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Outcomes of Interferon/Ribavirin Therapy in Patients with HCV Defined by Expression of Plasma Soluble Human Leukocyte Antigen-G but Not IL-37

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AG 1 **Shi-xiong Ding**
B 1 **Jian-bo Ma**
B 2 **Yao-ren Hu**
CD 2 **Ai-rong Hu**
CD 2 **Qiang Shen**
EF 1 **Guo-sheng Gao**

1 Department of Laboratory Medicine, The Affiliated Ningbo No. 2 Hospital, College of Medicine, Ningbo University, Ningbo, Zhejiang, P.R. China
2 Institute of Liver Disease, The Affiliated Ningbo No. 2 Hospital, College of Medicine, Ningbo University, Ningbo, Zhejiang, P.R. China

Corresponding Author: Shi-Xiong Ding, e-mail: shixiongdng@163.com
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Background: Chronic hepatitis C virus (HCV) infection leads to life-threatening complications worldwide. Immunomodulation signals the response to virus clearance. The immune-suppressive molecule human leukocyte antigen-G (HLA-G) has been shown to function in inhibiting both innate and adaptive immune responses. The objective of this study was to investigate the expression of HLA-G and IL-37 in sustained virological response (SVR) and non-SVR HCV-positive patients before and after complete treatment with a combination of pegylated interferon (IFN) and ribavirin (RBV).





Material/Methods: Our study included 132 chronic hepatitis C patients who received combined therapy with IFN- α and RBV. Both SVR and non-SVR patients were included. The end-of-treatment response was defined as undetectable HCV RNA at week 48. Patients with end-of-treatment response were detected by HCV RNA at 24 weeks after therapy. The expression levels of HLA-G and IL-37 at the end and 24 weeks after treatment were detected by ELISA.

Results: Plasma HLA-G and IL-37 were significantly increased in HCV-infected patients compared with healthy individuals before treatment. Furthermore, HLA-G in SVR patients was noticeably decreased after treatment, while HLA-G in non-SVR patients had no changes after treatment. Additionally, both in SVR and non-SVR patients, the expression of IL-37 was remarkably reduced compared with baseline after treatment.

Conclusions: These findings suggest that elevation of HLA-G and IL-37 in HCV may play an important role in response to combined therapy with IFN- α and RBV. Monitoring the expression of HLA-G during therapy could contribute to adjusting the treatment program of HCV-infected patients.

MeSH Keywords: **Hepatitis, Autoimmune • HLA Antigens • Ribavirin**

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Background

More than 180 million people worldwide are chronically infected with the hepatitis C virus (HCV) [1]. Chronic HCV infection leads to life-threatening complications, such as liver cirrhosis and hepatic carcinoma [2,3]. In the clinical guidelines, the current standard therapy for chronic HCV infection is a combination of pegylated interferon- α (PEG-IFN- α) plus ribavirin (RBV), but approximately 30% of treated patients relapse [4,5]. RBV is a synthetic guanosine analogue. Several mechanisms of action have been proposed to explain its anti-HCV effect, including direct inhibition of the viral RNA polymerase, inosine-5'-monophosphate dehydrogenase inhibition, hypermutagenesis, and immune modulation [6].

The progression of chronic HCV is closely associated with chronic inflammation, which is related to the efficiency of the immune response. Cytokines and serum soluble factors play important roles in immune response and treatment outcome of HCV infection [7,8].

Human leukocyte antigen G (HLA-G) is a nonclassical major histocompatibility complex class I gene encoding for a protein with a cytoplasmic tail [9]. As a tolerogenic molecule, HLA-G has been shown to function in inhibiting both innate and adaptive immune responses [10,11]. This molecule triggers the apoptosis of T and NK cells via CD8-like classical class I soluble molecules [12], and it inhibits the proliferation of B lymphocytes and the differentiation of these cells and their immunoglobulin secretion [13]. In our previous research, increased shedding HLA-G expression in HCV patients may play a role in the persistency of HCV infection [14], but recent studies on HLA-G are either for diagnosis or as a therapeutic tool/target to predict outcomes.

