFN-γ secreting T cells [76]. In a phase II study in sixty HLA-A2-positive chronic HCV infected patients who did not respond to standard interferon-based treatment, IC41 was well tolerated and T cell proliferation was detected in 67% of patients. About one third of them developed sustained T cell responses that lasted for six months after the last vaccination. However, only three patients' HCV serum RNA declined despite a detection of strong T cell responses, indicating that there is a limitation in the control of HCV viral load by T cells alone [18, 77]. Further optimization of the vaccine regimen may be considered, such as using stronger adjuvants, changing vaccination routes, trying different prime-boost schedules or incorporating the vaccine with combination therapy of standard interferon and ribavirin [75]. One optimized trial was performed in 50 HCV-infected patients. Using a biweekly vaccination schedule, 24% of patients showed a viral load decline 24 weeks after the last IC41 vaccination [78]. This study provided hope that the synthetic peptide vaccine could be beneficial to viral load control.

Besides IC41, many other clinical and pre-clinical trials are in progress. Phase I study of a personalized peptide vaccine, which contains four HLA-A24 restricted peptides, was shown to elicit peptide specific T cell responses in most patients who received the vaccine and viral load decline was also observed in three patients [79]. More recently, one peptide derived from HCV core protein (sequence: YLLPRRGPRL) was tested in a phase I clinical trial. Both core-specific cellular and humoral responses were detected and two responders had over 1 log decline in HCV RNA levels [80].

Taken together, peptide-based vaccine is a practical, cost-efficient, safe and well tolerated vaccine strategy that can elicit strong T cell responses against HCV infection. Other peptide-based vaccine approaches, such as using multi-linear epitopes, fusion of VLP-forming elements, combining with other types of vaccines (like antibody-based vaccine), using carrier systems (like influenza virosome or liposome) may provide more options for the epitope-based vaccines [75].

7. SUMMARY

As HCV infection has become a heavy burden to the global health care system, more studies to investigate the mechanisms of immune protection, drug therapies and vaccines are in urgent need. Currently, antibody responses, T cell responses (both $CD4^+$ and $CD8^+$) and innate immune responses are all believed to play essential roles in viral control during HCV infection. HCV-1b is an intensely studied subtype in HCV research area, for it has more challenges than other genotypes. Some T cell epitopes of HCV-1b have been identified, yet more are still to be discovered. This line of research will provide support to the development of a peptide-based vaccine.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

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