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Original Article

Comparison of the inhibitory and stimulatory effects of Core and NS3 candidate HCV vaccines on the cellular immune response

Kiandokht Borhani[1], Taravat Bamdad[1], Ava Hashempour[2], Amir Salek Farrokhi[3], Javad Moayedi[2]

[1]Department of Virology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran; [2]Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran; [3]Department of Immunology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

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Abstract: Currently, hepatitis C virus (HCV) infects nearly 3% of the global population, the majority of whom are chronically infected; however, hepatitis C vaccines are still in the developmental stage. Numerous studies suggest that the spontaneous resolution of HCV infection and the design of its vaccine are reliant on vital contributions from CTL cell responses and T regulatory cells. Multiple researchers have identified both Core and nonstructural protein 3 (NS3) proteins as crucial immune genes and potential candidates for HCV DNA vaccine design. In this study, Core and NS3 were subcloned and inserted into pcDNA3.1 to construct HCV DNA vaccines administered in mouse models. Furthermore, the effects of Core and NS3 on the induction of CTL and NK were compared in spleen mouse models using the LDH method. Additionally, flow cytometry was employed to investigate the percentage of T regulatory cells (Treg cells) and cells expressing PD-1 in the spleens of the mouse models. Our data indicated that pcDNA3.1+NS3 and pcDNA3.1+Core could enhance CTL and NK activity in mouse models. Importantly, the Treg and PD-1 analysis in mouse models revealed a substantial reduction in the proportions of CD4+/CD25+/Foxp3+ T cells and PD-1+ cells in experimental subjects treated with HCV NS3 along with 5 mg/kg of lenalidomide, utilized as a novel adjuvant, compared to those administered an equivalent dosage of lenalidomide in conjunction with HCV Core. In conclusion, our observations indicated that the NS3-HCV gene had a limited impact on the activation of inhibitory factors. Therefore, NS3 is considered a more suitable candidate for DNA vaccine design compared to Core HCV.

Keywords: HCV, Core, NS3, vaccine, CTL cell, T regulatory cells

Introduction

HCV is among the main factors in acute and chronic liver diseases such as hepatocellular carcinoma and cirrhosis [1-3]. According to studies conducted by the WHO, 170 million people currently suffer from HCV, and 3 to 4 million, mostly in developing countries, are newly affected by this disease each year globally. Despite significant efforts in producing an HCV vaccine, the development of such a vaccine has encountered several obstacles, namely a lack of animal models, genetic heterogeneity, and several effective immune escape strategies. Although neutralizing antibodies against HCV can be identified within 7-8 weeks post-infection, they cannot successfully protect the infected person against reinfection. However, cellular immunity is capable of clearing HCV infection, implying its importance in spontaneous resolution of acute HCV and long-term protection from persistent infection [4-6]. Furthermore, regulatory T cells have a pathologic role in viral infection, especially in chronic infections and vaccine design in humans. It has been shown that the number of circulating CD4+/CD25+/Foxp3 cells in HCV carriers is larger than that of healthy persons. Treg cells also inhibit CTL cells and IFN-γ secretion, which results in delayed virus clearance and persistent infection. In most cases, CD8 cytotoxic T-lymphoid (CTL) responses were associated with the control of infectious agents, especially against viral infection or tumor Ag-derived pep-

late the Treg activity in vivo and improve immunogenicity of the vaccine.

Based on the literature above, NK cells provide an early defense against pathogens, especially in viral infection. Moreover, these cells can rapidly attack the sites of virus entry and play a critical role in the restriction of acute viral infections [33]. In this research, the results obtained from animal models showed that NS3 and Core HCV vaccine candidates were able to boost the NK cell cytotoxicity. Leblanc et al. showed that lenalidomide was able to stimulate the NK cell activity by increasing the Thelper1-type cytokine response, such as IFN-γ and IL-2 [34, 35]. Another study indicated that lenalidomide, as an immunomodulatory drug, promoted the NK cell activity in vitro [36]. Our experiments illustrated that NK cell activity could notably increase after immunization with Core HCV DNA or NS3 HCV DNA vaccine plus 5 mg/kg lenalidomide in mouse models.

Furthermore, it has been reported that the upregulated expression of PD-1 was part of the major factor leading to T-cell exhaustion, especially in LCMV mouse models and chronic infections such as HBV, HIV, and HCV [37]. Researchers have shown that additional PD-L1 blockade, along with HBV vaccination, yielded a higher efficiency of the vaccine through increasing T-cell functional capacities [38, 39]. The results of this study showed that treatment with HCV Core could remarkably increase the expression of PD-1 in comparison to HCV NS3 treatment in the spleen of mouse models. However, we demonstrated that 5 mg/kg of lenalidomide, along with the HCV NS3 vaccine, could greatly decrease PD-1+ expression in comparison to the HCV Core vaccine in mouse models. In addition, researchers have shifted their focus to the application of adjuvants to improve the efficiency of the HCV vaccine and enhance the cellular response. In this research, we used 5 mg/kg lenalidomide along with pcDNA3.1(+)/NS3 and pcDNA3.1(+)/Core and observed that lenalidomide dramatically increased the CTL activity response in mouse models. Knobloch et al. [40] showed that lenalidomide stimulated different parts of the immune system in tumor mouse models by improving anti-inflammatory cytokines such as IL-6, IL-1, and IL-10 in human PBMC samples. In another study, it was reported that CTL responses increased in mice models that received HCV-NS3 along with lenalidomide, suggesting that lenalidomide can promote the CTL activity in response to the vaccine candidate [22].

Previous studies suggest that lenalidomide can decrease the Treg cells and enhance T helper 1 by producing cytokines in myeloma mouse models [22]. Several publications have shown that the percentage of CD4+/CD25+/Foxp3+ T cells decreased in T cells obtained from healthy donors by stimulation of anti-CD3/CD28 in the presence of lenalidomide [41]. We found that treatment with 5 mg/kg lenalidomide along with HCV NS3 or HCV Core vaccine candidates induced an inhibitory effect on the CD4+/CD25+/Foxp3+ T cells more efficiently than HCV vaccines alone. Moreover, the analysis of flow cytometry data on the Treg cells in the spleen of the mice revealed that the percentages of CD4+/CD25+/Foxp3+ T cells were significantly different from those of HCV Core and HCV NS3 plus injection of 5 mg/kg lenalidomide. Interestingly, examination of the CD4+/CD25+/Foxp3+ T cells in mouse models showed significantly lower percentages in cases that received HCV NS3 plus 5 mg/kg lenalidomide compared with the cases that received HCV Core plus 5 mg/kg lenalidomide.

Conclusion

Our data suggest that the NS3 sequence of the HCV genome is an attractive candidate for DNA vaccine design. At the same time, it has a limited impact on the activation of inhibitory factors in comparison to Core HCV. This study concluded that adjuvants were paramount components in vaccines in terms of enhancing and directing immunity to vaccine antigens. Overall, lenalidomide, along with the NS3 HCV DNA vaccine, might serve as an effective adjuvant candidate to improve the efficiency of vaccine, in comparison with Core antigen. Finally, the adjuvant lenalidomide decreased the activated Tregs more effectively in the NS3-vaccinated mice.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ava Hashempour, Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Tel: +98-7137386272; Fax: +98-7137386272; E-mail: thashem@sums.ac.ir

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