In vivo, expression of IL-37 reduces local and systemic inflammation in concanavalin A-induced hepatitis [15]. Accumulating evidence shows that IL-37 may play a significant role in the immune response of chronic hepatitis B virus (CHB) patients with HBeAg seroconversion. The serum levels of IL-37 are associated with liver damage in CHB patients [16], but there have been few reports on the relationship between IL-37 and chronic HCV infection.

In the present study, we found that elevation of HLA-G and IL-37 in HCV may play an important role in response to combined therapy with IFN- α and ribavirin. Monitoring the expression of HLA-G during therapy could contribute to adjusting the treatment program of HCV patients.

Material and Methods

Study population

A total of 132 blood samples were collected from patients chronically infected with HCV, and 112 age- and sex-matched, unrelated, healthy blood donors were included as normal controls. Specimens were collected from the Affiliated Ningbo No. 2 Hospital in China between February 2011 and November 2014. No patients received anti-HCV agents before sampling, and we excluded individuals with concomitant HIV and HBV infection and hepatitis due to reasons other than hepatitis C. The diagnosis complied with the diagnostic criteria of the 2000 Xi'an Viral Hepatitis Management Scheme issued by the Chinese Society of Infectious Diseases and Parasitology, and the Chinese Society of Hepatology, of the Chinese Medical Association. The study protocol was approved by the institutional ethics committee (NO. KYLL2010039). The characteristics of subjects enrolled in the study are shown in Table 1.

Study design

All patients with HCV genotype 1 infection received conventional IFN- α (80 μ g per week, subcutaneously, 3 times a week) and ribavirin 1000 mg (<75 kg) or 1200 mg (>75 kg) daily. The end of treatment response was defined as undetectable HCV RNA at week 48. Patients with end-of-treatment response and undetectable HCV RNA at 24 weeks after therapy were defined as having sustained virological response (SVR). Non-SVR was defined as a case in which HCV RNA had been undetectable at the end of treatment, but was detectable 24 weeks after treatment.

Serum biochemical indexes measurement

AxSYM HCV 3.0 (Abbott Laboratories, Wiesbaden-Delkenheim, Germany) was used to identify patients seropositive for anti-HCV antibody. The HCV-RNA values were detected by Cobas TaqMan HCV assay (Roche Diagnostics, Indianapolis, USA) according to the manufacturer's instructions. Other serum biochemical indexes were measured by automated biochemical analyzer (Hitachi, Japan).

HLA-G enzyme-linked immunosorbent assay

HLA-G were measured using HLA-G-specific enzyme-linked immunosorbent assay (Exbio, Prague, Czech Republic) as described previously [HIM]. Each plasma sample (100 μ l) was measured in triplicate. The optical densities were measured at 450 nm (Spectra Max250, Molecular Devices, Sunnyvale, USA). The final concentration was determined by optical density according to the standard curves.

Table 1. Clinical baseline characteristics of the enrolled 132 patients with HCV and 112 normal controls.

Variable	Control group (n=112)	HCV group (n=132)
Age, y	42.88±12.48	42.65±13.16
Gender, male/female	55/57	73/59
Globin, g/L	16.07±6.78	31.15±7.12
Albumin, g/L	37.67±5.67	42.75±5.92
Total bilirubin, µmol/L	7.65±2.01	16.47±6.13
AST, IU/L	14.41±5.81	66.65±22.22
ALT, IU/L	22.79±4.49	87.75±16.65
WBC counts, ×10 ⁹ /L	5.79±1.51	6.35±2.36
Hemoglobin, g/L	141.65±31.29	145.46±16.89
Platelet counts, ×10 ⁹ /L	134.46±38.08	165.07±44.40
HCV RNA (log ₁₀), IU/ml	–	6.21±1.08

HCV – hepatitis C virus; ALT – alanine aminotransferase; AST – aspartate transaminase; WBC – white blood cell. Data are expressed as means ± standard deviation (SD).

