Table 1 Clinical diagnosis of HCV carriers identified among blood donors at baseline

Features	Total ($n = 1019$)	Men ($n = 478$)	Women ($n = 541$)	Differences (men *vs* women)
Age	45.3 ± 11.1	43.5 ± 11.0	46.9 ± 11.4	$P < 0.01$
Liver disease				
No abnormalities	483 (47.4%)	174 (36.4%)	309 (57.1%)	$P < 0.001$
Chronic hepatitis	529 (51.9%)	299 (62.6%)	230 (42.5%)	$P < 0.001$
Treated	242 (45.7%)	136 (45.5%)	106 (46.1%)	
Followed up	222 (42.0%)	133 (44.5%)	89 (38.7%)	
Lost	65 (12.3%)	30 (10.0%)	35 (15.2%)	
Cirrhosis	5 (0.5%)	3 (0.6%)	2 (0.4%)	NS
HCC	1 (0.1%)	1 (0.2%)	0	NS
Acute hepatitis	1 (0.1%)	1 (0.2%)	0	NS

HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NS, not significant.

IFN treatment

IFN was used according to regular protocol. Types of IFN were recombinant IFN-α2a, recombinant IFN-α-2b and IFNα. For example, recombinant IFN-α2a at a daily dose of 6–9 million international units (MIU) was given during the initial 2 weeks, followed by 3 MIU three times a week until 24 weeks after the start of IFN (total dose: 300–342 MIU). Sustained virological response (SVR) to IFN was diagnosed 24 weeks after the completion of treatment by the elimination of HCV-RNA from serum detectable by PCR. Biochemical response with normalization of aminotransferases, without loss of serum HCV-RNA, was not regarded as a response to IFN.

Statistical analyses

Categorical variables were compared between groups by the χ^2 test or Fisher's exact test. Kaplan–Meier life tables were used in assessing the risk of developing HCC with reference to gender, age and liver disease at the baseline, as well as treatment with IFN, using UMP version 5 software (SAS Institute, Tokyo, Japan).

RESULTS

Liver disease in 1019 HCV carriers found at blood donation

TABLE 1 LISTS liver disease diagnosed in 1019 HCV carriers stratified by gender. Overall, they were 45.3 ± 11.1 years old and included 478 (46.9%) men. Less than half had no abnormalities in the liver (483 [47.4%]), while cirrhosis had already developed in five (0.5% [three men included]) and HCC in one (0.1% [man]); acute hepatitis C was diagnosed in one (0.1%). Less frequent were no abnormalities in the liver (36.4% *vs* 57.1%, $P < 0.01$) in men than in women, and chronic hepatitis was found more often (62.6% *vs* 42.5%, $P < 0.01$) in men than in women. Among 529 carriers who were diagnosed as chronic hepatitis, 242 carriers (45.7%) were diagnosed as requiring a treatment at the first medical consultation. There were no differences between men and women in the ratio of treated HCV carriers.

Differences were noted in the influence of age on the baseline liver disease between men and women (Table 2). Among HCV carriers aged ≤39 years and 40–49 years, chronic hepatitis was more common in men than in women ($P < 0.01$). For those aged 50–59 years and ≥60 years, however, clinical diagnoses were no different between men and women.

Comparison of clinical outcomes between HCV carriers with and without IFN treatment

Of the 1019 HCV carriers, 408 (40.0%) were followed for 5 years or longer with date of the last visit and final diagnosis being specified. Among them, 197 (48.3%) carriers received IFN therapy (one course of 24-weeks IFN). There were no significant differences in the age (46.0 ± 10.2 *vs* 47.3 ± 11.2 years) or sex (men accounting for 41% *vs* 51%) between carriers with and without IFN. However, the observation period was longer in carriers with IFN than without IFN (9.2 ± 1.7 [range: 5.0–11.7] *vs* 8.8 ± 1.9 [5.1–11.9] years, $P < 0.05$). Figure 1 compares clinical outcomes between HCV carriers with and without IFN. HCV-RNA was not detectable in serum 24 weeks after the completion of treatment in the 61 (31.0%) carriers who had received

© 2007 The Japan Society of Hepatology

Table 2 Baseline clinical diagnoses of the 476 men and 541 women found with serum HCV-RNA at blood donation stratified by age

Age	Men	Women	Differences
≤39 years	(*n* = 180)	(*n* = 123)	$P < 0.01$
No abnormalities	64 (35.6%)	82 (66.7%)	
Chronic hepatitis	116 (64.4%)	41 (33.3%)	
Cirrhosis	0	0	
40–49 years	(*n* = 146)	(*n* = 153)	$P < 0.01$
No abnormalities	51 (34.9%)	89 (58.2%)	
Chronic hepatitis	94 (64.4%)	64 (41.8%)	
Cirrhosis	1 (0.7%)	0	
50–59 years	(*n* = 111)†	(*n* = 199)	NS
No abnormalities	45 (40.5%)	104 (52.3%)	
Chronic hepatitis	65 (58.6%)	94 (47.2%)	
Cirrhosis	1 (0.9%)	1 (0.5%)	
≥60 years	(*n* = 39)‡	(*n* = 66)	NS
No abnormalities	14 (35.9%)	34 (51.5%)	
Chronic hepatitis	24 (61.5%)	31 (47.0%)	
Cirrhosis	1 (2.6%)	1 (1.5%)	

†Case of acute hepatitis excluded.
‡Case of hepatocellular carcinoma excluded.
HCV, hepatitis C virus; NS, not significant.

IFN, including 13 of the 48 (27.1%) without abnormalities in the liver and 48 of the 148 (32.4%) with chronic hepatitis at the baseline. In contrast, HCV-RNA disappeared in only one of the 128 (0.8%) carriers without IFN therapy; he did not have abnormalities in the liver at the baseline. Thus, HCV-RNA was cleared from serum much more frequently with than without IFN treatment (61/197 [31.0%] *vs* 1/211 [0.5%], $P < 0.0001$).

Cirrhosis developed anew in 15 carriers including five of the 211 (2.4%) without IFN and 10 of the 197 (5.1%) with IFN (Table 3). All the 10 carriers developing cirrhosis, despite receiving IFN, were non-responders (one partial responder included). Genotypes of HCV were 1b in eight of the 11 (73%) cirrhotics for whom they were determined, and 2a or 2b in the remaining three. Likewise, HCC developed in 14 carriers including six of the 211 (2.8%) without IFN and eight of the 197 (4.1%) with IFN (Table 4). Of the eight carriers developing HCC, in spite of IFN treatment, six were non-responders (one partial responder included) and one did not complete the full course of IFN. The remaining one (case 13) developed HCC 6 years after he achieved complete response to IFN; he received partial hepatectomy. Of the 10 carriers developing HCC for whom genotypes were examined, eight (80%) were infected with HCV of genotype 1b.

Factors influencing the development of HCC

Hepatocellular carcinoma developed in 14 of the 408 (3.4%) HCV carriers who had been followed for 5 years or longer, including six without and eight with IFN therapy (Table 4). As most carriers developing HCC did not respond to IFN, they were analyzed collectively with the carriers in whom HCC occurred in the absence of IFN therapy. By analysis of Kaplan–Meier life tables (Fig. 2a–c), baseline cirrhosis ($P < 0.0001$), age at the detection of HCV carrier state ($P < 0.002$) and the male gender ($P < 0.01$) enhanced the development of HCC. IFN did not significantly influence the development of HCC, however (data not shown).

Risk of developing HCC in carriers who were initially diagnosed with chronic hepatitis, stratified by the age, is shown in Figure 3. Cumulative HCC risk during 10 years was estimated at 18.7% (95% confidence interval: 1.9–35.5%) for carriers aged 60 years or older, 8.7% (0–17.5%) for those in their fifties and 7.2% (0–15.5%) for those in their forties when they presented with chronic hepatitis at blood donation.

DISCUSSION

IN PREVIOUS STUDIES, we have identified HCV carriers at the time of blood donation in the Japanese

© 2007 The Japan Society of Hepatology

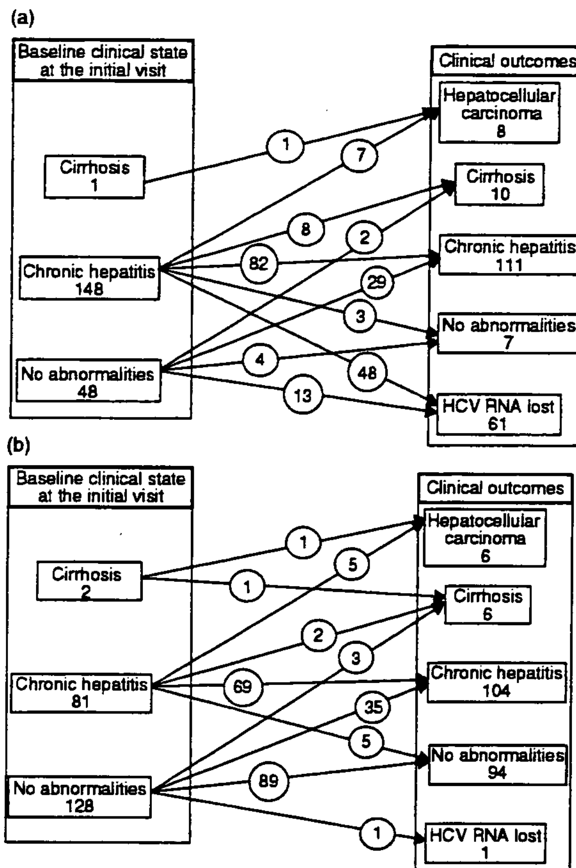

Figure 1 (a) Clinical outcomes of the 197 hepatitis C virus (HCV) carriers with interferon (IFN) therapy during follow ups of 5 years or longer stratified by the baseline clinical state. (b) Clinical outcomes of the 211 carriers without IFN therapy during follow ups of 5 years or longer stratified by the baseline clinical state.

Red Cross (JRC) Hiroshima Blood Center and followed them for evolution of liver disease at local hepatology centers.[17–19] Here we report the final results on 1019 HCV carriers compiled during the past 15 years. Such attempts may shed light on the morbidity of HCV carriers in the community who have no symptoms or knowledge of their infection. Unlike patients with HCV infection who visit hospitals[10,11] or pregnant women accidentally infected by contaminated anti-D gammaglobulin,[13,14] who account for only a minority of total HCV infection, asymptomatic carriers represent the great majority of HCV infection in the community. Asymptomatic HCV carriers were incidentally identified at blood donation; the present study clarified the clinical natural course of HCV carriers who were found without symptoms at the occasion of blood donation. This study is a population-based study by contrast with a hospital-based case study.

