Hepatitis G virus genomic RNA is pathogenic to Macaca mulatta

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Hepatitis G virus (HGV), once named GB virus C (GBV-C), is a positive-sense single-stranded RNA virus whose genetic structure resembles hepatitis C virus and is considered to be a member of Flaviviridae family[1-3]. Early researches support that HGV is associated with post-transfusion hepatitis and other acute or chronic liver-diseases[4-5], but subsequent works question the pathogenicity of HGV[6-8]. Actually, HGV infection is found widely in human population, with frequencies of active or past infection ranging from 5% to 15%-9-10. HGV infection is frequently persistent and associated with high-level of circulating viremia[11-12]. Until now, many efforts have been made on the expression and replication of HGV in vitro, but few researches have focused on the association of HGV with liver diseases[12-16]. Since HGV does not infect routine experimental animals and is difficult to replicate in vitro, the pathogenicity, pathogenesis, replication and expression of HGV in host are not clear[17-18]. Although experimental HGV infection with HGV RNA-positive plasma has been reported in chimpanzees and rhesus monkeys, the results are controversial[19-21].

The genome of positive strand RNA virus functions as mRNA, from which all viral proteins necessary for virus propagation are translated. Thus, genomic RNA as well as RNA transcripts from full-length cDNA clones, should be infectious. In fact, it has been proved in cell cultures and animal inoculation studies of HAV and HCV using full-length RNA transcripts[22-24]. In this laboratory, we have constructed a full-length HGV cDNA clone (pHGVqz), which is proved to be infectious in vitro[25-29]. pHGVqz is deposited in the GenBank with the accession number of AF081782 and contains 9373 bp in length[30,31]. In the present study, the full-length HGV genome was transcribed from this clone and intra-hepatically injected into the liver of Macaca mulatta to study its pathogenicity[32,33].

MATERIALS AND METHODS

Experimental animals

Three Macaca mulattas (BY1, M, 2 years, 2 kg; BM1, F, 10 m, 1.0 kg; BB1, F, 1 years, 1.5 kg) were used in this study. These
animals were purchased from Chinese Academy of Sciences (Shanghai) and maintained under conditions that met all requirements for use in an approved facility. The animals were not inoculated with any material from animal or human, prior to this study. Serum samples and liver biopsies were taken as negative controls before inoculation. The Macaca mulattas were observed for several months before use and proved to be healthy with normal ALT, negative for HGV RNA, anti-HAV, anti-HBV, anti-HCV and anti-HGV.

In vitro transcription
phGVqz was linearized with Xba I, and transcribed with T7 RNA polymerase, according to the manufacturer’s instructions (Riboprobe transcription system, Promega, Madison, WI). The products were digested with 1 u/μg RQ1 DNase at 37 °C for 15 min, extracted with phenol/chloroform and precipitated with ethanol. The HGV RNA transcripts were stored at -70 °C for use.

Methods of infection
Laparotomy was performed and HGV RNA transcripts (90 μg) were injected into 6 sites of the exposed liver of BY1. One milliliter of HGV RNA-positive serum taken from BY1 at the ninth month post-inoculation was intravenously injected into BM1, and then BB1 was intravenously injected with 1 mL of BM1 serum at the seventh month post-inoculation. Serum samples were collected weekly for the first part of the study after injection, and less frequently thereafter, and monitored for HGV RNA, liver enzymes [alanine aminotransferase (ALT)] and anti-HGV antibodies (HGV IgG ELISA, Sinoclone Ltd, Hongkong). The cut-off value of ALT was 40 IU/L. Liver tissues were taken regularly for the examination of inflammatory changes.

Detection of HGV RNA
Total RNA was extracted from serum or liver tissues using TRIzol LS or TRIzol reagent. RNA pellet was resuspended in 25 μL of RNase-free water. HGV RNA was amplified by RT-PCR with the primer from the 5’-NTR of HGV and with an external primer pair of 5’-ACCGACG-CCTATCTAAGTGA-3’ and 5’-CTTGGAGTCCCTTCCAGGCC-3’, and an internal primer pair of 5’-GACAGGGTTTGGTGCTGAATCC-3’ and 5’-AGAGAGAAGTTGAGGGCCGC-3’. Reverse transcription was performed at 42 °C for 1 h in 20 μL reaction volume using avian myeloblastosis virus reverse transcriptase (Promega) and external antisense primer (for the detection of genomic RNA) or sense primer (for the detection of minus strand RNA). cDNA was amplified with internal primer pair for 35 cycles with denaturation at 94 °C for 30 s, primer annealing at 56 °C for 30 s, and DNA amplification at 72 °C for 45 s. The amplified product was analyzed by electrophoresis through 15 g/L agarose gel containing ethidium bromide followed by UV transillumination. Each set of experiments included a positive control and a negative control.

