Long-term persistent GBV-B infection and development of a chronic and progressive hepatitis C-like disease in marmosets

Yuki Iwasaki1,2†, Ken-ichi Mori3†, Koji Ishii4, Noboru Maki3, Sayuki Iijima1, Tomoyuki Yoshida5, Sachi Okabayashi5, Yuko Kataki5, Young-Jung Lee1, Akatsuki Saito5, Hiromi Fukai3, Nobuyuki Kimura1, Naohide Ageyama1, Sayaka Yoshizaki1, Tetsuro Suzuki4, Yasuhiro Yasutomi1, Tatsuoj Miyamura4, Mari Kannagi2 and Hirofumi Akari1,5*

1 Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Tsukuba, Japan
2 Department of Immunotheerapeutics, Graduate School of Medicine and Dentistry, Tokyo Medical and Dental University, Tokyo, Japan
3 Advanced Life Science Institute, Wako, Japan
4 Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan
5 Primate Research Institute, Kyoto University, Inuyama, Japan
6 Corporation for Production and Research of Laboratory Primates, Tsukuba, Japan

†Yuki Iwasaki and Ken-ichi Mori have contributed equally to this work.

It has been shown that infection of GB virus B (GBV-B), which is closely related to hepatitis C virus, develops acute self-resolving hepatitis in tamarins. In this study we sought to examine longitudinally the dynamics of viral and immunological status following GBV-B infection of marmosets and tamarins. Surprisingly, two of four marmosets but not tamarins experimentally challenged with GBV-B developed long-term chronic infection with fluctuated viremia, recurrent increase of alanine aminotransferase and plateaued titers of the antiviral antibodies, which was comparable to chronic hepatitis C in humans. Moreover, one of the chronically infected marmosets developed an acute exacerbation of chronic hepatitis as revealed by biochemical, histological, and immunopathological analyses. These results demonstrated for the first time that GBV-B could induce chronic hepatitis C-like disease in marmosets and that the outcome of the viral infection and disease progression may depend on the differences between species and individuals.

Keywords: GBV-B, HCV, marmoset, tamarin, hepatitis C

INTRODUCTION

Among the known viruses, GB virus B (GBV-B) is closely related to hepatitis C virus (HCV), with 25–30% homology at the amino acid level, and is tentatively classified in the genus of Flavivirus family (Muerhoff et al., 1995; Simons et al., 1995; Olha et al., 1996). Due to limited epidemiological analyses, the natural host(s) and prevalence of GBV-B have remained to be determined.

Hepatitis C virus is a major causative agent for non-A, non-B hepatitis. HCV is globally disseminated and estimated to be carried by more than 170 million people (Chisari, 2005; Lavanchy, 2009). Most HCV-infected individuals develop chronic liver diseases such as liver cirrhosis and hepatocellular carcinoma (Hoofnagle, 1997; Seeff and Hoofnagle, 2002; Rehermann and Nascimbeni, 2005). Since standard therapy with PEGylated interferon and ribavirin is effective for only about 50% of patients, it is crucial to develop more effective therapeutics (Feld and Hoofnagle, 2005; Melnikova, 2008). The only validated animal model for HCV infection is the chimpanzees. This model has been valuable for determining important aspects of this disease, including the relationship between the virus and the antiviral immune responses of the host and the process of viral pathogenesis (Bukh, 2004; Akari et al., 2009; Boonstra et al., 2009). However, chimpanzees are endangered and present ethical complications and the availability of these experimental animals is severely restricted.

When tamarins (members of the New World monkeys) are infected with GBV-B, they generally develop acute viremia and self-resolving hepatitis as indicated by increases in the levels of serum enzymes such as alanine aminotransferase (ALT) (Bukh et al., 1999; Beames et al., 2000; Beames et al., 2001; Sbardellati et al., 2001; Lanford et al., 2003; Martin et al., 2003; Bright et al., 2004; Jacob et al., 2004; Nam et al., 2004; Kyuregyan et al., 2005; Ishii et al., 2007; Weatherford et al., 2009). Thus, the monkeys have been proposed as a surrogate model of HCV infection of chimpanzees and humans. However, a major hurdle for the development of a monkey-based surrogate model is the difficulties encountered in obtaining chronically infected monkeys that exhibit progression of chronic hepatitis C-like diseases (Martin et al., 2003; Nam et al., 2004; Takikawa et al., 2010).