First, more than half of the 1019 HCV carriers (mean age 45.3 ± 11.1 years) identified at blood donation had liver disease; no abnormalities in the liver were diagnosed in 483 (47.4%) of them. Chronic hepatitis was more frequent in men than in women (62.6% [299/478] *vs* 42.5% [230/541], $P < 0.01$). Cirrhosis had developed already in three (0.6%) men and two (0.4%) women, and HCC in one (0.1%) man. Subjectively, they were healthy enough to offer blood donation. Liver disease would have progressed to chronic hepatitis insidiously in many HCV carriers in their forties. Distribution of chronic hepatitis was age dependent. Among HCV carriers aged ≤39 and 40–49 years, it was more frequent in men than in women (64.4% *vs* 38.0% and 64.4% *vs* 41.8%, respectively, $P < 0.01$ for each); gender differences were smaller in those aged 50–59 and ≥60 years (58.6% *vs* 47.2% and 61.5% *vs* 47.0%, respectively), however.

Second, IFN can improve the course of asymptomatic HCV infection. HCV-RNA was cleared from the circulation in 61 of the 197 (31.0%) carriers who had received IFN, as against only one of the 211 (0.5%) who had not. Responders to IFN included 48 of the 148 (32.4%) carriers with chronic hepatitis and 13 of the 48 (27.0%) without abnormalities in the liver at the baseline. It remains debatable whether or not IFN should be given to HCV carriers without liver disease.[20–23] Loss of HCV infection would be auspicious, by any standard, and may deserve consideration unless IFN is contra-indicated.

Third, cirrhosis developed in 15 (3.6%) and HCC in 14 (3.4%) of the 408 HCV carriers during follow ups for 5 years or longer; genotype 1b predominated in carriers who developed with cirrhosis (8/11 [73%]) or HCC (8/10 [80%]). Carriers who developed cirrhosis or HCC despite receiving IFN were largely non-responders. During the study period, the standard IFN for 24 weeks was approved in Japan. With recent remarkable advances in antiviral therapy, represented by pegylated IFN combined with ribavirin, the response has been improved to 50% even in patients infected with HCV of genotype 1b.[24,25] There is a possibility, therefore, that cirrhosis and HCC in the 29 HCV carriers would have been prevented, at least in part, should they have received sophisticated antiviral treatment.

Fourth, risk factors for the development of HCC were identified in the followed HCV carriers. The initial diag-

© 2007 The Japan Society of Hepatology

Table 3 Development of cirrhosis in 15 HCV carriers during follow ups for 5 years or longer

Case no.	Age/Sex	Baseline diagnosis	Cirrhosis (years elapsed)	IFN therapy	HCV genotype
1	52/M	No abnormalities	4	No	1b
2	50/F	No abnormalities	8	No	1b
3	49/F	Chronic hepatitis	10	No	1b
4	63/F	Chronic hepatitis	3	No	1b
5	59/M	No abnormalities	9	No	1b
6	36/M	Chronic hepatitis	10	Yes (NR)	1b
7	40/M	Chronic hepatitis	6	Yes (NR)	ND
8	41/M	Chronic hepatitis	5	Yes (NR)	ND
9	42/M	No abnormalities	5	Yes (NR)	ND
10	45/F	Chronic hepatitis	3	Yes (NR)	2b
11	48/F	Chronic hepatitis	3	Yes (NR)	2a
12	51/F	Chronic hepatitis	0	Yes (NR)	1b
13	55/F	Chronic hepatitis	5	Yes (PR)	ND
14	56/M	Chronic hepatitis	7	Yes (NR)	1b
15	59/F	No abnormalities	7	Yes (NR)	2a

HCV, hepatitis C virus; IFN, interferon; ND, not determined; NR, no response to IFN; PR, partial response with the normalization of alanine aminotransferase without loss of HCV-RNA.

nosis of cirrhosis, old age at detection of HCV-RNA and male risk factor were significant risk factors for HCC, in corroboration with previous reports.[5,26] Among carriers presenting with chronic hepatitis, the cumulative incidence of HCC during 10 years was significantly higher ($P < 0.01$) in those aged ≥60 years at the baseline (18.7% [95% confidence interval: 1.9–35.5%]) than in those aged 50–59 years (8.7% [0–17.5%]) or 40–49 years (7.2% [0–13.3%]) by analysis in Kaplan–Meier life tables. Hence, it would be imperative to detect HCV infection early in life and treat liver disease before it has progressed, especially in men.

Finally, the present results would justify the campaign for preventing HCC arising in asymptomatic carriers. Encouraged by these results on HCV carriers identified at blood donation, the Japanese government started a 5-year program since the fiscal year 2002 to identify ongoing HCV infection in the recipients of health check-

Table 4 Development of HCC in 14 HCV carriers during follow ups of 5 years or longer

Case no.	Age/Sex	Baseline diagnosis	Years until HCC	IFN therapy	HCV genotype
1	46/M	Chronic hepatitis	7	No	1b
2	41/M	Chronic hepatitis	19	No	1b
3	58/M	Chronic hepatitis	4	No	ND
4	62/F	Chronic hepatitis	5	No	1b
5	61/M	Cirrhosis	7	No	ND
6	60/M	Chronic hepatitis	11	No	1b
7	40/M	Chronic hepatitis	10	Yes (NR)	2b
8	52/M	Cirrhosis	9	Yes (NR)	1b
9	53/M	Chronic hepatitis	10	Yes (PR)	ND
10	52/F	Chronic hepatitis	10	Yes (NR)	1b
11	59/M	Chronic hepatitis	5	Yes (NR)	1b
12	61/M	Chronic hepatitis	7	Yes†	1b
13	63/M	Chronic hepatitis	8	Yes (CR)	2b
14	65/F	Chronic hepatitis	10	Yes (NR)	ND

†Did not receive the full course of interferon (IFN).
CR, complete response with the loss of HCV-RNA from serum; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ND, not determined; NR, no response to IFN; PR, partial response with the normalization of alanine aminotransferase without loss of HCV-RNA.

© 2007 The Japan Society of Hepatology

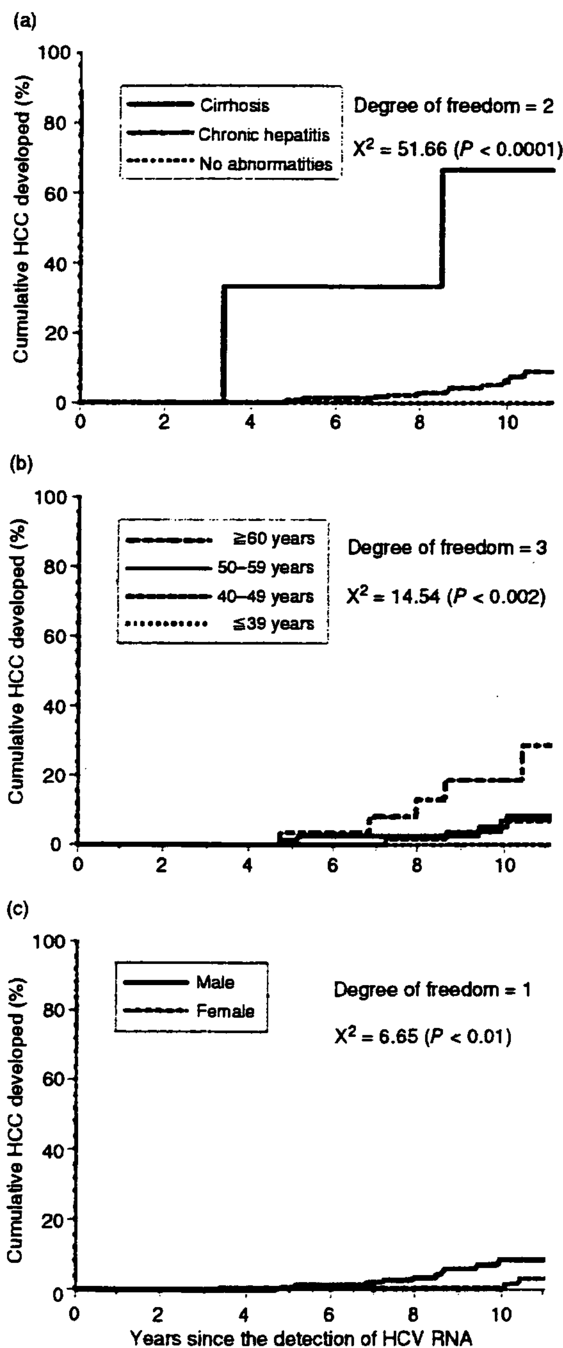

Figure 2 Risk of hepatocellular carcinoma (HCC) increasing with time in the individuals ($n = 949$) found with hepatitis C virus (HCV) infection on blood donation. Influence of liver disease at the (a) baseline, (b) age at the diagnosis of HCV infection and (c) genders is shown.

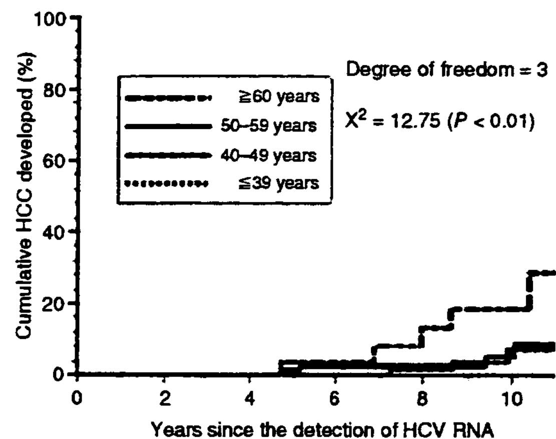

Figure 3 Risk of hepatocellular carcinoma (HCC) increasing with time in the HCV carriers ($n = 498$) identified at blood donation who were diagnosed with chronic hepatitis at the baseline.

ups offered to individuals older than 40 years at a 5-year interval until they enter their seventies.[16] In the present study, however, only 1097 of the 3377 (32.5%) individuals found with HCV infection at blood donation visited hepatology specialists in Hiroshima Prefecture during 1991–2001. Further efforts are crucial for orienting the general population to take tests for HCV infection, and when found with it, consult specialists and receive IFN treatment as required. The results of the present study promise that such endeavors are rewarding, by deceasing morbidity and mortality of persistent HCV infection, along with lessened economic burdens on the nation.

REFERENCES

1 Cohen J. The scientific challenge of hepatitis C. *Science* 1999; 285: 26–30.
2 Afdhal NH. The natural history of hepatitis C. *Semin Liver Dis* 2004; 24: S3–8.
3 Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; 20: 17–35.
4 Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006; 3: 47–52.
5 Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002; 36: S21–9.
6 Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; 36: S35–46.
7 Thomas DL, Astemborski J, Rai RM *et al.* The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 2000; 284: 450–6.