HGV specific probe tagged with fluorescence (nt 136-1112, 5’-FAM-CAGGTTTGAGTCTGT-TAAATCCGG-TAMRA-3’) was synthesized by Shenyou Biotechnology Company, Shanghai. TaqMan™ PCR detection kit (Perkin–Elmer Applied Biosystems) was used to quantitate HGV genomic RNA and minus RNA in liver tissues of the infected Macaca mulattas.

Histological inspection and immunohistochemical staining
Autopsy tissues from Macaca mulatta were fixed with 100 mL/L formaldehyde and embedded in paraffin routinely. Each paraffin-embedded specimen was sliced into 5-μm thick sections, which were stained with hematoxylin and eosin (HE). The inflammatory changes of liver tissue sections were observed under a light microscope.

Paraffin-embedded liver sections (5 μm) were dewaxed in xylol and re-hydrated through a series of ethanol dilutions and then disposed with H2O2 to inactivate endogenous peroxidase, digested for 30 min with 1 g/L trypsin (GIBCO, Gaithersburg, MD). Monoclonal anti-HGV E2 (kindly provided by Dr. Engel, Roche Diagnostics, Germany), anti-mouse IgG conjugated with hors eradish peroxidase (HRP) and the substrate H2O2-diaminobenzidine (DAB)/Ace were added to the sections step by step. The normal liver tissue sections were used as negative controls.

In situ hybridization
Full-length HGV cDNA containing plasmid pHGVqz was digested with Pst I, and the 3 642 bp fragment containing a 470 bp of HGV 5’-NCR and vector sequence was recovered and self-ligated. In this new constructed plasmid, HGV cDNA fragment was flanked with the T7 (5’ to the cDNA insert) and SP6 (3’ to the insert) RNA promoters. The recombinant plasmid was digested with Xba I or EcoRI and the linearized plasmid was transcribed in vitro with T7 (Xba I linearized plasmid) or SP6 (EcoRI linearized plasmid) RNA polymerases in the presence of digoxigenin 11-UTP (DIG DNA labeling and detection kit, Roche Diagnostics, Germany) to generate probes of sense and anti-sense polarity, respectively.

The 10-μm thick frozen liver sections fixed with 40 mL/L paraformaldehyde/0.1 mol/L PBS at 4 °C for 1 h, were rinsed with 0.1 mol/L glycine/PBS and 4 mL/L Triton X-100/PBS. After digestion with protease K (1 mg/mL) at 37 °C for 10 min, the sections were acetylated in 5 mL/L acetic anhydride in 0.1 mol/L triethanolamine (pH 8.0) for 20 min at room temperature. The slices were rinsed in 2×SSC (standard saline citrate) and quickly dehydrated in ethanol. After that, 20 μL of hybridization mixture consisting of 50 mM L formamide, 100 mM L dextran sulfate, 1×Denhardt solution, 10 mmol/L Tris–HCl pH 8.0, 0.3 mmol/L NaCl, 1 mmol/L EDTA pH 8.0, 10 mmol/L DNA of salmon sperm, and 5 ng of heat-denatured labeled probe were placed on each slice, sealed with rubber solution. The sections and probe were denatured together at 80 °C for 10 min. The slices were incubated at 56 °C for 16 h. After hybridization, slices were washed for 1 h at 56 °C with 3×SSC, digested with RNase A (20 mg/mL), and rinsed in 1.5×SSC and 0.75×SSC at 50 °C for 1 h each. The digoxigenin-labeled hybrids were detected with a digoxigenin antibody–alkaline phosphatase conjugate and an enzyme substrate chromogen (nitroblue tetrazolium-5-bromo-4-chloro-3-indolylphosphate) according to the manufacturer’s instructions (Dig nucleic
Acid detection kit; Roche Diagnostics, Germany). The results were documented under a light microscope. The negative controls included sections without probes and sections with normal liver tissues.