Abbreviations: ALT, alanine aminotransferase; GBV-B, GB virus B; HCV, hepatitis C virus; HE, hematoxylin and eosin; p.i., post infection.
It has recently been shown that marmosets, another member of New World monkeys, are susceptible to GBV-B infection and develop relatively lower levels of acute viremia (10^5–10^6 copies/ml) as compared with that in tamarins (10^8–10^9 copies/ml) (Lanford et al., 2003; Bright et al., 2004; Woollard et al., 2008; Weatherford et al., 2009), although it remains elusive whether the marmosets could permit persistent GBV-B infection. Considering that the viral loads in the acute phase of experimental HCV infection of chimpanzees that consequently develop persistent infection are generally 10^7 copies/ml or less (Fernandez et al., 2008; Weatherford et al., 2009), it is possible that the lower viral loads in the acute phase is preferable for the establishment of viral persistence. We thus initiated studies of the dynamics of viral and immunological status following GBV-B infection of tamarins and marmosets in a longitudinal follow-up study. We show here for the first time that GBV-B infection produces a chronic and progressive hepatitis C-like disease in marmosets as demonstrated by fibrosis and a recurrent ALT increase and that one of the marmosets experienced acute exacerbation of chronic hepatitis as indicated by piecemeal necrosis and an ALT flare >4 years after infection.

MATERIALS AND METHODS

ANIMALS

Adult red-handed tamarins (Saguinus midas) and common marmosets (Callithrix jacchus) were housed in individual cages at the Tsukuba Primate Research Center. All animal studies were conducted in accordance with the protocols of experimental procedures that were approved by the Animal Welfare and Animal Care Committees of the National Institute of Biomedical Innovation and the National Institute of Infectious Diseases.

GBV-B INFECTION IN TAMARINS AND MARMOSETS

GBV-B infectious serum obtained from a tamarin (1.3 × 10^8 viral RNA copies per inoculum) was injected into each tamarin and marmoset intrahepatically as previously described (Ishii et al., 2007). We confirmed that the inoculum contained no mutations as compared with the original sequence. Of note, an anti-luciferase siRNA in a cationic liposome formulation was administered to one animal under anesthesia and the plasma samples were evaluated during the period of experimental infection in Cj05-004, respectively. Throughout this article, the viral genome sequences in each time point are as follows: AB630358, AB630359, and AB630360 for 45, 104, and 229 weeks after infection in GBV-B-core and NS3 proteins.

QUANTIFICATION OF GBV-B GENOMIC RNA

GBV-B RNA was isolated from the plasma samples by using a QIAamp MinElute Virus Spin kit (QIAGEN) and was quantified by real-time PCR using the 5'-exonuclease PCR (TaqMan) assay system (Ishii et al., 2007). The primers 558F [5'-AACGACAGCAATGCGAAGTC] and 626R [5'-CATCATGATACCCAGCAATTTTGT] and the probe 579P [5'-FAM-AGCCGCATGCTCGGCTCTGTA-TAMRA] (Beames et al., 2000) were obtained from Sigma-Aldrich. The cutoff value was 10^3 copies/ml. All the specimens were evaluated in duplicate and the average values were calculated.

DETECTION OF ANTIBODIES AGAINST GBV-B CORE AND NS3 PROTEINS BY ELISA

Tamarin and marmoset plasma samples were evaluated for anti-GBV-B core and NS3 antibodies by ELISA as described previously (Ishii et al., 2007).

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSES

Liver samples obtained by necropsy from the GBV-B-infected marmoset were examined histopathologically as previously described (Ishii et al., 2007). For standard histological examination, the sections were subjected to hematoxylin and eosin (HE) staining. Masson's trichrome staining was also performed to estimate the development of fibrosis according to a standard laboratory protocol. To detect the viral protein in tissues, we employed a mouse anti-core monoclonal antibody, 5A10, that we generated. In brief, Mice were immunized with the GBV-B core protein expressed in E. coli (Ishii et al., 2007). Hybridoma cells producing an anti-core mAb were screened by both the core-expressing 293T cells and the liver sections of an acutely GBV-B-infected tamarin. Liver samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections were deparaffinized by pretreating with 0.5% periodic acid and then subjected to antigen retrieval with citric acid buffer and heating in an autoclave for 10 min at 121°C. The sections were then incubated free floating in primary antibody solution (5A10; 1:50 dilution) overnight at 4°C. Following brief washes with wash buffer, the sections were sequentially incubated with a biotinylated goat anti-mouse IgG (1:400 dilution), followed by addition of a streptavidin–biotin–horseradish peroxidase complex (sABC kit; DAKO, Denmark). Immunoreactive elements in the sections were visualized by treatment with 3,3′-diaminobenzidine tetradoxide (Dojin Kagaku, Japan), together with counterstaining with hematoxylin.