© 2007 The Japan Society of Hepatology

8 Wiley TE, Brown J, Chan J. Hepatitis C infection in African Americans: its natural history and histological progression. *Am J Gastroenterol* 2002; 97: 700–6.
9 Tanaka J, Kumada H, Ikeda K *et al.* Natural histories of hepatitis C virus infection in men and women simulated by the Markov model. *J Med Virol* 2003; 70: 378–86.
10 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; 12: 671–5.
11 Tong MJ, el-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med* 1995; 332: 1463–6.
12 Vogt M, Lang T, Frosner G *et al.* Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N Engl J Med* 1999; 341: 866–70.
13 Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Engl J Med* 1999; 340: 1228–33.
14 Wiese M, Grungreiff K, Guthoff W, Lafrenz M, Oesen U, Porst H. Outcome in a hepatitis C (genotype 1b) single source outbreak in Germany - a 25-year multicenter study. *J Hepatol* 2005; 43: 590–8.
15 Okamoto H, Okada S, Sugiyama Y *et al.* Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5'-noncoding region. *Jpn J Exp Med* 1990; 60: 215–22.
16 Okamoto H, Sugiyama Y, Okada S *et al.* Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol* 1992; 73: 673–9.
17 Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. *Oncology* 2002; 62: S8–17.
18 Yoshizawa H, Tanaka J. A national project for the management of viral hepatitis toward prevention of hepatocellular carinoma in Japan. In: Morrissey RF, ed. *International Kilmer Conference Proceedings*, Vol. 8. Laval: Polyscience Publications, 2004; 247–64.
19 Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. *Intervirology* 2006; 49: 7–17.
20 Hui CK, Monto A, Belaye T, Lau E, Wright TL. Outcomes of interferon alpha and ribavirin treatment for chronic hepatitis C in patients with normal serum aminotransaminases. *Gut* 2003; 52: 1644–8.
21 Mamori S, Suzuki F, Hosaka T *et al.* Interferon monotherapy for patients with chronic hepatitis C and normal serum aminotransferase levels at commencement of treatment. *J Gastroenterol* 2004; 39: 776–82.
22 Persico M, Persico E, Suozzo R *et al.* Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. *Gastroenterology* 2000; 118: 760–4.
23 Shiffman ML, Stewart CA, Hofmann CM *et al.* Chronic infection with hepatitis C virus in patients with elevated or persistently normal serum alanine aminotransferase levels: comparison of hepatic histology and response to interferon therapy. *J Infect Dis* 2000; 182: 1595–601.
24 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–82.
25 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–65.
26 Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; 362: 2095–100.

© 2007 The Japan Society of Hepatology

ONCOLOGY REPORTS 18: 1177-1181, 2007

Epidemiological survey of oral lichen planus among HCV-infected inhabitants in a town in Hiroshima Prefecture in Japan from 2000 to 2003

YUMIKO NAGAO[1], YOSHINARI MYOKEN[2], KEIKO KATAYAMA[3], JUNKO TANAKA[3], HIROSHI YOSHIZAWA[3] and MICHIO SATA[1]

[1]Department of Digestive Disease Information and Research, Kurume University School of Medicine, Kurume, Fukuoka; [2]Department of Oral Surgery, Hiroshima Red Cross and Atomic Bomb Survivors Hospital; [3]Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan

Received June 18, 2007; Accepted July 27, 2007

Abstract. The objective of our study was to evaluate the natural history of oral lichen planus (OLP) and other extrahepatic manifestations in the inhabitants of an area in Japan that is hyperendemic for hepatitis C virus (HCV) infection. Over 4 years, 224 adult inhabitants with HCV infection were examined for OLP by a single oral surgeon. All subjects were interviewed regarding the natural history of other extrahepatic manifestations they had developed. The antibodies to HCV (anti-HCV) and serum HCV RNA were determined. Anti-HCV were detected in sera from 224 subjects (100%); HCV RNA in 210 (93.8%). Of the 224, 88 had at least 1 oral examination for OLP during the 4-year period. In 2000, 2001, 2002 and 2003, OLP was observed in 8.5 (5/59), 14.8 (8/54), 20 (11/55) and 21.4% (12/56) of subjects, respectively. OLP prevalence increased as the subjects grew older. The incidence of OLP over the 4 years among all subjects with HCV infection was 17.0% (15/88, 2 men and 13 women). None experienced natural healing or the development of malignant transformations. Between 2000 and 2003, there was an increase in the prevalence of type 2 diabetes mellitus (DM), thyroid dysfunction, skin disease, renal disease and hypertension. Screening for extrahepatic manifestations should be conducted in patients with risk factors for HCV infection.

Correspondence to: Dr Yumiko Nagao, Department of Digestive Disease Information and Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
E-mail: nagao@med.kurume-u.ac.jp

Abbreviations: HCV, hepatitis C virus; OLP, oral lichen planus; HCC, hepatocellular carcinoma; anti-HCV, antibodies to HCV; DM, diabetes mellitus

Key words: lichen planus, hepatitis C virus, extrahepatic manifestations

Introduction

Hepatitis C virus (HCV) infection is a major health problem in Japan. It is highly prevalent in subjects with chronic liver disease and is strongly associated with hepatocellular carcinoma (HCC). HCV-related HCC accounts in large part for the recent increase in HCC and now constitutes about 80% of all HCC cases in Japan. HCV also incites many extrahepatic manifestations (1,2) of which lichen planus is the most common (3,4). Other associated diseases include cryoglobulinaemic nephropathy and glomerulonephritis (5), thyroid dysfunction (6), porphyria cutanea tarda (7) and type 2 diabetes mellitus (DM) (8).

We previously reported that the incidence of oral lichen planus (OLP) in subjects with HCV infection was significantly higher than in those without HCV. We reached this conclusion by mass screening 685 inhabitants of a hyperendemic area, H town, located in the Fukuoka prefecture of Northern Kyushu, Japan (Fig. 1) for HCV infection (9). The prevalence of other extrahepatic manifestations in subjects with antibodies to HCV (anti-HCV) was higher than in those without HCV (10).

We also conducted an epidemiological study of another HCV hyperendemic area, O town, in the northwest of the Hiroshima prefecture in Honshu, Japan (Fig. 1). The presence of HCV-associated extrahepatic manifestations was found in 66.1% (39/59) of those screened (11). These findings suggest that the high prevalence of various extrahepatic manifestations among HCV-infected subjects is not unique to specific areas.

In the present investigation, we annually examined extrahepatic manifestations in the inhabitants of O town from 2000 to 2003. The aim of this study was to evaluate the natural history of OLP and other extrahepatic manifestations in individuals with HCV infections.

Patients and methods

Patients. From 2000 to 2003, we studied a total of 224 adult inhabitants of O town, a hyperendemic area of HCV infection. All were HCV carriers, though the causes of viral transmission were unknown. In 2000, 2001, 2002 and 2003,

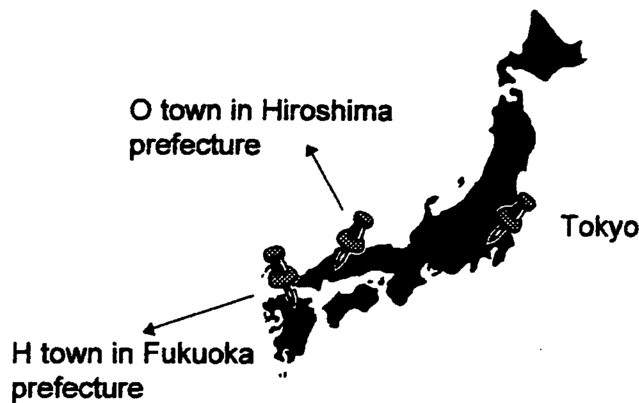

Figure 1. The research area. The location of O town in the northwest region of the Hiroshima prefecture in Honshu, Japan.

we examined 59, 54, 55 and 56 inhabitants, respectively (Table I). A single oral surgeon examined subjects for oral membrane diseases. A topographic classification of the oral mucosa, with location codes indicated, is shown in Fig. 2 (12). The diagnosis of OLP was made based on clinical and histopathological features.

All subjects were interviewed in person by 2 trained interviewers. We inquired about the following: cigarette smoking habits, present health condition and the presence of extrahepatic manifestations of HCV infection such as type 2 DM, rheumatoid arthritis, thyroid dysfunction, skin disease, renal disease, hypertension and extrahepatic malignant tumors.

Informed consent was obtained from all subjects once the purpose and methods of the study were explained.

Examination for anti-HCV and HCV RNA in serum. Sera were examined for the presence or absence of HCV. Anti-HCV were measured by a second-generation, enzyme-linked immunosorbent assay (Abbott HCV PHA 2nd Generation, Dainabot Co., Ltd., Tokyo, Japan). HCV RNA in the sera was detected using the Amplocore HCV test (Nippon Roche, Tokyo, Japan).

Examination of the prevalence of extrahepatic manifestations from 2000 to 2003. We have previously reported on the prevalence of extrahepatic manifestations in HCV infection, including OLP, for inhabitants of the same town (11). We now examined the prevalence of these extrahepatic manifestations from 2000 to 2003.

Results

Anti-HCV were detected in the sera of 224 subjects (100%) and HCV RNA in 210 subjects (93.8%), as shown in Table I. Of the 224, 88 had at least 1 oral examination over the course of the 4 years of the study (34 men and 54 women).

Table I shows the prevalence of OLP in all subjects. In 2000, 2001, 2002 and 2003 it was 8.5 (5/59), 14.8 (8/54), 20 (11/55) and 21.4% (12/56), respectively. The prevalence of OLP in HCV RNA positive subjects in 2000, 2001, 2002 and 2003 was 8.8 (5/57), 16 (8/50), 21.6 (11/51) and 23.1% (12/52), respectively. The prevalence of OLP increased with age. The incidence of OLP among all subjects with HCV infection over the 4-year period was 17.0% (15/88, 2 men and 13 women). A history of smoking was found in 1/15 OLP cases (6.7%) among inhabitants. Of the 15 cases, 2 had medical checkups once a year, 3 had them 3 times a year, 9 had them twice a year and 1 had a checkup just once in the 4-year period from 2000 to 2003 (Table II). No one had visited a clinic for the treatment of their OLP prior to our discovery of their OLP lesions. By far the most common site for OLP was the buccal mucosa. The predominant type in 53.3% of the 15 cases (8/15) was the reticular form of the disease. In 46.7% (7/15) it was the erosive form. Fig. 3 shows the erosive form (inhabitant No. 6 in Table II). Reticular lesions were generally asymptomatic. Two of the 15 cases had aggravated oral symptoms during the 4-year period. None experienced natural healing or developed malignant transformation.

From 2000 to 2003, there was an increase in the prevalence of type 2 DM, thyroid dysfunction, skin disease, renal disease and hypertension (Table I).

Discussion

HCV carriers in Japan are presumed to number 2 million (13). The growing incidence of HCC is expected to reach a plateau by around the year 2015. However, there are many people who are not aware that they are infected, some of whom will advance to liver cirrhosis or HCC (14). The incidence of HCC varies greatly among different regions. Epidemiological studies conducted by the Japanese Ministry of Health, Labour and Welfare showed that the mortality rate associated with HCC was high in several prefectures in Western Japan. Areas with high rates of anti-HCV, such as the Saga prefecture (3.9%), Hiroshima (1.8%), Fukuoka (1.7%) and Kagawa (1.7%), had high death rates for primary liver cancer of 43.1, 39.6, 39.8 and 31.9 per 100,000 people, respectively. These rates were higher than the national average (15).

