

GB Virus C/Hepatitis G Virus Does Not Induce Expression of p44 Antigen in Chimpanzee Hepatocytes

Yohko K. Shimizu*, Minako Hijikata and Hiroshi Yoshikura¹

*Department of Respiratory Diseases, Research Institute, International Medical Center of Japan,
Toyama 1-21-1 Shinjuku-ku, Tokyo 162-8655 and
¹National Institute of Infectious Diseases,
Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640*

Communicated by Hiroshi Yoshikura

(Accepted June 1, 2001)

The cytoplasmic antigen, p44, was originally discovered in hepatocytes of chimpanzees experimentally infected with the parenterally transmitted form of non-A, non-B hepatitis virus (NANBHV) (1). Expression of the antigen, found in parallel with unique ultrastructural alterations (2), appeared to be a host-response to infection with NANBHV or hepatitis delta virus, but not to infection with hepatitis A virus, hepatitis B virus, or enterically transmitted NANBHV. Until hepatitis C virus (HCV) was molecularly cloned in 1989, showing that most parenterally transmitted NANBHV infections, p44 was useful as a reliable marker for the NANBHV (now HCV) infection. The gene encoding p44 was subsequently isolated and shown to be a member of the family of interferon- α/β inducible genes (3). It is now speculated that p44 is possibly one of the mediators involved in the antiviral action of interferon.

Recently, GB virus C (also called as hepatitis G virus) (GBV-C/HGV), which is closely related to but distinct from HCV, was discovered. Although they share little sequence homology, HCV and GBV-C/HGV have a quite similar genetic organization, with the exception that the latter almost lacks sequences encoding a core protein. We were interested to know if GBV-C/HGV induces p44, as both viruses presumably replicate via double-stranded RNA replicative intermediate, an interferon inducer.

When we retrospectively examined samples from the NANBHV transmission studies conducted in the 1980s, we

found chimpanzees whose sera were positive for GBV-C/HGV RNA by RT-PCR. Utilizing the liver biopsy specimens collected from these chimpanzees, we investigated appearance of the p44 antigen and the related tubular structures in hepatocytes.

The inoculum used for the transmission study was obtained from an implicated donor. A 61-year-old woman received thrombocytes from her son, a healthy individual negative for hepatitis B surface antigen (HBsAg) and antibody to hepatitis B surface antigen (anti-HBs), in the course of a splenectomy in 1981. She developed acute NANB hepatitis; her level of serum transaminase (ALT) rose 10 days after transfusion and peaked at 15 days. Serum was again collected from the donor 3 months after donation, and 1 ml of 10^{-2} , 10^{-4} , and 10^{-6} dilution was inoculated into chimpanzee #1310, #429, and #125, respectively. All of the chimpanzees developed hepatitis as evidenced by the elevated level of serum ALT; the peak was at week 2 for #1310, at week 12 for #429, and at week 5 for #125.

Serum samples and liver biopsy specimens collected at intervals from chimpanzees #429 and #125 were available for the present assays. For both chimpanzees, all of the serum samples were negative for HCV RNA as measured by RT-PCR, as well as anti-C100 HCV antibody as measured by ELISA, throughout the observation period of 35 weeks. GBV-C/HGV RNA, as detected by RT-PCR with primers detecting the 5' non-coding region of the viral genome, became transiently positive at 1-2 weeks and then continuously positive at 10-35 weeks in chimpanzee #429 (Fig. 1A). In chimpanzee #125, GBV-C/HGV RNA was detected at 3-8 weeks, then reappeared