IL-37 enzyme-linked immunosorbent assay

Plasma cytokine concentrations were determined in triplicate with a commercially available human IL-37 by ELISA Kit (R&D Systems, USA). Each sample was tested according to the manufacturer's instructions. The final concentration was determined by optical density according to the standard curves.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 5. We performed one-way analysis of variance (ANOVA) followed by *t* test for comparisons. Differences with *P*<0.05 were considered as statistically significant.

Results

Characteristics of HCV-infected patients and normal controls

Characteristics of the HCV-infected patients and controls are shown in Table 1. In this study, 132 HCV-infected patients (male/female: 73/59; mean age: 42.65 years) and 112 age- and sex-matched, unrelated, healthy individuals (male/female: 55/57; mean age: 42.88 years) were included. The HCV-RNA (log₁₀) load in HCV-infected patients was 6.21±1.08 copies/ml.

HLA-G and IL-37 are increased in the plasma of HCV patients

Before combination therapy, HLA-G expression is dramatically increased in HCV-infected patients over that in normal controls (1.88±0.07 vs. 74.24±3.09 ng/L, *p*<0.01; Figure 1A). In the same class, the plasma level of IL-37 in chronic HCV patients were higher than in healthy people at baseline (47.64±5.92 vs. 89.10±15.04 pg/ml, *p*<0.05; Figure 1B).

HLA-G and IL-37 affecting treatment outcome

At 48 weeks after combination therapy, 104 patients (79%) were HCV RNA-negative (SVR), while the other 28 were positive (non-SVR). The data revealed that the plasma HLA level was significantly higher in non-SVR patients than in SVR patients after treatment. In SVR patients, after combination therapy, the HLA level was lower than at baseline, whereas it was not significantly different in non-SVR patients under therapy compared with baseline (Figure 2A). After combination therapy, plasma IL-37 level was the same in non-SVR patients and in SVR patients; however, the expression of IL-37 in SVR and non-SVR patients was remarkably reduced compared with baseline after treatment (Figure 2B).

Discussion

HCV infection is a global health problem. Most HCV-infected individuals become chronic hepatitis patients, which progressively

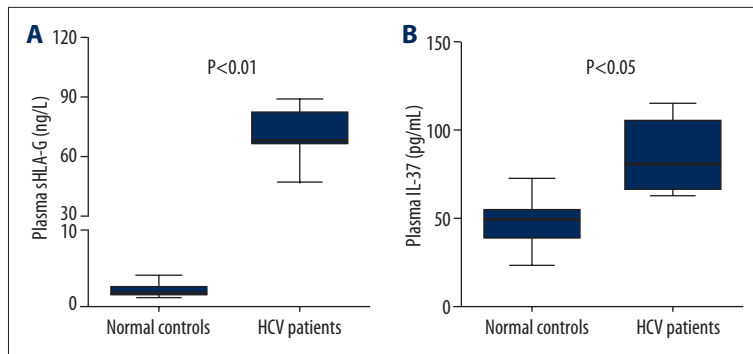


Figure 1. Plasma HLA-G and IL-37 levels increased in HCV patients compared to normal controls measured by specific ELISA. (A) Plasma HLA-G level; (B) Plasma IL-37 level. Mean plasma levels with min to max.

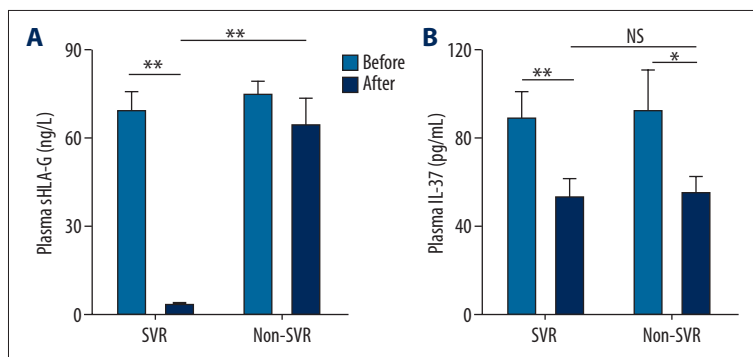


Figure 2. Plasma HLA-G and IL-37 levels decreased in HCV patients after IFN- α and RBV combined treatment. (A) Plasma HLA-G level; (B) Plasma IL-37 level. SVR: sustained virological response. Mean plasma levels \pm SD, * p<0.05; ** p<0.01.