HCV is associated with a wide range of extrahepatic manifestations. Zignego *et al* classified the extrahepatic manifestations of HCV into 4 main categories (16). The first category (A) includes extrahepatic manifestations characterised by a very strong association to HCV and supported by both epidemiological and pathogenetic evidence. Category A comprises mixed cryoglobulinaemia. The second category (B) includes disorders which are significantly associated with HCV infection, supported by adequate data. Category B comprises B-cell non-Hodgkin's lymphoma, monoclonal gammopathies, porphyria cutanea tarda and lichen planus. The third category (C) includes manifestations whose association with HCV still requires confirmation and/or a more detailed characterisation of similar pathologies of different aetiology or idiopathic nature. Finally, the fourth category (D) includes only anecdotal observations.

Lichen planus is a chronic inflammatory disease of the skin and mucous membranes that frequently involves the oral mucosa. In Japan, the age-adjusted incidence rate of OLP is 59.7 per 100,000 males and 188.0 per 100,000 females (17).

Table I. Prevalence of extrahepatic manifestations in adult inhabitants with HCV infection.

	2000	2001	2002	2003
Subjects	59	54	55	56
Age (mean years ± SD)	70.7±7.2	71.2±7.2	72.0±6.5	73.4±6.8
Sex (M/F)	21/38	22/32	23/32	24/32
% with history of smoking	18.6 (11/59)	11.1 (6/54)	12.7 (7/55)	14.3 (8/56)
% positive for anti-HCV	100 (59/59)	100 (54/54)	100 (55/55)	100 (56/56)
% positive for HCV RNA	96.6 (57/59)	92.6 (50/54)	92.7 (51/55)	92.9 (52/56)
Extrahepatic manifestations				
% positive for oral lichen planus	8.5 (5/59)	14.8 (8/54)	20.0 (11/55)	21.4 (12/56)
Age (mean years ± SD)	74.8±5.2	74.3±5.7	73.1±5.1	74.7±5.8
Sex (M/F)	1/4	2/6	2/9	2/10
% positive for anti-HCV	8.5 (5/59)	14.8 (8/54)	20.0 (11/55)	21.4 (12/56)
% positive for HCV RNA	8.8 (5/57)	16.0 (8/50)	21.6 (11/51)	23.1 (12/52)
% positive for DM	15.3 (9/59)	24.1 (13/54)	20.0 (11/55)	19.6 (11/56)
Age (mean years ± SD)	67.9±7.2	68.8±7.9	69.5±7.9	68.6±7.4
Sex (M/F)	5/4	10/3	8/3	7/4
% positive for anti-HCV	15.3 (9/59)	24.1 (13/54)	20.0 (11/55)	19.6 (11/56)
% positive for HCV RNA	14 (8/57)	20.0 (10/50)	15.7 (8/51)	15.4 (8/52)
% positive for rheumatoid arthritis	1.7 (1/59)	1.9 (1/54)	5.5 (3/55)	5.4 (3/56)
Age (mean years ± SD)	67.9±7.2	70.0±0	70.0±0.8	73.0±2.9
Sex (M/F)	5/4	0/1	1/2	1/2
% positive for Anti-HCV	15.3 (9/59)	1.9 (1/54)	5.5 (3/55)	5.4 (3/56)
% positive for HCV RNA	14 (8/57)	2.0 (1/50)	5.9 (3/51)	5.8 (3/52)
% positive for thyroid dysfunction	0	3.7 (2/54)	3.6 (2/55)	8.9 (5/56)
Age (mean years ± SD)	-	67.0±1.0	68.0±1.0	72.0±3.3
Sex (M/F)	-	1/1	1/1	1/4
% positive for anti-HCV	-	3.7 (2/54)	3.6 (2/55)	8.9 (5/56)
% positive for HCV RNA	-	4.0 (2/50)	3.9 (2/51)	9.6 (5/52)
% positive for skin disease	5.1 (3/59)	11.1 (6/54)	7.3 (4/55)	16.1 (9/56)
Age (mean years ± SD)	70.3±7.3	71.3±5.8	70.8±5.2	74.1±5.9
Sex (M/F)	0/3	1/5	1/3	4/5
% positive for anti-HCV	5.1 (3/59)	11.1 (6/54)	7.3 (4/55)	16.1 (9/56)
% positive for HCV RNA	5.3 (3/57)	12.0 (6/50)	7.8 (4/51)	15.4 (8/52)
% positive for renal disease	1.7 (1/59)	5.6 (3/54)	0	1.8 (1/56)
Age (mean years ± SD)	76.0±0	76.0±2.2	-	86.0±0
Sex (M/F)	1/0	1/2	-	1/0
% positive for anti-HCV	1.7 (1/59)	5.6 (3/54)	-	1.8 (1/56)
% positive for HCV RNA	1.8 (1/57)	6.0 (3/50)	-	1.9 (1/52)
% positive for hypertension	28.8 (17/59)	40.7 (22/54)	43.7 (24/55)	55.4 (31/56)
Age (mean years ± SD)	71.0±6.9	70.9±6.3	72.6±6.0	74.4±6.7
Sex (M/F)	6/11	7/15	10/14	13/18
% positive for anti-HCV	28.8 (17/59)	40.7 (22/54)	43.6 (24/55)	55.4 (31/56)
% positive for HCV RNA	26.3 (15/57)	42.0 (21/50)	41.8 (13/51)	57.7 (30/52)
% positive for extrahepatic malignant tumor	11.9 (7/59)	13 (7/54)	9.1 (5/55)	7.1 (4/56)
Age (mean years ± SD)	74.4±3.5	76.3±3.7	77.2±4.2	79.3±2.2
Sex (M/F)	2/5	3/4	3/2	3/1
% positive for anti-HCV	11.9 (7/59)	13 (7/54)	9.1 (5/55)	7.1 (4/56)
% positive for HCV RNA	12.3 (7/57)	14 (7/50)	9.8 (5/51)	7.7 (4/52)

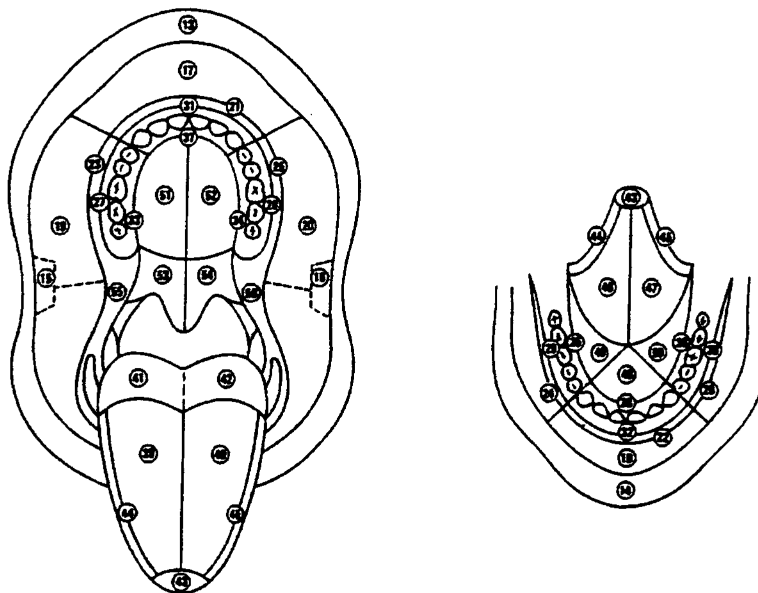

Figure 2. Topography of the oral mucosa modified from Roed-Petersen *et al* (12). The numbered locations are referred to in Table II.

Table II. Site involvement and clinical form of oral lichen planus (OLP) in subjects with HCV infection.

No.	Sex	Type	Smoking history	2000	2001	2002	2003	Course of OLP
1	F	Reticular	Negative	30	30	24, 30	24, 30	No change
2	M	Reticular	Negative	20	20	20	20	No change
3	F	Erosive	Negative	15, 16, 19-21, 23, 25, 27, 28, 31, 33, 34, 37, 39, 40, 46, 47	15, 16, 19-21, 23, 25, 27, 28, 31, 33, 34, 37, 39, 40, 46, 47	Not screened	Liver cirrhosis death	Unknown
4	F	Erosive	Negative	14, 19, 20	Not screened	Not screened	14, 19, 20	Exacerbation
5	F	Erosive	Negative	20, 45	Not screened	20, 45	Not screened	Unknown
6	F	Erosive	Negative	Not screened	19, 20, 47	Not screened	14, 19, 20, 46, 47, 51	Exacerbation
7	F	Reticular	Negative	ND	19, 55	Not screened	19, 55	No change
8	F	Reticular	Negative	Not screened	20, 26, 30	20, 26, 30	20, 26, 30	No change
9	M	Reticular	Negative	ND	46	29, 30, 35, 36, 46	29, 30, 35, 36, 46	No change
10	F	Reticular	Negative	Not screened	26, 30	26, 30	26, 30	No change
11	F	Erosive	Negative	ND	ND	14	14	No change
12	F	Erosive	Negative	Not screened	Not screened	17	17	Alleviation
13	F	Reticular	Negative	ND	Not screened	30	30	No change
14	F	Reticular	Negative	ND	ND	29	29	No change
15	F	Erosive	Positive	Not screened	Not screened	14-16, 19, 20, 24, 26, 32, 44, 45, 51, 52	Not screened	Unknown

ND, not detected. The numbers below the dates refer to locations in the oral mucosa as seen in Fig. 2.

Figure 3. A representative oral erosive lichen planus on the right buccal mucosa.

We conducted an epidemiological investigation to ascertain the possible correlation between OLP and HCV infection in patients living in Western Japan (9-11,18), where the prevalence of HCV infection is the highest in the country (15,19). We found the incidence of OLP in our patients to be higher than in the general population. OLP aside, the prevalence of other extrahepatic manifestations in subjects with anti-HCV was also higher than in those without HCV (10).

We previously reported a study on an HCV hyperendemic area, O town, with a population of approximately 3,900 in the northwest region of the Hiroshima prefecture in Honshu, Japan (11). The incidence there of subjects with 1 or more extrahepatic manifestations of HCV was 66.1%. In the present investigation, we examined extrahepatic manifestations in the same place over a 4-year period. Inhabitants with HCV infection had various extrahepatic manifestations, including OLP. The prevalence of OLP increased with the age of the subjects. This is consistent with an earlier study of inhabitants of the Fukuoka prefecture (20).

Patients with HCV-associated HCC in Japan are aging. People with chronic HCV infection should be monitored and followed carefully for extrahepatic manifestations. It is necessary for physicians and dentists to have an increased awareness of OLP in order for it to be detected at an early stage and treated promptly.

Acknowledgments

As a project for establishing new high technology research centers, this study was supported by a Grant-in-Aid for Scientific Research (C) (No.18592213) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by the Second Award Fukuoka Field of Medicine from the Medical Care Education Research Foundation.