RESULTS

HGV genomic RNA

As seen in Figure 1, the full-length HGV cDNA clone could be transcribed in vitro. The majority of HGV genome RNA was about 9,400 nt in length. Compared to the T7 linear control, the integrity of HGV genomic RNA was good and the yield was about 0.8 μg/μL. HGV RNA transcripts were examined by RT-PCR with or without RTase and the expected fragment was about 233 bp. The absence of residual plasmid DNA was confirmed by the negative results in the PCR analysis when the RT step was omitted (data not shown).

Elevated ALT and positive Anti-HGV

In Macaca mulatta BY1, the ALT level elevated intermittently from wk 4 post-inoculation and kept high for quite a long time lasting 37 wk after inoculation; the peak was 418 IU/L at wk 83 post-inoculation. While in BM1 and BB1, ALT levels elevated 29 and 3 wk after inoculation, respectively (Figure 2). Serum anti-HGV was calculated based on the cut-off values (0.222). Anti-HGV was detectable from wk 39-50 and 64-66 post-inoculation in BY1, at wk 32 and 40-48 post-inoculation in BM1, from wk 6 to 12 post-inoculation in BB1 (Figure 2).

HGV RNA

The expected PCR product was about 233 bp. Positive control and negative samples were set up in each reaction. In Macaca mulatta BY1, serum HGV RNA turned positive from wk 8, kept up for 13 wk and kept intermittently positive thereafter. BY1 was observed for 90 wk then, HGV RNA could also be found in the serum. In BM1, serum HGV RNA became positive from wk 3 to 24, and kept intermittently positive thereafter. While in BB1, serum HGV RNA was only detectable at wk 3 and 4 (Figure 2).

Quantitative PCR showed that the copies of genomic RNA and minus RNA in the liver were 6.24×10⁴ and 9.76×10⁵/g in BY1, 2.24×10⁴ and 1.56×10⁶/g in BM1, 1.76×10⁴ and 2.88×10⁵/g in BB1, respectively.

Histological changes

The histological appearances were normal in all three Macaca mulattas before infection. All of them developed mild inflammatory changes in the liver after the infection (Table 1). Lymphocyte infiltration, focal necrosis, hydropic degeneration and mild proliferation of Kupffer’s cells were predominant in the early time and the normal structure of liver lobule was destroyed. When the time of infection was prolonged, the infiltration of lymphocytes and proliferation of fibroblasts became apparent in the portal areas.

Table 1 Pathological finding in hepatocytes of infected Macaca mulattas

| Macaca mulatta | Weeks post-inoculation | Histology               | Immuno-histochemistry | In situ hybridization |
|----------------|------------------------|-------------------------|-----------------------|----------------------|
| BY1            | 0                      | Normal                  | -                     | -                    |
|                | 18                     | Chronic mild hepatitis  | +                     | +                    |
|                | 48                     | Chronic mild hepatitis  | +                     | +                    |
|                | 68                     | Chronic mild hepatitis  | +                     | +                    |
| BM1            | 0                      | Normal                  | -                     | -                    |
|                | 18                     | Mild hepatitis          | +                     | +                    |
|                | 32                     | Chronic mild hepatitis  | +                     | +                    |
| BB1            | 0                      | Normal                  | -                     | ND                   |
|                | 18                     | Mild hepatitis          | +                     | ND                   |

+, positive; -, negative; ND, not done.

Immunohistochemical inspection and in situ hybridization

The immunohistochemistry and in situ hybridization in liver tissues were negative before inoculation. HGV E2 protein and HGV mRNA were detectable in hepatocytes of BY1, BM1 and BB1 (Table 1). HGV E2 antigen-positive hepatocytes...
were distributed sparsely in liver lobules, mainly in the cytoplasm of hepatocytes. The negative controls in the experiments were normal (Figure 3). The results of immunohistochemistry and in situ hybridization were basically coincident.

**DISCUSSION**

HGV is a newly identified causative agent of post-transfusion non-A-E hepatitis[1-3]. Although whether HGV could lead to human hepatitis is still controversial, HGV RNA does exist in the sera of both blood donors and various hepatitis patients[6-8]. Besides the epidemiological and clinical studies, some primate animal models have been used to study the pathogenicity of HGV[20-23]. When 2 chimpanzees are inoculated with patient’s serum containing $10^8$ copies of HGV RNA, viremia occurs at wk 10 and 11 after inoculation but neither of the chimpanzees developed hepatitis[20]. However, elevated ALT, HGV RNA and anti-HGV are detectable[21-23], since the materials used are from patients, which could not exclude the possibility that the undiscovered infectious agents may interfere with the results of experiments.

