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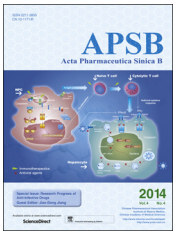

ORIGINAL ARTICLE

Replication priority of hepatitis C virus genotype 2a in a Chinese cohort

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Abstract HCV genotypes have been documented in clinical practice. The aim of this study was to determine the replication priority of different HCV genotypes in a Chinese HCV positive cohort. Serum samples from 491 apparently healthy Chinese blood donors testing positive for HCV antibodies and naive to antiviral drug therapy were tested. Genotyping analysis showed that genotypes 1b and 2a were predominant and accounted for 77.6% of the HCV infections. Among the genotype groups, individuals infected with genotype 2a had an HCV RNA viral load (10^8 copies/mL) about 200-fold (lg, 2.3) greater than those infected with other genotypes (10^4–10^5 copies/mL) indicating a replication priority of genotype 2a. However, there was no correlation between HCV genotype and antibody response suggesting that the amplification advantage of genotype 2a results from a favorable interaction with the host cellular environment. In conclusion, HCV genotypes 1b and 2a are the predominant genotypes in China and genotype 2a possesses a significant replication priority compared

Abbreviations: EDTA, ethylenediaminetetraacetic acid; GPT, glutamate-pyruvate transaminase; HCV, hepatitis C virus; NS3, NS4 and NS5, non-structure protein 3, 4 and 5; RdRp, RNA dependent RNA polymerase; SVR, sustained virological response

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with the other genotypes. This suggests the existence of host cellular factors that may act as drug-targets for entirely clearing HCV infection in the future.

1. Introduction

Hepatitis C virus (HCV) belongs to the family *Flaviviridae* and is a single-stranded RNA virus with genetic variability. The genetic diversity extends to seven major genotypes with 30–35% difference at the nucleotide level[1]. In relation to the response to antiviral therapy, HCV genotypes 2 and 3 show a greater therapeutic response to interferon/ribavirin regimen than the other genotypes especially in the initial stages of treatment[2,3]. Epidemiologically, genotype 1 is the most widespread globally and is the most prevalent of the seven; genotypes 2 and 3 are common in the far East; genotype 4 has been documented in the Middle East and North Africa, genotype 5a in South Africa and genotype 6 in Southeast Asia[4–6]. HCV genotypes are also associated with disease progression; for instance, Japan-specific HCV genotype 1b (J subtype) shows a low pathogenicity[7]. To further understand the biological significance of HCV genotypes, we investigated the prevalence, viral replication priority and characteristics of the antibody response for each of the genotypes in blood samples from an HCV positive Chinese cohort of blood donors not previously treated with any antiviral agent. The results provide an opportunity to examine the HCV replication capacity of the different genotypes in an infection course free of drug intervention.

2. Materials and methods

2.1. Serum samples

Serum samples from 491 seemingly healthy blood donors (297 male, 194 female; age range 18–60; average age 36.8) collected at the Langfang Blood Center (about 200 miles South of Beijing, China) were selected based on a positive test for HCV antibodies and no history of antiviral drug therapy (*i.e.* this was the first time HCV antibodies were detected). Of the 491 individuals, 166 showed an elevated liver transaminase indicating an abnormality in their liver function. Blood was collected into EDTA tubes followed by serum isolation by centrifugation at 1000 rpm for 10 min at room temperature. Serum samples were stored at −80 °C prior to testing. The study was approved by the Research Committees of the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, and the National Institutes for Food and Drug Control.

2.2. HCV testing

Serum HCV antibodies were detected using the Abbott HCV antibody detection kit (Axsym System HCV version 3.0). Quantitation of HCV RNA was done using the Cobas HCV RNA kit (Roche, New Jersey). HCV genotyping was carried out using a kit from Ningbo Ruixin Biotech Inc. (Ningbo, China) and a Boao-5800 microarray reader (Beijing, China). To explore the antibody response characteristics of each HCV genotype, the antibody reaction to the HCV epitope antigens Core, NS3, NS4 and NS5 was examined using an HCV antibody detection kit from Jin-Wei-Kai Biotech Inc. (Beijing, China). All tests were performed in triplicate according to the manufacturers' instructions. The antibody response was taken as positive when the average S/C value >1. The antibody positive rate to HCV epitopes of serum was calculated as positive number detected/total number detected × 100%.

