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Characteristics of the cellular immune response in HIV/HCV patients with hemophilia during peginterferon/ribavirin therapy in southern China☆,☆☆

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ABSTRACT

Objective: The objectives of the study are to characterize the cellular immune response in hepatitis C virus (HCV) genotype 1 and HIV co-infected patients with hemophilia in southern China during treatment with interferon and ribavirin and to study its correlation with the virologic response (VR). Thirty-six HCV genotype 1 and HIV co-infected patients with hemophilia in southern China were enrolled into the study. Using an ELISpot assay, HCV antigen-specific interferon (IFN) γ, interleukin (IL) 2, IL-4, and IL-10 secreting cells were measured in peripheral blood mononuclear cells. Single nucleotide polymorphisms of IL28B were determined, and immunological, virologic, and clinical variables were collected to identify factors associated with HCV-sustained VR (SVR) at week 72 after treatment. At baseline, there were no significant differences in IFN-γ and IL-2 mediated immune responses in subjects with VR versus non-responders. Higher IL-10 specific responses to NS3 were observed in VR patients. Subjects who had significant decreases in IL-10 responses at week 72 compared with baseline for NS3 and NS5 were more likely to be VR. In SVR, IL-2 production decreased moderately, and the levels of IL-4 were low throughout. The main correlation for SVR in genotype-l infected subjects was sustained HCV-specific IFN-γ responses through the whole 72-week period. In subjects with HIV and HCV co-infection combined with hemophilia, IL28B genotype CC, a decrease in HCV specific IL-l0 and IL-2 responses, and the maintenance of IFN-γ responses during treatment were associated with a 12- or 72-week VR.

1. Background

Hepatitis C is a major cause of morbidity and death in HIV-infected individuals (Bambha et al., 2012; Mocroft et al., 2012). As reported, 9–40% HIV patients are co-infected with hepatitis C virus (HCV) (Greub et al., 2000), and HCV infection rate reaches 85% in HIV patients with hemophilia (Sterling et al., 2010). Co-infection with HIV accelerates the progression to liver cirrhosis. Cellular immune responses, crucial for the control of HCV infection (Rohrbach et al., 2010), are weak in chronic hepatitis C and are further impaired in HIV co-infected individuals. The cytokine profile plays an important role in treatment outcome of HCV infection and probably modulates the immune response against HCV.

In chronic HCV infection, Type 1 helper T cells produce interleukin (IL) 2, IL-12, and interferon (IFN) γ and play crucial roles in effective antiviral defense. Type 2 helper T cells secrete IL-4, IL-5, IL-10, IL-6, and IL-9 and can act as counterparts down-regulating antiviral activity by inhibiting T helper type 1 cytokine production (Bergamini et al., 2001; Capa et al., 2007).

Combination antiviral therapy for HCV reduces liver-related mortality in HIV/HCV co-infected individuals. Potential favorable effects of combination antiviral therapy on the course of hepatitis C include a reduction in immune activation and an increase in cellular immune responses. It is unclear to what extent a successful combination antiviral therapy restores HCV-specific immune responses. HCV genotype 1b is the most common HCV genotype in China and accounts for 80% of chronic hepatitis, cirrhosis, and hepatic carcinoma caused by HCV. Although there are some reports in HCV/HIV-infected patients without hemophilia, there are few studies on the cellular response in HCV/HIV-infected hemophilia patients and how it is related with the sustained virologic response (SVR). Haemophiliacs infected with HCV/HIV may have more rapid progression to liver failure and death than non-haemophilics with HCV/HIV (Qin et al., 2005; Shire et al., 2006), and the reasons are not fully understood, highlighting the need for research in this field. Besides cellular immune response, variation in the IL28B gene on chromosome 19, which encodes the type III interferon (IFN-λ3), is associated with SVR in patients with HCV genotype 1 (Alestig et al., 2011; Hayes et al., 2011 Feb), and other immunological and clinical variables are also related to SVR.

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☆☆ Authors' contributions: LY designed the experiment and drafted the manuscript. WJR collected the specimen and performed the statistical analysis. LJ carried out most experiments and participated in its design. All authors read and approved the final manuscript.

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In this study, we investigated the T-cell responses in the peripheral blood mononuclear cells (PBMCs) of persons with HIV/HCV co-infection with hemophilia in southern China entering an anti-HCV treatment trial. We also examined the relationships between type 1 and type 2-like cytokine responses and virologic response (VR) in order to assess the relative role of immune responses on histologic outcome and HCV RNA levels.

2. Methods

2.1. Patients

Thirty-six HIV/HCV co-infected patients with hemophilia in Shanghai Public Health Clinical Center from 2007 to 2009 were enrolled in this study, their ages ranging from 19 to 51 years old, sex ratio of 1:1, all the patients were HBV DNA negative and infected with HCV genotype 1b.