HGV is RNA virus, the genomic RNA serves as template both for viral replication and for protein translation. In order to eliminate other infectious factors from HGV-positive human plasma, we decided to study the pathology and replication of HGV using full-length genomic HGV RNA transcribed from HGV cDNA clone. We constructed a full-length genomic HGV cDNA clone (pHGVqz) in our laboratory, which provided us a good starting material for this study. HGVqz represents the full-length genome of HGV, 9373 bp in length, and consists of a 5'-noncoding region, an open reading frame, and a 3'-noncoding region. The HGV genomic cDNA was cloned into EcoR I and Xba I sites of pGEM-3zf (+) vector, and immediately downstream of the T7 promoter, which ensured that the T7 promoter started transcription from the exact 5'-end, stopped at the exact 3'-end of HGV, and produced the authentic HGV RNA transcripts[27,28,30,31]. Before in vitro transcription, the plasmid was linearized with Xba I. The successful construction of full-length genomic cDNA clone allowed us to avoid the defect of infection with positive plasma. Furthermore, chimpanzee or Macaca mulatta infected with RNA transcripts derived from a single cDNA clone can provide more detailed information on pathogenicity, pathogenesis and evolution of the virus.

In this study, Macaca mulatta BY1 was intra-hepatically injected with HGV RNA transcripts from pHGVqz, Macaca mulatta BM1 was intravenously inoculated with HGV RNA-positive serum collected from BY1 and Macaca mulatta BB1 was infected with serum from BM1. Our data showed that serum HGV RNA of the 3 experimental animals turned positive between the 3rd and 8th wk post-inoculation and existed for quite a long time, suggesting that HGV can not only replicate in Macaca mulatta, but also transmit to normal Macaca mulattas. Quantitive PCR results of both liver and serum (data not shown) showed that HGV RNA decreased with the infection passage, possibly because the virulence reduced during the passage of infection. Intermittently elevated serum ALT level was detectable in all 3 Macaca mulattas without direct association with HGV RNA. Anti-HGV, detectable in the inoculated Macaca mulatta, suggests HGV protein is expressed and immune responses are induced in the Macaca mulattas. Compared to normal liver before inoculation, the 3 Macaca mulattas developed mild hepatitis. HGV E2 protein and mRNA were also detectable using immunohistochemistry and in situ hybridization. Serological and histological changes in 3 Macaca mulattas proved that HGV might exist in Macaca mulattas and have potential infectivity.

Although the replication mechanism of HGV is unknown, it is presumed that HGV replicates in the same manner as other positive-stranded RNA flaviviruses. We examined HGV minus strand RNA in the liver to see whether the liver was the replicate site of HGV. In our study, HGV minus RNA was found in the liver of all 3
Macaca mulattas, which was 10-fold more than genomic RNA. Until now, the tissue tropism of HGV is unclear. Some studies reported that HGV minus RNA is found in borne marrow cells and peripheral blood mononuclear cells (PBMC), but not in liver[26-28]. Contradictory results have been reported that HGV minus RNA is detectable in liver and PMBC samples from chronically HGV-infected patients[29-30]. These discrepancies could be partially explained if there are HGV variants with different tissue tropism. Fogeda et al[7] reported that both hepatotropic and lymphotropic HGV variants exist in infected hosts. Replication of different tropic variants determines the distribution of serum HGV, and only a fraction of HGV variants present in serum is able to infect and replicate in PBMC. in vitro. In our study, the expression and replication of HGV were detectable in the livers of experimental animals, suggesting that the HGV RNA genome transcribed in vitro has the liver tropism, indicating that HGV RNA genome may be pathogenic to Macaca mulattas and can lead to viremia and inflammatory changes of liver.

In conclusion, HGV genomic RNA is infectious. HGV RNA exists and replicates in Macaca mulattas, and is capable of causing hepatitis in infected Macaca mulattas. Macaca mulatta is susceptible to HGV infection and may be used as an animal model for studying HGV replication and selecting anti-viral drugs.