*Corresponding author: E-mail: yshimizu@ri.imcj.go.jp

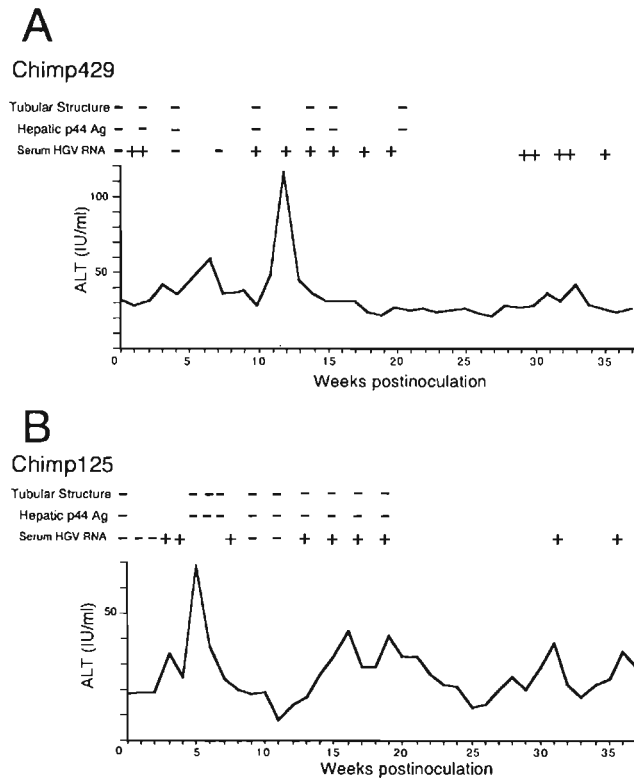


Fig. 1. Clinical course of GBV-C/HGV-infected chimpanzees.

at 13 weeks and remained positive thereafter (Fig. 1B). Liver biopsies collected at pre- and post-inoculation until week 20 were examined for the appearance of the p44 antigen by immunofluorescence using a mouse monoclonal anti-p44 antibody (4) and for the cytoplasmic tubular structures in the hepatocytes by electron microscopy. Neither was detected (Figs. 1A and 1B).

It remains unclear whether the liver is the main target for GBV-C/HGV replication. Fogeda et al. (5) analyzed viral sequences recovered from sera, livers, and peripheral blood mononuclear cells from chronically infected patients and demonstrated the existence of different GBV-C/HGV variants with different tropism. We obtained similar data by comparing the 5' non-coding sequences in the serum and in the liver of chimpanzee #429 obtained on the same day (at

week 12). These observations, together with the ALT elevation in infected chimpanzees, indicated actual viral replication in the liver.

Despite its similarity to HCV, GBV-C/HGV was unable to induce neither p44 nor tubular structures which are constantly induced by HCV. Because the gene encoding p44 is interferon-inducible, the production of p44 after HCV infection has been explained by the production of interferon which is induced by double-stranded RNA replicative intermediates. However, GBV-C/HGV's failure to induce p44 apparently does not fit this hypothesis, since GBV-C/HGV in the same family also is expected to replicate via a double-stranded RNA intermediate. These considerations lead us to speculate that the double-stranded RNA intermediates may not be the major inducer of p44 even in HCV. The question then remains as the identity of the inducer? The major difference in the genetic constitution of the two viruses is the near deletion of a core protein in GBV-C/HGV. The possibility of the role of a core protein in p44 induction remains to be tested.

REFERENCES

1. Shimizu, Y. K., Oomura, M., Abe, K., Uno, M., Yamada, E., Ono, Y. and Shikata, T. (1985): Production of antibody associated with non-A, non-B hepatitis in a chimpanzee lymphoblastoid cell line established by in vitro transformation with Epstein-Barr virus. *Proc. Natl. Acad. Sci. USA*, 82, 2138-2142.
2. Shimizu, Y. K., Feinstone, S. M., Purcell, R. H., Alter, H. J. and London, W.T. (1979). Non-A, non-B hepatitis: ultrastructural evidence for two agents in experimentally infected chimpanzees. *Science*, 205, 197-200.
3. Kitamura, A., Takahashi, K., Okajima, A. and Kitamura, N. (1994): Induction of the human gene for p44, a hepatitis C associated microtubular aggregate protein, by interferon- α/β . *Eur. J. Biochem.*, 224, 877-883.
4. Maeda, T., Honda, Y., Hanawa, M., Yamada, E., Ono, Y., Shikata, T. and Shimizu, Y. K. (1989): Production of antibodies directed against microtubular aggregates in hepatocytes of chimpanzees with non-A, non-B hepatitis. *J. Gen. Virol.*, 70, 1401-1407.
5. Fogeda, M., Lopez-Alcorocho, J. M., Bartolome, J., Arocena, C., Angeles, M. and Carreno, V. (2000): Existence of distinct GB virus-C/Hepatitis G virus variants with different tropism. *J. Virol.*, 74, 7936-7942.