DETERMINATION OF THE GBV-B SEQUENCE

Viral RNA was isolated from the plasma of GBV-B-infected marmosets as described above. GBV-B cDNA was synthesized using SuperScript reverse transcriptase III (Invitrogen) with random hexamer primers (Invitrogen). The resulting cDNAs were used to obtain PCR amplification products of lengths of 0.5–1 kb, using GBV-B-specific primers and LA-Taq DNA polymerase (TaKaRa). The PCR products were then purified from the gel using a QIAquick gel extraction kit (QIAGEN), and the purified amplimers were sequenced directly using a CEQ-2000XL analysis system (Beckman) with a DTCS quick start kit and GBV-B-specific primers according to the manufacturer's instructions. Sequence data were analyzed using the Sequencher 4.8 (Gene Codes) and Mac Vector 10.6 (MacVector) software packages. The GenBank accession numbers of the viral genome sequences in each time point are as follows: AB630358, AB630359, and AB630360 for 45, 104, and 135 weeks after infection in Cj05-002; AB630361, AB630362, AB630363, and AB630364 for 33, 88, 141, and 229 weeks after infection in Cj05-004, respectively. Throughout this article, the amino acids are numbered according to the full-length genome sequence of isolate pGBB (GenBank accession number AF179612).
RESULTS
GBV-B INFECTION IN TAMARINS AND MARMOSETS
Four tamarins and four marmosets were intrahepatically inoculated with GBV-B and the growth kinetics and pathogenesis of the virus were compared. In tamarins, the peak viral loads in plasma reached $10^9$–$10^{10}$ copies/ml in the acute phase and the viremia was maintained for an average of 3 months in parallel with increases in plasma ALT levels (Figure 1A). Antibodies reactive with the viral core and NS3 proteins were developed in all of the tamarins as the plasma viral loads were reduced and the antibody titers reached maximum levels concurrently with the complete loss of detectable viral RNA (Figure 1A). In contrast, two of four marmosets infected with GBV-B developed chronic infection while the others exhibited a phenotype similar to that of the tamarins (i.e., subacute clearance of the viremia followed by antibody responses). One exception is that lower plasma viral loads ($10^7$–$10^8$ copies/ml) were observed in the marmosets relative to those of the tamarins (Figure 1B). The details of the chronically infected marmosets are described below.

Case 1: Cj05-002 (Figures 1B and 2). The viral RNA was undetectable until week 4 post infection (p.i.) and then gradually increased to a peak at week 18 p.i. ($3 \times 10^8$ copies/ml). Subsequently, this case retained intermittent viremia during the observation period of week 180 p.i., while the intervals between the viremia phases were prolonged. Importantly, the titers of anti-core and anti-NS3 antibodies reached a persistent plateau at 6 months and 1 year p.i., respectively. In addition, ALT levels were recurrently increased without observation of other clinical symptoms.

Case 2: Cj05-004 (Figures 1B and 2). During the acute phase of infection, the level of viremia was relatively low and transient, followed by a 1-year period when the virus was essentially undetectable. Irrespective of the very low viral load, the titer of anti-NS3 but not anti-core antibody steadily increased and reached a plateau at week 42 p.i. Moreover, an occasional but obvious increase in the level of ALT was observed during this period. We thus suspected that antigenic stimulation by a lower level of viral growth in the liver, which remained below detectable levels in blood, might lead to the induction of the anti-NS3 antibody and the recurrent ALT increase. Subsequently, viremia became detectable at week 58 p.i. and $10^4$–$10^5$ copies/ml of the viral RNA persisted until week 108 p.i. Thereafter, an abrupt increase of the anti-core antibody was detected, concomitant with augmentation of the viral load of $10^{5.5}$ copies/ml on average and recurrent increases in the ALT level. Eventually, the individual was euthanized at week 229 p.i. because of poor prognosis since the ALT value drastically increased by 161-fold, which was accompanied by a dramatic decrease of platelet counts and a deteriorating general status. Histopathological analyses of the necropsy samples demonstrated that the liver developed diffuse piecemeal necrosis with infiltration of lymphocytes and