References

1. Gumber SC and Chopra S: Hepatitis C. A multifaceted disease. Ann Intern Med 123: 615-620, 1995.
2. Zignego AL and Brechot C: Extrahepatic manifestations of HCV infection: facts and controversies. J Hepatol 31: 369-376, 1999.
3. Jubert C, Pawlotsky JM, Pouget F, *et al*: Lichen planus and hepatitis C virus-related chronic active hepatitis. Arch Dermatol 130: 73-76, 1994.
4. Pawlotsky JM, Dhumeaux D and Bagot M. Hepatitis C virus in dermatology. A review. Arch Dermatol 131: 1185-1193, 1995.
5. Johnson RJ, Gretch DR, Yamabe H, *et al*: Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. N Engl J Med 328: 465-470, 1993.
6. Huang MJ, Wu SS and Liaw YF: Thyroid abnormalities in patients with chronic viral hepatitis. Hepatology 20: 1651-1652, 1994.
7. Piperno A, D 'Alba R, Roffi L, Pozzi M, Farina A, Vecchi L and Fiorelli G: Hepatitis C virus infection in patients with idiopathic hemochromatosis (IH) and porphyria cutanea tarda (PCT). Arch Virol (Suppl. 4): S215-S216, 1992.
8. Petit JM, Bour JB, Galland-Jos C, *et al*: Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. J Hepatol 35: 279-283, 2001.
9. Nagao Y, Sata M, Fukuizumi K, Tanikawa K and Kameyama T: High incidence of oral precancerous lesions in a hyperendemic area of hepatitis C virus infection. Hepatol Res 8: 173-177, 1997.
10. Nagao Y, Sata M, Fukuizumi K, Ryu F and Ueno T: High incidence of oral lichen planus in an HCV hyperendemic area. Gastroenterology 119: 882-883, 2000.
11. Nagao Y, Tanaka J, Nakanishi T, *et al*: High incidence of extrahepatic manifestations in an HCV hyperendemic area. Hepatol Res 22: 27-36, 2002.
12. Roed-Petersen B and Renstrup: A topographical classification of the oral mucosa suitable for electronic data processing. Its application to 560 leukoplakias. Acta Odontol Scand 27: 681-695, 1969.
13. Higuchi M, Tanaka E and Kiyosawa K: Epidemiology and clinical aspects on hepatitis C. Jpn J Infect Dis 55: 69-77, 2002.
14. Niederau C, Lange S, Heintges T, *et al*: Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 28: 1687-1695, 1998.
15. Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A and Tanaka E: Hepatocellular carcinoma: recent trends in Japan. Gastroenterology 127 (Suppl. 1): S17-S26, 2004.
16. Zignego AL, Ferri C, Pileri SA, Caini P and Bianchi FB for the Italian Association of the Study of Liver (A.I.S.F.) Commission on Extrahepatic Manifestations of HCV Infection: Extrahepatic manifestations of hepatitis C virus infection: a general overview and guidelines for a clinical approach. Dig Liver Dis 39: 2-17, 2007.
17. Nagao T, Ikeda N, Fukano H, Hashimoto S, Shimozato K and Warnakulasuriya S: Incidence rates for oral leukoplakia and lichen planus in a Japanese population. J Oral Pathol Med 34: 532-539, 2005.
18. Nagao Y, Sata M, Tanikawa K, Itoh K and Kameyama T: Lichen planus and hepatitis C virus in the northern Kyushu region of Japan. Eur J Clin Invest 25: 910-914, 1995.
19. Hayashi J, Yoshimura E, Nabeshima A, *et al*: Seroepidemiology of hepatitis C virus infection in hemodialysis patients and the general population in Fukuoka and Okinawa, Japan. J Gastroentol 29: 276-281, 1994.
20. Nagao Y, Kawaguchi T, Tanaka K, Kumashiro R and Sata M: Extrahepatic manifestations and insulin resistance in an HCV hyperendemic area. Int J Mol Med 16: 291-296, 2005.

TRANSFUSION COMPLICATIONS

Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C

Yutaka Komiya, Keiko Katayama, Hisao Yugi, Masaaki Mizui, Harumichi Matsukura, Tetsushi Tomoguri, Yuzo Miyakawa, Ayako Tabuchi, Junko Tanaka, and Hiroshi Yoshizawa

BACKGROUND: In planning optimal hepatitis B virus (HBV) blood screening strategies, the minimum infectious dose and early dynamics of HBV need to be determined for defining the window period for HBV DNA as well as for hepatitis B surface antigen (HBsAg).

STUDY DESIGN AND METHODS: Pairs of chimpanzees were inoculated with preacute-phase inocula containing HBV of genotype A or genotype C to determine the minimum infectious dose, and two pairs of chimps infected with the lowest infectious dose of genotypes A and C were followed for HBV markers.

RESULTS: The minimum 50 percent chimpanzee infectious dose (CID_{50}) was estimated to be approximately 10 copies for genotype A and for genotype C. In the two chimps inoculated with the lowest infectious dose, the HBV DNA window was 55 to 76 days for genotype A and 35 to 50 days for genotype C, respectively. The HBsAg window was 69 to 97 days for genotype A and 50 to 64 days for genotype C, respectively. The doubling times of HBV DNA were 3.4 days (95% confidence interval [CI], 2.6-4.9 days) for genotype A and 1.9 days (95% CI, 1.6-2.3 days) for genotype C. When comparing the replication velocity of HBV DNA between the two genotypes, the doubling time of genotype C was significantly shorter than that of HBV genotype A ($p < 0.01$).

CONCLUSION: Although the CID_{50} of approximately 10 copies was similar for the two HBV genotypes, the doubling time and pre-HBV nucleic acid amplification technology (<100 copies/mL) window period in chimps infected with the lowest infectious dose seemed to be shorter for genotype C than for genotype A.

Posttransfusion infection with hepatitis B virus (HBV) has decreased dramatically since screening for hepatitis B surface antigen (HBsAg) was introduced in the early 1970s. The number of reported posttransfusion hepatitis B cases has been further reduced after screening for antibody to HBV core (anti-HBc) was implemented in the late 1980s in the United States and Japan.[1,2] Japan introduced HBV DNA screening by nucleic acid amplification technology (NAT) in minipools (MPs) in 1999. Since introduction of MP-NAT, more than 500 seronegative donations with detectable HBV DNA have been interdicted, although there are still units of blood in an early or late phase of HBV infection

ABBREVIATIONS: CID_{50} = 50 percent chimpanzee infectious dose; CLIA = chemiluminescent immunoassay; JRC = Japanese Red Cross; MP(s) = minipool(s).

From the Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima; Primate Park, Sanwa Kagaku Kenkyusho, Ltd, Kumamoto; the Department of NAT, Central Blood Institute, Japanese Red Cross Society, Tokyo; the Department of Laboratory Medicine, Japanese Red Cross Hiroshima Blood Center, Hiroshima; the Department of Research and Development of Reagents, Japanese Red Cross Osaka Blood Center, Osaka; and the Miyakawa Memorial Research Foundation, Tokyo, Japan.

Address reprint requests to: Hiroshi Yoshizawa, MD, Department of Epidemiology, Infectious Disease Control and Prevention, Graduate School of Biomedical Sciences, Hiroshima University, Kasumi 1-2-3, Minami-Ku, Hiroshima 734-8551, Japan; e-mail: eidcp@hiroshima-u.ac.jp.

This work was supported in part by grants from the Japanese Ministry of Health, Labor and Welfare.

Received for publication May 7, 2007; revision received July 30, 2007, and accepted August 2, 2007.

doi: 10.1111/j.1537-2995.2007.01522.x

TRANSFUSION 2008;48:286-294.

with low viral load that can escape detection by NAT.[3] Interestingly, the lookback program of the Japanese Red Cross (JRC) demonstrated that low viral load donations in the window phase were more than 10-fold more often implicated in HBV transmission reports than were occult carriers with anti-HBc titers below the exclusion limit of the anti-HBc hemagglutination inhibition screening assay.[3] Bearing in mind the relatively high infectivity of HBV in the window phase, exact knowledge on early dynamics of HBV replication is important for residual risk estimations.[4-7] It will determine the threshold of NAT in identifying blood donors during the preacute phase of HBV infection, which is important for planning and executing evidence-based hepatitis B blood screening strategies.[7,8] In this context, the relative infectivity of HBV in the early window phase is an important factor for measuring the effect of NAT screening systems on the residual risk of HBV transmission by blood transfusion.[7-9]

Chimpanzees are the only experimental animals susceptible to human hepatitis viruses and have been very useful in transmission studies.[10] As early as the mid-1970s, it was demonstrated that blood units from HBV carriers, especially those positive for the presence of hepatitis B e antigen (HBeAg), have a tremendously high infectious potential and can transmit infection to chimps by intravenous inoculation with 1 mL of plasma diluted to $1:10^8$.[11] Now that NAT enables detection of HBV DNA in blood donors even in individual-donation format, it can be estimated by mathematical modeling what the residual risk would be depending on the minimum copy number required for infection.[9] More conservative risk modeling assumes that a single copy of HBV, if it successfully reaches a hepatocyte in susceptible hosts, may be enough to establish infection.[5] To pursue strategies for preventing HBV infections by blood transfusions, additional information on the infectivity of HBV is crucially required. It is imperative to define not only the minimum copy number of HBV DNA or number of virions required for transmission of HBV, but also the early dynamics of HBV replication in the circulation of infected hosts. This can be established more accurately in chimpanzee experiments. In this report on experimental transmission of hepatitis B in chimps, the minimum infectious dose was determined separately for HBV of genotypes A and C, and the copy number of HBV DNA for establishing infection was defined for each of them. Moreover, the doubling time and logarithmic time of HBV DNA were determined by following the viral dynamics in the preacute phase of chimpanzees who had received the minimum infectious dose of HBV.

MATERIALS AND METHODS

Chimpanzees

Six chimps entered this study. Their age, sex, and weight, as well as the HBV inocula that they received are listed in Table 1 along with the infection outcome. Every chimp was kept in an individual cage and received humane care in accordance with all relevant requirements for the use of primates in an approved institution. None of the six chimps had serologic or molecular biologic evidence of past or present HBV infection prior to the inoculation. They were also not infected with hepatitis C virus (HCV) and human immunodeficiency virus type-1. Chimps were inoculated intravenously while they were under anesthesia by intramuscular injection with ketamine hydrochloride. After the inoculation, serum samples were obtained once a week or more frequently as required, until 16 weeks or longer. They were tested for HBV DNA, HBsAg, anti-HBc, anti-HBs, and alanine aminotransferase as well as aspartate aminotransferase.