Clinical characteristics of the study group are described in Table 1. Age and ethnicity were similar among the patient groups.

All patients were treated by combined antiviral therapy for HCV. They were given PEG-IFN-alfa-2a 180 μg, once a week, subcutaneously (200 mg/d) and ribavirin orally (1000 or1200 mg/d, based on body weight). At 12 weeks, HCV RNA levels were detected for all patients. Treatment was stopped in patients with a decrease in HCV RNA level of <2 log; others were treated for 48 weeks. Patients were then followed for at least 24 weeks after treatment, and if at week 72, HCV RNA levels of the patient were undetectable, the patient gained a SVR; the others were classified as non-responders (NR). At baseline and at week 72, we assessed the cellular immune response, IL28B genotype, HCV RNA levels, alanine aminotransferase, liver biopsy, and inflammatory scores in the patients.

2.2. Recombinant HCV proteins

The recombinant HCV proteins used were derived from HCV genotype 1b and included core protein [amino acid (amino acids) 1–115], and non-structural (NS) proteins NS3 (amino acids 1007–1534), and NS5 (amino acids 2622–2868) at 1 mg/mL (Mikrogen, Munich, Germany).

2.3. ELISPOT assay

The ELISPOT is performed individually for each patient. PBMCs were isolated by Ficoll-Hypaque (Amersham Biosciences, Piscataway, NJ, USA) density gradient centrifugation and cryopreserved. Enzyme-linked immunosorbent spot assays were performed as described previously (Harcourt et al., 2006).

Antigens used in cell proliferation were recombinant HCV proteins, as described above. Phytohaemagglutinin (1 μg/mL, PHA; Murex Biotech Limited, Dartford, UK) was used as polyclonal control.

The ELISPOT assay was used to determine IFN-γ, IL-10, IL-2, and IL-4 secreting cells. Briefly, plates were coated with primary monoclonal antibody (mAb) (anti-IFN-γ, anti-IL-2, anti-IL-4, and anti-IL-10 [Endogen, Woburn, MA, USA]), at a concentration of 10 mg/mL. 2.5×10^5 PBMC were cultured for 40 h in the presence of the recombinant HCV protein core and NS proteins NS3 and NS5 at 1 mg/mL. Positive control wells consisted of PHA (5 mg/mL; Sigma, St. Louis, MO, USA) and *Candida* cellular antigen (20 mg/mL; Greer Labs, Lenoir, North Carolina, USA). Negative control wells were media alone and buffer. After 48 h, the PBMCs were removed, and 50 mL of biotin-conjugated secondary mAb (anti-IFN-γ or anti-IL-10 [Endogen] or anti-IL-2, IL-4 [BD Pharmingen, San Diego, CA, USA]) were added at 0.2 mg/mL. Plates were developed, and the numbers of spots per well were scored using a dissection microscope. The averaged numbers of spot-forming cells (SFC) in control wells were subtracted from antigen-stimulated wells to correct for spontaneous cytokine production.

2.4. Determination of the single nucleotide polymorphisms (SNPs) of IL28B

DNA was extracted using the automated MagNA Pure DNA extraction method (Roche Diagnostics, Madison, WI, USA). The rs129679860 SNP was genotyped using a custom TAQMAN genotyping assay (Applied Biosystems, Beijing, China) on DNA isolated from whole blood samples. The DNA was genotyped according to the manufacturer's instructions on a MX3005 thermocycler using MXpro software (Stratagene, Shanghai, China). The researchers responsible for genotyping procedures were unaware of other data from the patients.

2.5. Statistics

Frequencies of ELISPOT responses were analyzed by Fisher's exact test or the Chi square test. Statistical analyses were performed using SPSS 11.5 software. In all cases, 2-tailed *P*-values ≤0.05 were considered statistically significant.

Table 1
Demographic characteristics of HIV/HCV haemophilics at baseline and during the treatment.

	Baseline	Week 12 Early VR	Week 72 SVR	Week 72 No response
PEG-IFN + Ribavirin	36	12	10	25
Male	18	5	3	15
Female	18	7	7	11
Age in years				
≤40	23	8	7	16
>40	13	4	3	8
CD4 cell count ($\times 10^6$/L)				
100–299	10	1	1	9
300–500	17	4	3	14
>500	9	2	2	7
HCV-RNA(copies/L)	(5.57 ± 1.64) × 106	(3.7 ± 1.64) × 102	≤50	(2.8 ± 0.93) × 106
ALT	105.42 ± 27.71	37.53 ± 12.39	29.64 ± 10.23	74.26 ± 23.67
HIV RNA load undetectable (< 50 copies/ml)	5	2	2	3
IL28B genotype				
CC	17	8	7	10
CT	13	3	3	10
TT	6	1	0	6
Liver histology HAI A-D				
0–5 score	21	6	4	17
>5 score	15	7	6	9