![Figure 1](https://example.com/figure1.png)  
**Figure 1** | Infection profiles until week 70 p.i. of tamarins (A) and marmosets (B) inoculated intrahepatically with GBV-B infectious serum obtained from a tamarin. Plasma samples periodically obtained from the monkeys were evaluated for the copy numbers of GBV-B genomic RNA (green shaded area), ALT levels (red circles), and the antibody titers against GBV-B core and NS3 (blue and yellow triangles, respectively).

![Figure 2](https://example.com/figure2.png)  
**Figure 2** | Profiles of the marmosets persistently infected with GBV-B. Plasma samples periodically obtained from the marmosets were evaluated for the copy numbers of GBV-B genomic RNA (green shaded area), ALT levels (red circles), and the antibody titers against GBV-B core and NS3 (blue and yellow triangles, respectively). The cruciate mark in Cj05-004 indicates that the individual was euthanized due to a poor prognosis at week 229 p.i. At that time, the ALT value in plasma had increased by 161-fold.
formation of lymphoid follicles (Figure 3A, Appendix). The viral load in the liver was relatively high ($3.8 \times 10^4$ copies/mg tissue weight), which was similar to the viral load observed for tamarins acutely infected with GBV-B (Ishii et al., 2007). The high viral load in the liver was consistent with a large number of granular positive signals for the core protein, which was in similar manner with the core protein of HCV (Miyamari et al., 2007), as immunostained with an anti-GBV-B core monoclonal antibody (Figure 3B). Notably, Masson trichrome staining (Figures 3C,D) as well as Elastica van Gieson staining (Appendix) demonstrated that the liver also developed diffuse and abundant fibrosis. The disease of this marmoset was therefore diagnosed as a case of acute exacerbation of progressive chronic hepatitis by GBV-B infection.

ANALYSIS OF MUTATIONS IN GBV-B GENOMES

Next, we determined the dominant sequence of the viral genomes at weeks 45, 104, and 135 p.i. in Cj05-002 and weeks 33, 88, 141, and 229 p.i. in Cj05-004. As seen in Figure 4A, it was found that there was no specific region in which extensive nucleotide mutations occurred throughout the study periods and that the nucleotide mutation rates were $1.9-2.9 \times 10^{-3}$ and $1.5-3.6 \times 10^{-3}$ changes per site per year in Cj05-002 and Cj05-004, respectively (Table 1). In terms of amino acid substitution, we observed the following: (i) several back or sequential mutations (G250V > A, S731L > S, E2346G > E in Cj05-002; V254A > V, L285V > I, L285V > I, T735 > T, F2135L > F > S in Cj05-004) in both marmosets; (ii) highly selective non-synonymous mutations that were remarkable in E1, but such mutations were rarely observed in core (Figures 4 and 5); and (iii) the non-synonymous mutation rates were $1.8-4.0 \times 10^{-3}$ and $2.1-4.6 \times 10^{-3}$ substitutions per site per year in Cj05-002 and Cj05-004, respectively (Figures 4 and 5; Table 2). (iv) The non-synonymous changes detected mainly in NS5A and NS5B in both animals were also observed in a number of previous reports (Simons et al., 1995; Bukh et al., 1999; Sbardellati et al., 2001; Martin et al., 2003; Nam et al., 2004; Kyuregyan et al., 2005; Weatherford et al., 2009; Takikawa et al., 2010). It may be reasonable to consider that the molecular clone we employed (Bukh et al., 1999) was derived from a minor clone of mixed populations and emergence of a new mutations easily occurred as a mechanism of GBV-B adaptation to a new host, while it is also possible that “consensus” non-synonymous changes were due to either a result of a selection of the pre-existent minor variants. Taken together, these results suggest that efficient
and selective evasion from immune pressure in the two marmosets resulted in long-term persistent GBV-B infection accompanied by subsequent chronic hepatitis.