REFERENCES

1 Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK. Sequence and genomic organization of GBV-C, a novel member of the flaviviridae associated with human non-A-E hepatitis. J Med Virol 1996; 48: 60-67
2 Linnen J, Wages J, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayannis P, Fung K, Nakatsuji Y, Shih JW, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Imsay M, Lifson JD, Hess G, Foung SK, Thomas H, Bradley D, Margolis H, Kim JP. Molecular cloning and disease association of hepatitis G virus; a transfusion-transmissible agent. Science 1996; 271: 505-508
3 Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK. Isolation of novel virus-like sequences associated with human hepatitis. Nat Med 1995; 1: 564-569
4 Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JW, Kim JP. The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. N Engl J Med 1997; 336: 747-754
5 Alter MJ, Gallagher M, Morris TT, Moyer LA, Meeks EL, Krawczynski K, Kim JP, Margolis HS. Acute non-A-E hepatitis in the United States and the role of hepatitis G virus infection. Sentinel Counties Viral Hepatitis Study Team. N Engl J Med 1997; 336: 741-746
6 Sieffried C, Weber M, Bialleck H, Sieffried E, Schrezenmeier H, Roth WK. High prevalence of GBV-C/HGV among relatives of GBV-C/HGV-positive blood donors in blood recipients and in patients with aplastic anemia. Transfusion 2004; 44: 268-274
7 Abraham P, John GT, Raghuraman S, Radhakrishnan S, Thomas PP, Jacob CK, Sridharan G. GB virus C/hepatitis G virus and TT virus infections among high risk renal transplant recipients in India. J Clin Virol 2003; 28: 59-69
8 Sentjens R, Basaraz M, Simmonds P, Vrielink H, Reesink H. HCV/GB virus C transmission by blood components in patients undergoing open-heart surgery. Transfusion 2003; 43: 1479-1486
9 Hattori J, Ibe S, Nagai H, Wada K, Morishita T, Sato K, Utsumi M, Kaneda T. Prevalence of infection and genotypes of GBV-C/HGV among homosexual men. Microbiol Immunol 2003; 47: 759-763
10 Kleines M, Schellenberg K, Ritter K. Efficient extraction of viral DNA and viral RNA by the Chemagic viral DNA/RNA kit allows sensitive detection of cytomegalovirus, hepatitis B virus, and hepatitis C virus by PCR. J Clin Microbiol 2003; 41: 5273-5276
11 Lo SY, Ku CW, Ma HC, Li YH, Yu JH, Lin HH, Lai AC, Lee ML. Detection of serologic responses to GB virus C/hepatitis G virus infection. Int J Infect Dis 2002; 6: 223-227
12 Ren H, Zhu FL, Qi ZT. Studies of HepC2 cells infected with HGV RNA genome. Weishengxue Tongbao 2002; 29: 60-64
13 Zhu FL, Ren H, Zhu SY, Song YB, Qi ZT. High-level expression of GBV-C/HGV NS3 protein in SF9 insect cells using Bac-to-Bac vectors. Bingdu Xuebao 2000; 16: 273-275
14 Zhu FL, Ren H, Zhu SY, Xie N, Pan W, Dong H, Qi ZT. Expression of HGV NS3 protein in Baculovirus vector and its antigenicity. Dier Junyi Daxue Xuebao 2000; 21: 849-852
15 Nie QH, Hu DR, Li MD, Xie Q. Expression of virus-related antigens in the livers of patients co-infected with HGV and HCV. Shi jie Huanen Xiaohua Zazhi 2000; 8: 114-115
16 Zhu FL, Ren H, Song YB, Qi ZT. Expression of GBV-C/HGV NS3 protein in Escherichia coli. Zhonghua Weishengxue He Miaoxue Zazhi 1999; 19: 475-478
17 Orii K, Tanaka E, Rokubara A, Maruyama A, Ichijo T, Yoshizawa K, Kiyosawa K. Persistent infection mechanism of GB virus C/hepatitis G virus differs from that of hepatitis C virus. Intervirology 2000; 43: 139-145
18 De Filippi F, Fraquelli M, Conte D, Soffiredini R, Prati D, Ronchi G, Zanella A, Del Ninno E, Colombo M. High prevalence but low pathogenicity of hepatitis G virus infection in Italian patients with genetic haemochromatosis. Ital J Gastroenterol Hepatol 1998; 30: 529-533
19 Matzkiez IK, Behner U, Weizenegger M, Bartel J, Cullen P, Schaefer RM. Prevalence of hepatitis G in patients on chronic hemodialysis. Clin Lab 2000; 46: 247-250
20 Buhk J, Kim JP, Govindarajan S, Appar CL, Foung SK, Wages J Jr, Unj Aj, Shapiro M, Emerson SU, Purcell RH. Experimental infection of chimpanzees with hepatitis G virus and genetic analysis of the virus. J Med Virol 1998; 77: 855-862
21 Wang XT, Zhuang H, Li HM, Li K, Qi ZB, Zhu WF, Zhu YH, Sheng HY, Lin JX. Macaca mulatta as an animal model for GB virus C/hepatitis G virus. Zhongguo Weishengxue He Miaoxue Zazhi 1997; 17: 363-365
22 Mao P, He H, Hong S. Study on the experimental infection of hepatitis G virus in rhesus monkey. Zhonghua Shiyan He Linchuang Bingdu Xuebao 1998; 12: 258-260
23 Cheng Y, Zhang W, Li J, Li Ba, Zhao Jm, Gao R, Xin S, Mao P, Cao Y. Serological and histological findings in infection and transmission of GBV-C/HGV to macaques. J Med Virol 2000; 60: 28-33
24 Cohen JJ, Ticehurst JR, Feinstone SM, Rosenblum B, Purcell RH. Hepatitis A virus cDNA and its RNA transcripts are infectious in cell culture. J Virol 1987; 61: 3035-3039
25 Dash S, Halim AB, Tsuji H, Hiramatsu N, Gerber MA. Transmission of HepC2 cells with infectious hepatitis C virus genome. Am J Pathol 1997; 151: 363-373
26 Kolykhlov AA, Agapov BV, Blight KJ, Mihalik K, Feinstein SM, Rice CM. Transmission of hepatitis C by intravesical inoculation with transcribed RNA. Science 1997; 277: 570-574
27 Zhu FL, Qi ZT, Ren H, Song YB, Shao L. Splicing and cloning of the full-length genomic cDNA of GB virus C/hepatitis G virus. Dier Junyi Daxue Xuebao 1998; 19: 301-306
28 Shao L, Shinzawa K, Ishikawa K, Zhang X, Ishibashi M, Misawa H, Yamada N, Tokashi H, Takahashi T. Sequence of hepatitis G virus genome isolated from a Japanese patient with non-A-E hepatitis; amplification and cloning by long reverse transcription-PCR. Biochem Biophys Res Commun 1996; 228: 785-791
Hu WJ, Wang L, Wang W, Qi ZT. Expression of full length hepatitis G virus (HGV) genome in vitro. Weishengwuxue Zazhi 2002; 22: 4-6