Inocula containing HBV

The chimpanzees received four kinds of inocula (Table 1). Inocula I and III were fresh-frozen plasma (FFP) units from blood donors acutely infected with HBV genotypes A and C, respectively. Inocula II and IV were plasma samples from chimps infected with inoculum I of genotype A and inoculum III of genotype C, respectively (see Figs. 1A and 1B). Inocula II and IV were: 1) recovered in the preacute phase of HBV infection before immune responses of the host had developed; 2) positive for the presence of HBV DNA in the highest titer before anti-HBc developed; and 3) taken with utmost care to maintain the infectious activity and avoid attenuation during serial processing from blood collection until storage. Immediately after blood drawing

TABLE 1. Six chimpanzees and HBV inocula and HBV infection outcomes

Chimpanzee	Age, sex, weight	HBV DNA copies	Outcome
Inoculum I: FFP from a human donor in the preacute phase of HBV infection of genotype A			
1 Chimp 246	13 years, male, 60.7 kg	1 mL (6.9×10^4 copies/mL)	Infected
Inoculum II: Preacute-phase plasma of Chimp 246 containing HBV (2.6×10^6 copies/mL)			
2 Chimp 272	9 years, male, 58.7 kg	1 mL ($1:10^6$ dilution)	Not infected
3 Chimp 279	8 years, male, 51.4 kg	1 mL ($1:10^6$ dilution)	Not infected
3 Chimp 279	Reinoculation	1 mL ($1:10^5$ dilution)	Infected
4 Chimp 280	8 years, male, 39.4 kg	1 mL ($1:10^5$ dilution)	Infected
Inoculum III: FFP from a human donor in the preacute phase of HBV infection of genotype C			
2 Chimp 272	Reinoculation	5 mL (5.3×10^5 copies/mL)	Infected
Inoculum IV: Preacute-phase plasma of Chimp-272 containing HBV (3.0×10^6 copies/mL)			
5 Chimp 269	11 years, male, 62.5 kg	1 mL ($1:10^6$ dilution)	Not infected
6 Chimp 285	7 years, male, 41.1 kg	1 mL ($1:10^6$ dilution)	Not infected
5 Chimp 269	Reinoculation	1 mL ($1:10^5$ dilution)	Infected
6 Chimp 285	Reinoculation	1 mL ($1:10^5$ dilution)	Infected

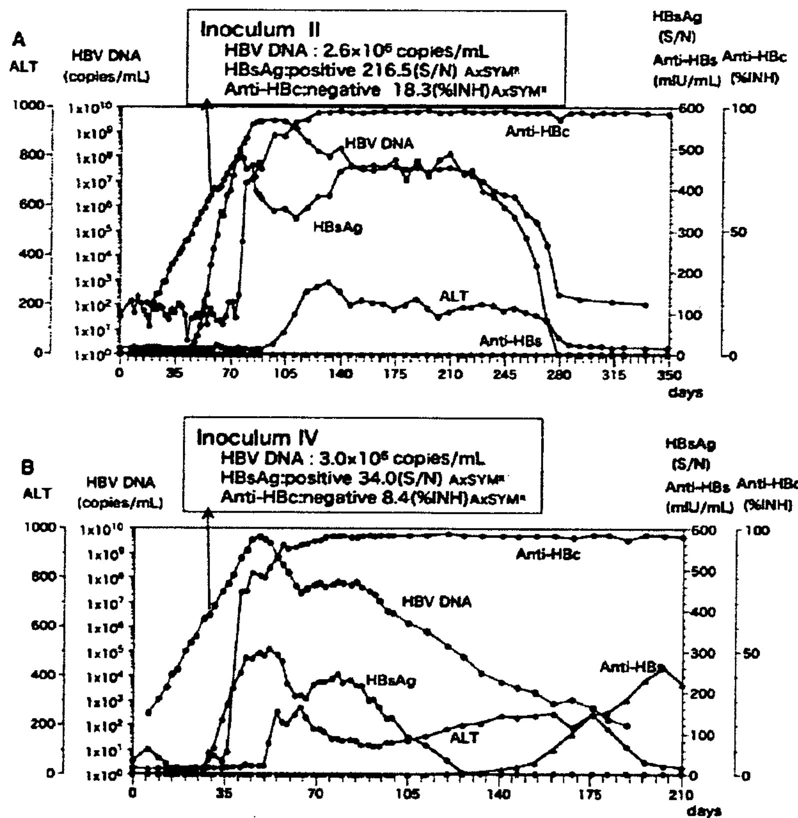

Fig. 1. Time course of HBV serum markers in chimps used as a source of inoculation of HBV genotype A and genotype C. (A) Chimp 246 was inoculated with human plasma of HBV genotype A. Inoculum II for chimp infectivity studies was harvested in the ramp-up phase of viremia before anti-HBc seroconversion at the time HBV DNA had reached a concentration of 2.6 × 10⁶ copies per mL and the HBsAg response had increased to a signal-to-noise (S/N) ratio of 216.5 (cutoff S/N = 2.0). (B) Chimp 272 was inoculated with human plasma of HBV genotype C. Inoculum IV was harvested in the ramp-up phase of viremia before anti-HBc seroconversion at the time HBV DNA had reached a concentration of 3.0 × 10⁶ copies per mL and HBsAg had increased to an S/N ratio of 34.0.

from chimps, plasma samples were separated. They were divided into 15 tubes in 1-mL aliquots, snap-frozen in liquid nitrogen, and kept in a deep freezer at −80°C until used for transmission experiments. For each experiment, the plasma in one tube was thawed gently by immersing it in a water bath at 37°C, and the required amounts were used.

Laboratory tests

HBsAg, anti-HBc, and antibody to HBsAg (anti-HBs) were determined by chemiluminescent immunoassay (CLIA) with commercially available kits (AxSYM, Abbott Japan, KK, Tokyo, Japan), with the index of 2.0 (signal-to-noise [S/N]), 50 percent inhibition, and 5.0 mIU per mL as cutoff values, respectively. Qualitative assay for HBV DNA was performed by polymerase chain reaction (PCR) with primers deduced from the S region of HBV DNA.[12] HBV DNA was quantitated by the PCR (TaqMan, Roche Diagnostics KK, Tokyo, Japan) with a sensitivity of 100 copies per mL. Quantitative assays for HBV DNA were performed simultaneously for an accurate comparison of data.

Calculation for doubling time and logarithmic time of HBV DNA

To calculate the doubling time and the logarithmic time (time for reaching 10 times the amount) of HBV DNA at ramp-up after the infection, HBV DNA copy numbers were evaluated statistically by log linear regression analysis. The comparison of regression slope (the doubling time and the logarithmic time) between HBV genotypes was evaluated by growth curve analysis (Vonesh-Carter-Ohtaki method).[13,14]

RESULTS

Inocula and copy numbers of HBV genotype A or genotype C recovered from chimpanzees in the preacute phase of infection

Chimp 246 was injected intravenously with 1 mL of FFP from a blood donor in the preacute phase of HBV infection (Table 1); the donor had been screened by NAT at a JRC Blood Center. His plasma sample contained 6.9×10^4 copies per mL of HBV DNA genotype A and was positive for the presence of HBsAg but negative for the presence of anti-HBc (inoculum I). Plasma was harvested from Chimp 246, in the preacute phase of HBV infection 57 days after inoculation (inoculum II). It contained 2.6×10^6 copies per mL HBV DNA and was positive for the presence of HBsAg but still negative for the presence of anti-HBc and anti-HBs (Fig. 1A).

Likewise, Chimp 272 was injected intravenously with 5 mL of FFP from a blood donor in the preacute phase of HBV infection who had been screened by NAT at JRC (Table 1). It contained 5.3×10^5 copies per mL of HBV DNA genotype C and was positive for the presence of HBsAg but negative for the presence of anti-HBc and anti-HBs (inoculum III). Thus, Chimp 272 was inoculated with

2.7×10^6 copies of HBV genotype C. The preacute plasma sample was collected from Chimp 272 29 days after challenge (inoculum IV). It contained 3.0×10^6 copies per mL HBV DNA and was positive for the presence of HBsAg but still negative for the presence of anti-HBc and anti-HBs (Fig. 1B).

Estimates of HBV DNA copy numbers in serial 1-in-10-fold dilutions and inocula below the HBV NAT detection limit

Serial 1-in-10 dilutions of inoculum II of genotype A were prepared in preinoculation serum sample from each chimp (e.g., Chimp 272, Chimp 279, and Chimp 280, respectively). Dilutions were delivered to three tubes each in 1-mL aliquots and snap-frozen in liquid nitrogen. Concentration of HBV DNA was determined in one of the three tubes in each dilution so as to guarantee copy numbers of HBV DNA in the other two vials that were inoculated into chimps. These samples had been stored in a deep freezer at –80°C until inoculation.

Table 2 shows the measured HBV DNA concentrations in 1-in-10 dilutions of inoculum II (genotype A). The quantitative HBV DNA results starting from 2.6×10^6 copies per mL in the undiluted sample varied between 2.0×10^5 to 2.3×10^5, 2.0×10^4 to 2.4×10^4, 1.6×10^3 to 2.0×10^3, and 1.7×10^2 to 2.8×10^2 copies per mL, respectively, in the 1:10, 1:10^2, 1:10^3, and 1:10^4 dilutions. These quantitative results are an indication of the accuracy of the dilution and assay procedure. On the premise that dilutions beyond 1:10^4 had been performed properly, further dilutions to 1:10^5 and 1:10^6 would have contained 16 to 28 and 1.6 to 2.8 HBV DNA copies per mL (ranges estimated by variations of HBV DNA measurements in lower dilutions), respectively, although they were below the detection limit of the PCR method used.

Likewise, serial 1-in-10 dilutions of inoculum IV (genotype C) were prepared in the plasma sample from Chimp 269 and Chimp 285. HBV DNA in 3.0×10^6, 3.5×10^5 to 3.8×10^5, 3.6×10^4 to 3.9×10^4, 3.6×10^3 to 4.6×10^3, and 4.3×10^2 to 4.6×10^2 copies per mL were detected in the original serum samples at 1:10, 1:10^2, 1:10^3, and 1:10^4 dilutions thereof, respectively (Table 3). Thus, further experiments were performed on the assumption that serial dilutions of 1:10^5 and 1:10^6 of inoculum IV would have contained 35 to 46 and 3.5 to 4.6 HBV DNA copies per mL, respectively.

Determination of the minimum copy number required for transmission of HBV genotype A or genotype C to chimpanzees

When Chimp 272 and Chimp 279 were inoculated intravenously with 1.0 mL of inoculum II diluted 1:10^6 (equivalent to 1.6 to 2.8 copies of HBV DNA in an in vitro assay), HBV infection did not develop in either of them during monitoring for 119 days (17 weeks) and thereafter. Chimp 279 was then rechallenged with 1.0 mL of inoculum II diluted 1:10^5 (equivalent to 16-28 copies). He then became infected and developed HBV DNA in his serum 55 days (8 weeks) after the inoculation. Chimp 280 was also inoculated intravenously with 1.0 mL of inoculum II diluted 1:10^5 (equivalent to 16 to 28 copies of HBV DNA). He developed HBV DNA in the circulation 76 days (11 weeks) after infection. In view of the incubation period of 55 to 76 days (8-11 weeks) for 1:10^5 dilution of inoculum II, HBV infection would probably not have occurred in chimps who received 1:10^6 dilution if they had been followed longer than 119 days (17 weeks).