DISCUSSION

In this study, we show for the first time that GBV-B is capable of eliciting a chronic and progressive hepatitis C-like disease in marmosets. Evidence for this condition is demonstrated by long-term persistent GBV-B infection, recurrent ALT increase, and fibrosis. Moreover, one of the chronically infected marmosets developed acute exacerbation of chronic hepatitis as indicated by diffuse piecemeal liver necrosis and an ALT flare, which is seen in patients with viral hepatitis (Perrillo, 1997). While the usefulness of the monkey model as a surrogate model for HCV infection has been under debate due to the virtual inability of GBV-B to cause chronic hepatitis C-like disease in tamarins, the present data demonstrate that the ability of GBV-B to induce the chronic disease is likely to be inherent depending on the differences between species and individuals.

It has been reported that tamarins generally permit extensive replication of GBV-B in the subacute phase of infection and develop acute hepatitis as shown by significant increases of serum enzymes such as ALT and isocitrate dehydrogenase. The viral load in marmosets seems to be lower than in tamarins (Lanford et al., 2001).

### Table 1: Summary of the nucleotide substitutions in GBV-B genome sequences amplified from plasma of the marmosets persistently infected with GBV-B.

| Genomic region | nt position | Cj05-002 | Cj05-004 |
|----------------|-------------|----------|----------|
|                |             | 45 weeks | 104 weeks | 135 weeks | 45 weeks | 104 weeks | 135 weeks |
| 5'UTR          | 1–445       | 2 (0.45) | 3 (0.67)  | 2 (0.45)  | 1 (0.22) | 0 (0)     | 3 (0.67)  |
| Core           | 446–913     | 0 (0)    | 1 (0.21)  | 1 (0.21)  | 1 (0.21) | 4 (0.85)  | 2 (0.43)  |
| E1             | 914–1489    | 1 (0.17) | 3 (0.52)  | 0 (0)     | 1 (0.17) | 3 (0.52)  | 1 (0.17)  |
| E2             | 1490–2449   | 0 (0)    | 5 (0.52)  | 1 (0.10)  | 0 (0)    | 2 (0.21)  | 1 (0.10)  |
| p13            | 2450–2641   | 2 (1.04) | 1 (0.52)  | 2 (1.04)  | 2 (1.04) | 1 (0.52)  | 0 (0)     |
| NS2            | 2642–3265   | 1 (0.16) | 5 (0.80)  | 1 (0.16)  | 1 (0.16) | 4 (0.64)  | 1 (0.16)  |
| NS3            | 3266–5125   | 1 (0.05) | 4 (0.22)  | 3 (0.16)  | 0 (0)    | 5 (0.27)  | 6 (0.32)  |
| NS4A           | 5126–5290   | 0 (0)    | 0 (0)     | 0 (0)     | 0 (0)    | 0 (0)     | 0 (0)     |
| NS4B           | 5291–6034   | 0 (0)    | 0 (0)     | 2 (0.27)  | 0 (0)    | 1 (0.13)  | 0 (0)     |
| NS5A           | 6035–7267   | 6 (0.49) | 4 (0.32)  | 2 (0.16)  | 4 (0.32) | 4 (0.32)  | 2 (0.16)  |
| NS5B           | 7268–9037   | 4 (0.23) | 5 (0.28)  | 3 (0.17)  | 2 (0.11) | 10 (0.56) | 1 (0.06)  |
| Total          | 9037        | 17 (0.19)| 31 (0.34) | 17 (0.19) | 12 (0.13)| 34 (0.38) | 17 (0.19) |

Mutation rate/year: 2.2 × 10^{-3}, 3.0 × 10^{-3}, 3.2 × 10^{-3}, 2.1 × 10^{-3}, 3.6 × 10^{-3}, 1.8 × 10^{-3}, 1.6 × 10^{-3}.