Qi ZT, Ren H, Zhu FL, Shao L, Pan W, Hu WJ, He JW, Miao XH, Du P. Construction and identification of a single stranded cDNA clone containing full-length genome of hepatitis G virus. Ross Gastroenterol Zh 2001; 2: 46-56

Qi ZT, Zhu FL, He JW, Shao L. Hepatitis G virus: A full-length cDNA clone. J Med Coll PLA 1998; 13: 101

Zhu FL, Qi ZT, Zhu SY, Ren H, Shao L. A preliminary study on Macaca mulatta infected by intrahepatic injection of GBV-C/HGV RNA transcripts produced in vitro. Bingdu Xuebao 2000; 16: 176-178

Ren H, Zhu FL, Zhu SY, Wang L, Qi ZT. Serological and histological changes of rhesus monkey infected with HGV RNA transcripts. Shijie Huaren Xiaohua Zazhi 2002; 10: 7-11

Tucker TJ, Smuts HE, Eedles C, Knobel GD, Eickhaus P, Robson SC, Kirsch RE. Evidence that the GBV-C/hepatitis G virus is primarily a lymphotropic virus. J Med Virol 2000; 61: 52-58

Laras A, Zacharakis G, Hadziyannis SJ. Absence of the negative strand of GBV-C/HGV RNA from the liver. J Hepatol 1999; 30: 383-388

Fan X, Xu Y, Solomon H, Ramrakhiani S, Neuschwander-Tetri BA, Di Bisceglie AM. Is hepatitis G/GB virus-C virus hepatotropic? Detection of hepatitis G/GB virus-C viral RNA in liver and serum. J Med Virol 1999; 58: 160-164

Fogeda M, Lopez-Alcorocho JM, Bartolome J, Arocena C, Martin MA, Carrero V. Existence of distinct GB virus C/hepatitis G virus variants with different tropism. J Virol 2000; 74: 7936-7942