2.3. Statistical methods

Differences in mean viral load among study groups were tested using the Student's t-test for equal or unequal variances depending on a preliminary F test for homogeneity of variance.

Table 1 Blood viral RNA load in individuals infected with different HCV genotypes.

Genotype	Group size[a] (female/male)	HCV RNA viral load (copy/mL)		P[c]
		Mean	SE[b]	
1a	33(13/20)	1.63×10^4	3.67×10^3	
1b	204(80/124)	6.17×10^5	3.70×10^5	0.006
2a	177(77/100)	1.12×10^8	4.89×10^7	
2b	33(11/22)	2.91×10^4	1.15×10^3	
3a	13(3/10)	1.67×10^4	6.66×10^3	
3b	9(3/6)	1.78×10^4	3.67×10^3	
6	12(2/10)	4.31×10^5	3.64×10^3	
Unidentified	10(5/5)	3.31×10^5	5.03×10^4	

[a]Number of subjects.
[b]Standard error.
[c]1b *vs* 2a, using unpaired Student's t-test.

3. Results

3.1. HCV prevalence

Of the 491 blood donors, infection with the different genotypes was as follows: 1a 33 (6.7%); 1b 204 (41.5%); 2a 177 (36.0%); 2b 33 (6.7%); 3a 13 (2.6%); 3b 9 (1.8%); 6 12 (2.4%); and unidentified 10 (2.0%). Thus the predominant HCV genotypes in this Chinese cohort were 1b and 2a accounting for a total of 77.6% of HCV infection. The results are consistent with previous reports[8,9].

3.2. HCV viral load

The viral load in serum samples was determined to investigate the correlation between HCV genotype and viral replication. As shown in Table 1, the highest viral load is seen in the genotype 2a group and is significantly greater than in the 1b group ($P<0.01$) by about 200-fold (lg, 2.3). The other genotypes had an average viral load below that of genotype 1b.

These results suggest that, although HCV genotypes 1b and 2a have almost identical infectivity in northern China, genotype 2a appears to have a greater ability to proliferate in the natural course of HCV infection. There could be at least two possible explanations for this: first, the antibody response could be different among the genotypes; and second, the advanced proliferation capacity of genotype 2a might result from a favorable interaction between 2a and host cellular factors.

3.3. HCV specific antibodies

We then aimed to explain why genotype 2a amplifies more than the other genotypes. The analysis was focused on the antibody response to the major HCV epitope antigens, Core, NS3, NS4 and NS5 and to determine the proportion of individuals in each genotype group who had positive antibody responses to each of the HCV epitope antigens. As shown in Fig. 1, the antibody positivity rates are 80%–97% for NS3, 84%–91% for Core, 10%–51% for NS4 and 10%–48% for NS5. The overall ranking of antibody positivity to the epitopes followed the order NS3 (95%) > Core (89%) > NS4 (47%) > NS5 (36%) and appears to apply to all genotypes. The results suggest that, among the HCV epitope antibodies, those against NS3 and Core are the early biomarkers of HCV infection. As individuals infected with different HCV genotypes exhibited a similar pattern of antibody response against the four HCV epitopes, HCV genotypes did not appear to correlate with the epitope-specific antibody responses. Thus, antibody response in HCV infection does not account for the replication priority of genotype 2a and suggests that intrinsic factors in genotype 2a infection play an important role after HCV enters the host cells.