Chimp 269 and Chimp 285 were inoculated with 1.0 mL of inoculum IV diluted 1:10^6 (equivalent to 3.5-4.6 copies of HBV DNA in an in vitro assay). During follow-up

TABLE 2. Quantification of HBV DNA in serial 1-in-10 dilutions of the standard serum for HBV genotype A (inoculum II)*

		Serial dilutions in preinoculation serum samples of each chimpanzee					
Chimpanzee	Undiluted	1:10	1:10^2	1:10^3	1:10^4	1:10^5	1:10^6
272	2.6×10^6	2.3×10^5	2.0×10^4	2.0×10^3	1.7×10^2	Not done	<100
279	2.6×10^6	2.0×10^5	2.4×10^4	2.0×10^3	2.4×10^2	<100	<100
280	2.6×10^6	2.3×10^5	2.3×10^4	1.6×10^3	2.8×10^2	<100	Not done

* Data are reported as copies per mL.

TABLE 3. Quantification of HBV DNA in serial 1-in-10 dilutions of the standard serum for HBV genotype C (inoculum IV)*

		Serial dilutions in preinoculation serum of each chimpanzee					
Chimpanzees	Undiluted	1:10	1:10^2	1:10^3	1:10^4	1:10^5	1:10^6
Chimp 269	3.0×10^6	3.8×10^5	3.9×10^4	3.6×10^3	4.6×10^2	<100	<100
Chimp 285	3.0×10^6	3.5×10^5	3.6×10^4	4.6×10^3	4.3×10^2	<100	<100

* Data are reported as copies per mL.

for 112 days (16 weeks), however, no HBV infection occurred in either of them. Subsequently, they were rechallenged with 1.0 mL of inoculum IV diluted 1:10^5 (equivalent to 35-46 copies of HBV DNA) 17 weeks after the initial inoculation. They developed HBV DNA in the circulation 35 and 50 days thereafter, respectively, indicating that both of them were infected. Therefore, the 50 percent chimp infectious dose (CID_{50}) for both genotype A and genotype C lies between the lowest infectious dose of approximately 30 copies and the subinfectious dose of approximately 3 copies or at approximately 10 HBV DNA copies.

HBV infection resolved in all six chimps and they never became carriers. Within a few weeks after the peak HBV DNA titer was reached, serum levels of transaminase increased slightly, within 3 times the upper limit of normal.

Replication velocity of HBV DNA in the preacute phase of infection

Doubling time and logarithmic time of HBV genotype A

Figure 2A illustrates the appearance of HBV genotype A in the circulation, when HBV DNA reached more than 10^2 copies per mL, as well as its early dynamics in Chimp 246, Chimp 279, and Chimp 280 during the preacute phase of exponential replication. HBV DNA emerged in the circulation earlier in Chimp 246 than the other two chimps, but

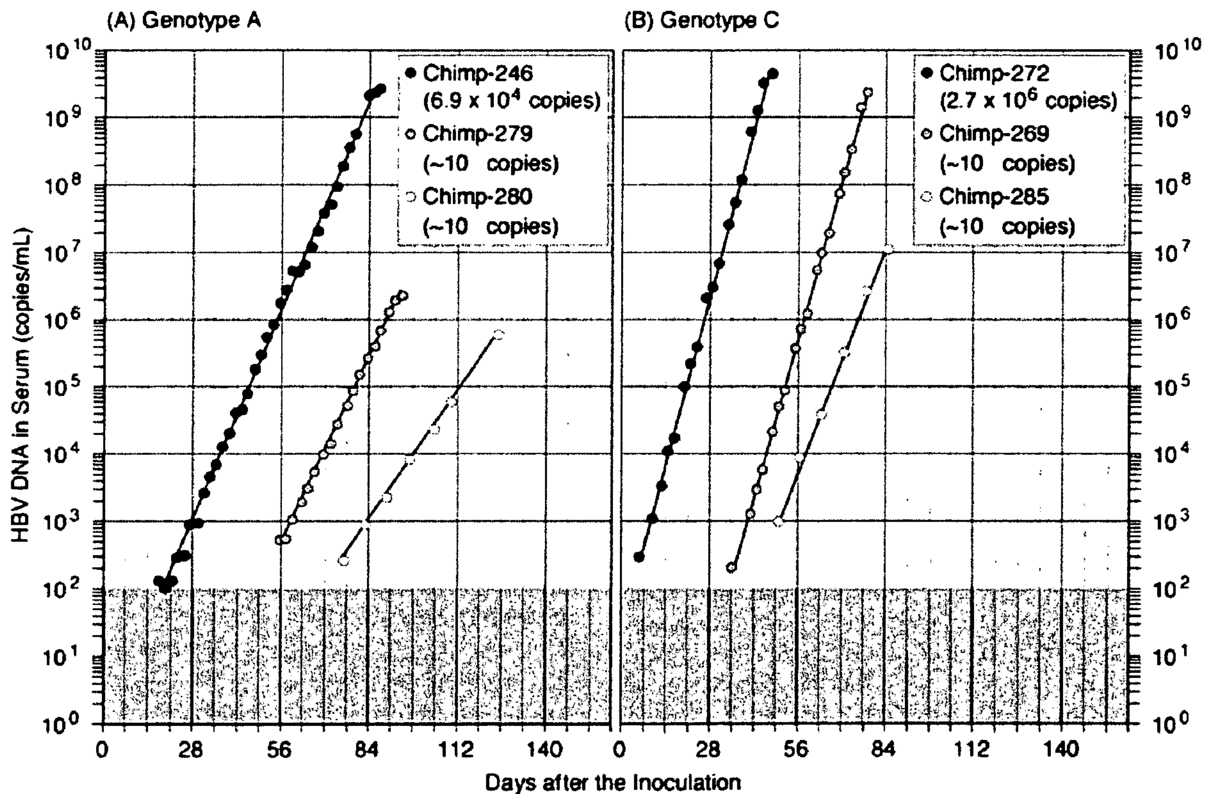

Fig. 2. Log-linear increase of HBV DNA in the circulation of chimpanzees during the early exponential replication phase. (A) Dynamics in the early ramp-up phase of viral DNA for three chimps inoculated with HBV genotype A: one chimp (Chimp 246) received 1 mL of human plasma containing 6.9 x 10^4 copies and the other two chimps (Chimps 279 and 280) received 1 mL of a 100,000 dilution of chimp plasma taken in the HBsAg ramp-up phase just before appearance of anti-HBc, which dilution contains a measured amount of 16 to 28 copies. (B) Graph summarizes the viral load dynamics for three chimpanzees inoculated with HBV genotype C: one chimp (Chimp 272) received 5 mL of human plasma with 2.7 x 10^6 copies of HBV DNA and the two other chimps (Chimps 269 and 285) received a measured amount of 35 to 46 copies (1:100,000 dilution) of preacute-phase chimpanzee plasma. Shaded areas are below the detection limit of NAT (<100 copies/mL). Only the phase of exponential replication is shown, and HBV DNA decreased after it reached peak values of 5.7 x 10^5 to 2.8 x 10^9 copies per mL in three chimps inoculated with HBV genotype A and 1.1 x 10^7 to 4.6 x 10^9 copies per mL in three chimps inoculated with HBV genotype C.

this animal had received more than a 1000-fold larger amount of copies of HBV than the other two chimps. Despite the 1000-fold higher infectious dose, the log-linear increase of HBV DNA in Chimp 246 was the same as in Chimp 279, who had received the minimum infectious dose. In Chimp 246, HBV DNA replicated exponentially from 21 to 97 days (3-13 weeks) until it peaked and then declined. Even though the same minimum infectious dose of HBV was inoculated, Chimp 279 developed detectable HBV DNA about 21 days (3 weeks) earlier than Chimp 280, in whom HBV replicated slightly slower. Despite differences in HBV doses and individual variation, the replication velocity was constant for HBV genotype A in the preacute phase of infection, before innate immune responses of the host developed, while the virus replicated at an exponential rate. The doubling time and the logarithmic time, in the early exponential viral replication phase, were calculated to be 2.7 to 4.4 and 9.0 to 14.7 days, respectively (see Table 4).

TABLE 4. Estimated doubling times and logarithmic times for HBV genotypes A and C with log-linear and growth-curve analysis

Genotype	Doubling time (days)	Logarithmic time (days)	$y = a \times \exp(b \times x)$ a	b	R^2
Genotype A					
Chimp 246	2.71	9.01	0.8491	0.2556	0.997
Chimp 279	3.05	10.14	0.0015	0.2271	0.998
Chimp 280	4.43	14.73	0.0022	0.1563	0.999
Genotype C					
Chimp 272	1.68	5.58	0.2074	0.413	0.998
Chimp 269	1.79	5.96	0.0002	0.3863	0.999
Chimp 285	2.5	8.31	0.0009	0.2771	0.997

TABLE 5. Comparing the replication velocity of HBV DNA between the two genotypes estimated by a growth curve

Genotype	Doubling time (95% CI), days	Logarithmic time (95% CI), days	$y = a \times \exp(b \times x)$ a*	b (95% CI)	p Value
A	3.44 (2.64-4.89)	11.42 (8.80-16.26)	2.299	0.2017 (0.14-0.26)	<0.01
C	1.9 (1.63-2.27)	6.3 (5.41-7.54)	2.299	0.3654 (0.31-0.43)	

* To evaluate the difference of "b" (that is, slope) between the two genotypes, the growth curve model is assuming that "a" is identical.[13]

Doubling time and logarithmic time of HBV genotype C

The replication velocity in the preacute phase of infection in chimpanzees inoculated with genotype C inocula was faster than in the chimps infected with HBV of genotype A (Fig. 2B). Again, slight variation in log-linear increase of HBV DNA was found, and HBV DNA appeared in serum earlier in Chimp 272 who was inoculated with a 100,000-fold higher infectious dose than was administered to Chimps 269 and 285. As seen in the chimps inoculated with HBV genotype A, HBV genotype C increased in a log-linear fashion in the absence of host immune responses. Doubling times of HBV DNA in the circulation of Chimp 272, Chimp 269, and Chimp 285 were calculated to be 1.7 to 2.5 days and logarithmic times were 5.6 to 8.3 days as determined with the regression formula shown in Table 4.

When comparing the replication velocity of HBV DNA between the two genotypes estimated by a growth curve, the difference was significant ($p < 0.01$, Table 5). That is, the doubling time of replications of HBV DNA with genotype A was estimated to be 3.44 days (95% confidence interval [CI], 2.64-4.89 days) and the logarithmic time was estimated to be 11.42 days (95% CI, 8.80-16.26 days). By contrast, those with HBV genotype C were estimated to be 1.90 days (95% CI, 1.63-2.27 days) and 6.30 days (95% CI, 5.41-7.54 days), respectively.