develops into liver fibrosis, liver cirrhosis, and hepatocellular carcinoma. We have recently reported that elevated sHLA-G expression in HCV patients was independent of viral genotype and viral RNA load, and an increase in sHLA-G may play a role in the persistence of HCV infection [14]. Accumulated data reveal the serum levels of IL-37 are associated with liver damage in CHB patients [16]. To determine whether expression of HLA-G and IL-37 during therapy could contribute to adjusting the treatment program of HCV patients, we analyzed the expression of HLA-G and IL-37 in sustained virological response (SVR) and non-SVR HCV-positive patients after treatment with a combination of IFN- α and RBV. According to the obtained data, plasma HLA-G and IL-37 were significantly increased in people with HCV compared with healthy individuals before treatment. Furthermore, HLA-G in SVR patients was noticeably decreased after treatment, while HLA-G in non-SVR patients had no changes after treatment. Additionally, both in SVR and non-SVR patients, the expression of IL-37 was remarkably reduced compared with baseline after treatment.

Various cytokines, such as IFNs, IL-10, IL-2, and TGF- β , influence HLA-G expression [17,18]. All these micro-environmental factors also play an effective role in the liver. Chies et al. reported the influence of HLA-G polymorphisms in human immunodeficiency virus infection and hepatitis C virus co-infection in Brazilian and Italian individuals [19]. There are no data currently available concerning the expression of HLA-G receptors in the liver, and studies on the expression of these receptors on Kupffer cells, hepatocytes, and hepatic stellate cells

have not been performed [20]. The number of HLA-G⁺ cells was significantly associated with fibrosis, and HLA-G secretion was significantly induced in human mast cells stimulated by IL-10 [21]. These data might support that HLA-G secretion in most hepatic diseases is correlated with the intensity of inflammation and immunity. Moreover, the HLA expression may be a contributing factor to aspirin resistance and platinum resistance [22,23]. This means that abnormal expression of HLA is a poor prognostic mechanism for drug response. According to our data, plasma HLA-G was significantly higher in people with HCV than in healthy individuals before receiving combination therapy. Furthermore, HLA-G in SVR patients was noticeably decreased after treatment, while HLA-G in non-SVR patients had no changes after treatment. These findings might provide an additional predictive factor for therapeutic effect of administered IFN- α and RBV.

IL-37 is a newly discovered cytokine of the IL-1 family that is expressed in a variety of tissues and cells, such as monocytes, natural killer (NK) cells, and stimulated B cells [24,25]. Expression of IL-37 in macrophages almost completely suppressed production of cytokines active in the initiation of the inflammatory reaction [26,27]. As an inhibitor of adaptive immunity, expression of IL-37 in DCs impaired activation of effector T-cell responses [28]. In our study, plasma IL-37 level was significantly increased in people with HCV compared with healthy individuals before IFN- α and RBV combined treatment. In both SVR and non-SVR patients, the expression of IL-37 was remarkably reduced compared with baseline after treatment.

These might be related to the action of IL-37 released or the modulation of hepatocyte inflammatory response.

Conclusions

The evolution of HLA-G level and IL-37 among HCV patients is associated with immune response. Treatment with IFN- α and RBV for 48 weeks led to significantly decreased IL-37 level compared to baseline. Moreover, HLA-G in SVR patients was noticeably decreased after treatment, while HLA-G in non-SVR patients remained at high levels after treatment. In this

study, we found the expression of HLA-G level and IL-37 was correlated with sustained virological response. These findings could provide an additional predictive risk factor of therapeutic outcomes of IFN- α and RBV administered in HCV patients. Future studies will focus on determining the major mechanism of HLA differential expression in SVR and non-SVR patients after combined therapy.

Conflict of interest statement

The authors report no conflicts of interest.

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