### Table 2: Summary of the amino acid substitutions in GBV-B genome sequences amplified from plasma of the marmosets persistently infected with GBV-B.

| Amino acid region | aa position | Cj05-002 | Cj05-004 |
|-------------------|-------------|----------|----------|
|                   |             | 45 weeks | 104 weeks | 135 weeks | 45 weeks | 104 weeks | 135 weeks |
| Core              | 1–156       | 0 (0)    | 0 (0)     | 0 (0)     | 0 (0)    | 0 (0)     | 0 (0)     |
| E1                | 157–348     | 1 (0.52) | 2 (1.04)  | 0 (0)     | 1 (0.52) | 3 (1.56)  | 1 (0.52)  |
| E2                | 349–613     | 0 (0)    | 2 (0.63)  | 0 (0)     | 0 (0)    | 1 (0.31)  | 0 (0)     |
| P13               | 669–732     | 1 (1.56) | 1 (1.56)  | 1 (1.56)  | 1 (1.56) | 0 (0)     | 0 (0)     |
| NS2               | 733–940     | 1 (0.48) | 2 (0.96)  | 0 (0)     | 1 (0.48) | 2 (0.96)  | 0 (0)     |
| NS3               | 941–1560    | 1 (0.16) | 0 (0)     | 1 (0.16)  | 0 (0)    | 2 (0.32)  | 2 (0.32)  |
| NS4A              | 1561–1615   | 0 (0)    | 0 (0)     | 0 (0)     | 0 (0)    | 0 (0)     | 0 (0)     |
| NS4B              | 1616–1863   | 0 (0)    | 0 (0)     | 0 (0)     | 0 (0)    | 0 (0)     | 0 (0)     |
| NS5A              | 1864–2274   | 4 (0.97) | 2 (0.49)  | 0 (0)     | 3 (0.73) | 1 (0.24)  | 2 (0.49)  |
| NS5B              | 2276–2864   | 2 (0.34) | 3 (0.51)  | 1 (0.17)  | 1 (0.17) | 5 (0.85)  | 1 (0.17)  |
| Total             | 2864        | 10 (0.38)| 12 (0.42) | 3 (0.10)  | 7 (0.24) | 14 (0.49) | 6 (0.21)  |

Mutation rate/year: 4.0 × 10^{-3}, 3.7 × 10^{-3}, 1.8 × 10^{-3}, 3.9 × 10^{-3}, 4.6 × 10^{-3}, 2.1 × 10^{-3}, 2.5 × 10^{-3}.
of the scarce viral loads over 1 year p.i., including the bipartite was observed to steadily increase after week 10 p.i. irrespective non-synonymous mutations will clarify this possibility.

In long-term persistent GBV-B infection and subsequent chronic evasion from immune pressures may result 1991; Fernandez et al., 2004), these results strongly suggest that the rates of both synonymous and non-synonymous mutations in the E1 and NS3 regions occurred throughout the observation periods in Cj05-004 with chronic GBV-B infection, which had not been identified previously (Simons et al., 1995; Bukh et al., 1999; Sbardellati et al., 2001; Martin et al., 2003; Nam et al., 2004; Kyuregyan et al., 2005; Weatherford et al., 2009; Takikawa et al., 2010). Together with the finding that the non-synonymous mutations per total amino acid numbers of each viral protein at multiple time points are illustrated (A) at weeks 45, 104, and 135 in Cj05-002; (B) weeks 33, 88, and 141 in Cj05-004. To compare the ratio of the non-synonymous mutations per total numbers of nucleotide mutations identified at weeks 45, 104, and 135 in Cj05-002 and at weeks 33, 88, and 141 in Cj05-004 in each viral gene. To compare the frequency between the two marmosets, the data at week 229 p.i. in Cj05-004 are omitted. (C) The accumulated frequency of the non-synonymous mutations per total amino acid numbers of each viral protein at multiple time points are illustrated (A) at weeks 45, 104, and 135 in Cj05-002; (B) weeks 33, 88, and 141 in Cj05-004. To compare the frequency between the two marmosets, the data at week 229 p.i. in Cj05-004 are omitted.

It is surprising that in Cj05-004, the antibody titer to NS3 was observed to steadily increase after week 10 p.i. irrespective of the scarce viral loads over 1 year p.i., including the bipartite periods of weeks 4–26 and 34–58 p.i. when the virus was undetectable (Figure 1). Considering that three spikes of ALT levels were observed during these periods, our results suggest that antigenic stimulation by the lower level of viral growth in the liver, which was below detectable levels in blood, may induce the antibody and cytotoxic T-cell responses. In addition, during longitudinal analyses of monkeys experimentally infected with GBV-B, it is important to comprehensively evaluate multiple parameters, including viral loads, serum enzymes, and antibodies against core and NS3 proteins, to define whether virus-infected monkeys that produce no detectable viremia for a period of time have cleared the virus or are experiencing a latent period of chronic infection.