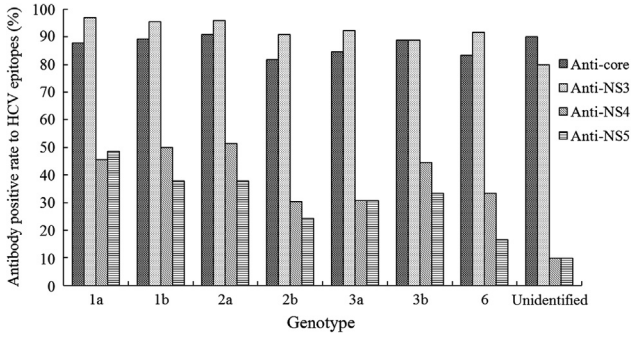

Figure 1 Antibody response to HCV epitopes in different genotype groups. The data show the antibody positivity rate to the four HCV epitopes in each genotype group.

3.4. HCV and liver damage

As hepatocyte apoptosis is involved in the pathogenesis of HCV infection[10,11], the high proliferation capacity of HCV genotype 2a may cause a rapid progression of liver damage. Indeed, we found that the GPT (glutamate-pyruvate transaminase) level in the group infected with genotype 2a was higher than in the genotype 1b group ($P<0.001$, 80 ± 16 *vs* 67 ± 8) indicating a faster progression of liver damage during the early stages of infection. However, viral load may not necessarily correlate with the blood level of liver transaminase and, indeed, the difference in GPT values may not be clinically significant.

4. Discussion

HCV is a positive single-stranded RNA virus belonging to the *Flaviviridae* family and the causative agent for hepatitis C around the world. Interferon in combination with ribavirin has become the standard pharmacotherapy for chronic hepatitis C over the past few decades[12]. However, three NS3-4A protease inhibitors (telaprevir, boceprevir and simeprevir) and one NS5B RNA dependent RNA polymerase (RdRp) inhibitor (sofosbuvir) have recently been approved by the US FDA for anti-HCV treatment[13,14] leading to the expectation that an all-oral, interferon-free combination of drugs will provide a cure for more than 90% of infections. Nevertheless, these new drugs have yet to make a significant impact on the prevalence of HCV infection[15] and it remains to be seen how they will deal with resistant HCV.

In patients with HCV infection, HCV genotype 2a is sensitive to standard-of-care treatment and sustained virological response (SVR) is higher in those infected with genotype 2a than in those infected with other genotypes[2,16]. The reason for this remains unclear. There are some reports that Interleukin-28B, or other IFN-stimulated genes (ISGs) may influence the SVR during treatment with interferons or interferon plus ribavirin[17,18] but this does not explain why they are not so efficient in treating those infected with other genotypes. Interestingly, the recently approved RdRp inhibitor sofosbuvir also showed higher response rates in those infected with genotype 2 than in those infected with other genotypes[19] suggesting viral factors are not alone in influencing therapeutic outcomes. Our results show that, although HCV genotypes 1b and 2a have equal prevalence in northern China, genotype 2a has a significant replication advantage which may partly explain its high sensitivity to combination therapy with alpha-interferon plus ribavirin or sofosbuvir plus ribavirin. Considering the small group size for those infected with genotypes 3a, 3b and 6 in the present study, a well-designed investigation with more subjects is needed to validate the results in these genotypes.

It is known that many factors can influence viral replication including immunity, age, hepatocellular damage and whether the infection is acute or chronic. The limited results presented here indicate that HCV genotype 2a has replication priority over other HCV genotypes during the natural course of HCV infection and

imply that, at least in Chinese subjects, the host hepatocyte environment is more supportive of genotype 2a than of genotype 1b or other HCV genotypes. In fact, in Huh-7 cell lines, the only permissive cell lines for HCV infection[20], genotype 2a did show a proliferation rate higher than that of other HCV genotypes[21,22] in agreement with our observations. The suggestion of more rapidly progressing liver damage in the genotype 2a group found here is also consistent with its higher replication level although the difference in GPT levels may not be clinically significant.

In conclusion, HCV genotypes 1b and 2a are the predominant HCV genotypes in our Chinese cohort of which genotype 2a has the replication priority in the natural course of HCV infection. This may result from a favorable interaction between genotype 2a and host cellular factors which, in turn, accelerates the progress of liver damage during the early stages of infection. These results may be useful in the search for new host cellular factors as drug-targets for entirely clearing HCV infection in the future.

Acknowledgments

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