Chronic hepatitis C-like diseases in marmosets
ACKNOWLEDGMENTS

We wish to thank T. Kurosawa, M. Fujita, and T. Ikoma for their helpful assistance and the members of Corporation for Production and Research of Laboratory Primates for technical assistance. Financial support: This work was supported by grants from the Ministry of Health, Labor, and Welfare of Japan (to Hirofumi Akari).

REFERENCES

Akari, H., Iwasaki, Y., Yoshida, T., and Iijima, S. (2009). Non-human primate surrogate model of hepatitis C virus infection. Microbiol. Immunol. 53, 53–57.

Beams, B., Chavez, D., Guerra, B., Notvall, L., Brasky, K. M., and Lanford, R. E. (2000). Development of a primary tamarin hepatocyte culture system for GB virus-B: a surrogate model for hepatitis C virus. J. Virol. 74, 11764–11772.

Beams, B., Chavez, D., and Lanford, R. E. (2001). GB virus B as a surrogate model for hepatitis C virus. IARJ J. 42, 152–160.

Boonstra, A., van der Laan, L. J., Vanwolleghem, T., and Janssen, H. L. (2009). Experimental models for hepatitis C viral infection. Hepatology 50, 1646–1655.

Bowen, D. G., and Walker, C. M. (2005a). Mutational escape from CIB+ T cell immune: HCV evolution, from chimpanzees to man. J. Exp. Med. 201, 1709–1714.

Bowen, D. G., and Walker, C. M. (2003b). Adaptive immune responses in acute and chronic hepatitis C virus infection. Nature 436, 946–952.

Bright, H., Carroll, A. R., Watts, P. A., and Fenton, R. J. (2004). Development of a GB virus B marmoset model and its validation with a novel series of hepatitis C virus NS5 protease inhibitors. J. Virol. 78, 2062–2071.

Bukh, J. (2004). A critical role for chimpanzee model in the study of hepatitis C. Hepatology 39, 1469–1475.

Bukh, J., Appar, C. L., and Yanagi, M. (1999). Toward a surrogate model for hepatitis C virus: An infectious molecular clone of the GB virus-B hepatitis agent. Virology 262, 470–478.

Bukh, J., Thimme, R., Meunier, J. C., Faulk, K., Spangenberg, H. C., Chang, K. M., Satterfield, W., Chisari, F. V., and Purcell, R. H. (2008). Previously infected chimpanzees are not consistently protected against reinfection or persistent infection after reexposure to the identical hepatitis C virus strain. J. Virol. 82, 8183–8195.

Burke, K. P., and Cox, A. L. (2010). Hepatitis C virus evasion of adaptive immune responses: a model for viral persistence. Immunol. Res. 47, 216–227.

Chisari, F. V. (2005). Unscrambling hepatitis C virus-host interactions. Nature 436, 930–932.

Feld, J. J., and Hoofnagle, J. H. (2005). Mechanism of action of interferon and ribavirin in treatment of hepatitis C virus (HCV). Nature 436, 967–972.

Fernandez, J., Taylor, D., Morhardt, D. R., Mihalki, K., Puig, M., Rice, C. M., Feinsteen, S. M., and Major, M. E. (2004). Long-term persistence of infection in chimpanzees inoculated with an infectious hepatitis C virus clone is associated with a decrease in the viral amino acid substitution and low levels of heterogeneity. J. Virol. 78, 9782–9789.

Hoofnagle, J. H. (1997). Hepatitis C: the clinical spectrum of disease. Hepatology 26, 158–205.

Ishi, K., Iijima, S., Kimura, N., Lee, Y. J., Ageyama, N., Yagi, S., Yamaguchi, K., Maki, N., Morii, K., Yoshizaki, S. Machida, S., Suzuki, T., Iwata, N., Sata, T., Terao, K., Miyamura, T., and Akari, H. (2007). GBV-B as a pleiotropic virus: distribution of GBV-B in extrahepatic tissues and evaluation of GBV-B in extrahepatic tissues linked with a GB hepatitis agent. J. Virol. 81, 1646–1655.

Michailov, M. I., Ross, S., Bukh, J., DeBiasi, R., Leary, T. P., Dawason, G. J., Desai, S. M., and Traboni, C. (2001). Generation of a GBV-C viral infection. Curr. Top. Microbiol. Immunol. 257S–61S.

Nam, J. H., Faulk, K., Engle, R. E., Gojobori, T., St, Claire M., and Bukh, J. (2004). In vivo analysis of the 3’ untranslated region of GB virus B after in vitro mutagenesis of an infections cDNA clone: persistent infection in a transfected tamarin. J. Virol. 78, 9389–9399.

Goettge, K., Popov, V., Rijnbrand, T., and Akari, H. (2007). GBV-B virus production. J. Virol. 81, 154–157.

Ogata, N., Alter, H. J., Miller, R. H., and Purcell, R. H. (1991). Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. Proc. Natl. Acad. Sci. U.S.A. 88, 3392–3396.

Ohashi, K., Mizokami, M., Lau, J.Y., Orito, E., Ikeo, K., and Gojobori, T. (1996). Evolutionary relationship of hepatitis C, pestiv-, flaviv-, plantviruses, and newly discovered GB hepatitis agents. FEBS Lett. 378, 232–234.

Perrillo, R. P. (1997). The role of liver biopsy in hepatitis C. Hepatology 26, 575–615.

Rehmann, B., and Nascimbeni, M. (2005). Immunology of hepatitis B virus and hepatitis C virus infection. Nat. Rev. Immunol. 5, 215–229.

Sbardellati, A., Scarselli, E., Verschoor, E., De Tomassi, A., Lazzaro, D., and Traboni, C. (2001). Generation of infectious and transmissible viruses from a GB virus B full-length consensus clone in tamarins. J. Gen. Virol. 82, 2437–2448.

Seeff, L. B., and Hoofnagle, J. H. (2002). National Institutes of Health Consensus Development Conference management of hepatitis C: 2002. Hepatology 36, 51–52.

Simons, J. N., Pilot-Matias, T. J., Leary, T. P., Dawson, G. J., Desai, S. M., Schlauder, G. G., Muerhoff, A. S., Leary, T. P., Dawason, G. J., Desai, S. M., and Traboni, C. (2001). Generation of infectious and transmissible viruses from a GB virus B full-length consensus clone in tamarins. J. Gen. Virol. 82, 2437–2448.

T., Kannagi M and Akari H (2011) Long-term persistent GBV-B infection and development of a chronic and progressive hepatitis C-like disease in marmosets. Front. Microbio. 2:240. doi: 10.3389/fmicb.2011.00240

This article was submitted to Frontiers in Virology, a specialty of Frontiers in Microbiology.

Copyright © 2011 Iwasaki, Mori, Koi, Ishii, Maki, Iijima, Yoshida, Okubayashi, Kakukai, Lee, Saito, Fukuikawa, K, Kojima, Aaya, Yokota, A, Sumitomo, Y, Miyamura, K, and Akari H. This is an open-access article subject to a non-exclusive license between the authors and Frontiers Media SA, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and other Frontiers conditions are complied with.
MATERIALS AND METHODS
Liver samples obtained by necropsy from the GBV-B-infected marmosets were histopathologically analyzed as described in Section “Materials and Methods.” Elastica–van Gieson staining was performed to evaluate fibrosis according to a standard laboratory protocol. To detect CD3 and CD20 antigens, liver samples were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Sections were deparaffinized by pretreatment with 0.5% periodic acid and then subjected to antigen retrieval with citric acid buffer and heating in an autoclave for 10 min at 121°C. Sections were then incubated free floating in the monoclonal antibody solution for CD20 (DAKO) and CD3 (DAKO) overnight at 4°C. Following brief washes with buffer, the sections were sequentially incubated with biotinylated goat anti-mouse IgG (1:400), followed by streptavidin–biotin–horseradish peroxidase complex (sABC kit; DAKO, Denmark). Immunoreactive elements were visualized by treating the sections with 3,3′-diaminobenzidine tetroxide (Dojin Kagaku, Japan). The sections were then counterstained with hematoxylin.

FIGURE A1 | Histopathological and immunohistochemical analyses of the liver from Cj05-004 at week 229 p.i.