TABLE 6. Window periods before HBV DNA and HBsAg developed in the circulation of chimpanzees inoculated with the minimum infectious dose of genotype A or genotype C

HBV inoculated	Chimp infected	Markers of HBV infection: HBV DNA (days)	HBsAg (days)
Genotype A	279	55	69
	280	76	97
Genotype C	269	35	50
	285	50	64

Window periods of HBV DNA and HBsAg in chimpanzees inoculated with the minimum infectious dose of HBV

After inoculation, the time before HBV DNA becomes detectable in the circulation by the single-sample NAT (with a sensitivity of 10^2 copies/mL) and the time before HBsAg was detected by CLIA after inoculation are listed in Table 6. The HBV DNA (<100 copies/mL) NAT window was 55 and 76 days, respectively, in Chimp 279 and Chimp 280 inoculated with the lowest infectious dose of HBV genotype A (approx. 30 copies). These NAT window periods were longer than the 35 and 50 days, respectively, found in Chimp 269 and Chimp 285 inoculated with the lowest infectious amounts of HBV genotype C (approx. 30 copies). Likewise, the HBsAg window was longer in Chimp 279 and Chimp 280 infected with genotype A than in Chimp 269 and Chimp 285 infected with genotype C (69 and 97 days, respectively, vs. 50 and 64 days, respectively).

DISCUSSION

Animal models sensitive to human hepatitis viruses offer robust advantages in obtaining basic data of viral infectivity.[10] By experimental infection of chimps with HCV, we have been able to determine the minimum infectious dose of HCV required for establishing infection.[15,16] The doubling time of HCV was determined to be 6.3 to 8.6 hours in two chimps inoculated with the minimum infectious dose of approximately 10 copies of HCV RNA. During the first 5 days after inoculation, HCV RNA did not increase above the NAT detection limit of 10^2 copies per mL in the circulation.[16] It would not be possible to detect HCV infection during the initial few days after exposure, even if 1-mL samples were used for individual NAT.

In this study, we have determined the minimum infectious dose for two standardized inocula containing defined copy numbers of HBV DNA. They were plasma passages of HBV in chimps harvested during the preacute phase of infection and had been processed with the utmost care for maintaining infectious activity. The minimum infectious dose of HBV or the dose where 50 percent of the chimps would be infected lies between 1-in-1 million and 1-in-100,000 dilution of the original inocula and is estimated to be of the order of 10 copies, as was the case for HCV.[15] On the basis of HBV DNA concentrations measured in serial dilutions of inocula (Tables 2 and 3), the minimum infectious dose can be determined to be 16 to 28 copies for HBV genotype A and 35 to 46 copies for HBV genotype C.

There are two definitions of the minimum infectious dose of HBV. Theoretically, it is a single copy of HBV. Not all HBV virions entering the circulation of recipients, however, will succeed in reaching hepatocytes, because some of them are phagocytized by circulating macrophages and Kupffer cells in the sinusoids of the liver. In a mathematical window-phase risk model, Weusten and colleagues[9] have proposed a minimum infectious dose approximately 10 copies of HBV, on the basis of the CID_{50}.[17-19] Recently the inocula derived from chronic HBV carriers used in older chimpanzee studies[17,18] were requantified by Hsia and coworkers[20] with real-time TaqMan PCR. The estimated HBV copy number per CID_{50} (geq) was 169 for genotype A *adw*, 78 for genotype D *ayw*, and 3 for genotype C *adr*, calculated by mathematical division, respectively. These viral load data, performed on cryopreserved aliquots from an inocula derived from a chronic HBV carrier (i.e., HBsAg- and anti-HBc–positive), were derived retrospectively several decades after the chimp titration studies. These results are different from the results obtained in our study, where the inocula was derived from the early ramp-up phase of viremia (HBsAg is positive but anti-HBc is negative) and the chimp titration and viral load analyses were performed prospectively. Hence, the minimum infectious dose defined as a single copy, proposed on a theoretical basis, would deserve revisiting in practical HBV infections. The window period of HBV infection changes with the size of the inoculum. The more copies of HBV inoculated therefore the shorter the incubation period in experimental transmission studies in chimps.[11] An inverse correlation is reported, also, between time before HBsAg appears in serum and the HBV dose in human beings.[21] In accordance with these reports, we also found that the NAT window was shorter in chimps receiving larger sizes of inocula both for genotypes A and C (Fig. 2). The NAT (<100 copies/mL) window period was approximately 1 week with an inoculum of 2.7×10^6 copies of genotype C, approximately 3 weeks with 6.9×10^4 copies of genotype A, 5 to 7 weeks when inoculating 35 to 46 copies of genotype C, and 8 to 11 weeks when inoculating 16 to 28 copies of HBV genotype A, while no infection was observed during 16 to 17 weeks of observation with an inocula of approximately 3 copies of genotype A or B. Theoretically, HBV infection might have become detectable after 17 weeks, but this is unlikely when extrapolating the data above. Inoculation with HBV in large amounts, as happens with transfusion with HBsAg-positive blood units, has been largely excluded since introduction of HBsAg testing in 1972. Barker and Murray[21] have shown that inoculation of lower infectious doses of HBV in the range of 10^4 to 10^7 diluted icteric plasma no longer caused clinical hepatitis in healthy individuals, while infection still occurred with up to a 10^7 diluted inoculum, as detected by an HBsAg complement fixation test. Our study showed that HBV DNA levels increase 6.5×10^3 to 2.2×10^5 copies per mL at the time of the first HBsAg-reactive sample in six chimpanzees in whom blood samples were taken at intervals of 2 to 7 days. These amounts are enough to cause clinical hepatitis B.[21] Indeed, Satake and coworkers[3] found that transmission of 5,000 to 50,000 copies of HBV by blood components with a low viral load in the pre-MP-NAT window phase could cause clinical hepatitis B. Transfusion-transmitted HBV after introduction of individual-donation or small-pool NAT (<10) is still possible, but would involve relatively low infectious doses of HBV of approximately 10 to 100 CID_{50}.

In the chimps inoculated with approximately 30 copies of HBV, the NAT window was determined by individual-donation NAT having a sensitivity of 10^2 copies per mL, while the HBsAg window was established by CLIA with the highest sensitivity presently available.[5,12] The NAT window was 55 to 76 days and HBsAg window was 69 to 97 days, respectively, in Chimp 279 and Chimp 280 who had been inoculated with approximately 30 copies of HBV genotype A. In contrast, the NAT window was 35 to 50 days and the HBsAg window was 50 to 64 days, respectively, for Chimp 269 and Chimp 285 inoculated with approximately 30 copies of HBV genotype C. Thus, neither

the NAT nor the HBsAg window phases overlapped between minimum-dose infections of HBV genotypes A and C; they were longer for genotype A than genotype C. It may be that the NAT window is longer for genotype A, prevalent in Western countries, than genotype C common in Japan. It cannot be excluded, however, that the results observed in our inoculation studies with a limited number of chimpanzees were influenced by the host rather than the genotype of the virus. The duration of the NAT and HBsAg windows are influenced at least by three factors: 1) the infectious dose, 2) individual variation among recipients, and 3) distinct HBV genotypes.

We found the replication velocity of HBV in the preacute phase of infection remarkably different between genotypes A and C. From three chimps infected with HBV genotype A, the doubling time was estimated to be 3.44 days (95% CI, 2.64-4.89 days) and the logarithmic time 11.42 days (95% CI, 8.80-16.26 days). From three chimps infected with HBV genotype C, the doubling time was estimated to be 1.90 days (95% CI, 1.63-2.27 days), and the logarithmic time 6.30 days (95% CI, 5.41-7.54 days). Also in chimeric mice with the liver replaced by human hepatocytes, genotype A was found to replicate much slower than genotype C in the initial weeks of HBV infection.[22]

The replication velocity of HBV in the circulation, indicated by the viral doubling time, is an important factor when calculating the window-period reduction provided by NAT screening systems. Biswas and colleagues[5] calculated a doubling time of 2.56 days (95% CI, 2.24-2.97 days) based on a seroconversion panel of 23 HBV infections. Yoshikawa et al.[4] followed 93 donors in preacute phase HBV infections who had been identified by the routine NAT screening program on 50-MPs at JRC Blood Centers. They estimated a median doubling time of HBV at 2.6 days (range: 1.3-15.2).

Kleinman and Busch[7] have assessed the HBsAg window period based on the HBV doubling time of 2.56 days documented by Biswas and colleagues.[5] They estimated an HBsAg window at 38.3 days (95% CI, 33.0-43.7 days) by the CLIA HBsAg seroconversion point at a concentration of 1650 copies per mL, while Minegishi and coworkers[12] determined the HBsAg seroconversion point at 2100 copies per mL. We found the HBsAg seroconversion with AxSYM occurred when the HBV DNA concentration reached a level of 6.5×10^3 to 2.2×10^5 in six chimpanzees. The differences in HBV levels at HBsAg seroconversion in CLIA may be related to the genotype, but also could reflect differences in the calibration of HBV quantitative assays in genome copies.

It is not known if the chimpanzee model is as susceptible for HBV infection as human beings. As a result, the minimum dose of HBV for transmitting infection to man is, in fact, not precisely known. Nevertheless, a minimum human infectious dose of approximately 10 HBV DNA copies, as indicated by our chimpanzee infectivity experiments, seems a reasonable assumption for modeling the HBV transmission risk in the pre-HBV-NAT window period.

ACKNOWLEDGMENTS

We thank members of two Japanese Red Cross Blood Centers for providing us with plasma samples in the HBsAg window and staff of Primate Park, Sanwa Kagaku Kenkyusho, in Kumamoto for caring for the chimps used in this study. We thank Dr Nico Lelie for his assistance in preparing the manuscript and thank Prof. Megu Ohtaki for his advice in analyzing the statistical difference of slopes in two genotypes. This work has been conducted as a part of viral hepatitis research by the taskforce under the auspices of the Ministry of Health, Labor and Welfare of Japan.

REFERENCES

1. Japanese Red Cross Non-A, Non-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. Lancet 1991;338:1040-1.
2. Kojima M, Shimizu M, Tsuchimochi T, Koyasu M, Tanaka S, Iizuka H, Tanaka T, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Posttransfusion fulminant hepatitis B associated with precore-defective HBV mutants. Vox Sang 1991; 60:34-9.
3. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low HBV-DNA levels identified in a look back program. Transfusion 2007;47:1197-205.
4. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.
5. Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW, Peddada L, Smith R, Schreiber GB, Epstein JS, Nemo GJ, Busch MP. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
6. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH; NHLBI-REDS NAT Study Group. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45: 254-64.
7. Kleinman SH, Busch MP. Assessing the impact of HBV NAT on window period reduction and residual risk. J Clin Virol 2006;36 Suppl 1:S23-9.
8. Yugi H, Mizui M, Tanaka J, Yoshizawa H. Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing—Japanese experience. J Clin Virol 2006;36 Suppl 1:S56-64.

9. Weusten JJ, van Drimmelen HA, Lelie PN. Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. Transfusion 2002;42:537-48.
10. Prince AM, Brotman B. Perspectives on hepatitis B studies with chimpanzees. ILAR J 2001;42:85-8.
11. Shikata T, Karasawa T, Abe K, Uzawa T, Suzuki H, Oda T, Imai M, Mayumi M, Moritsugu Y. Hepatitis B e antigen and infectivity of hepatitis B virus. J Infect Dis 1977;136: 571-6.
12. Minegishi K, Yoshikawa A, Kishimoto S, Yugi H, Yokoya N, Sakurada M, Kiyokawa H, Nishioka K. Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. Vox Sang 2003;84: 287-91.
13. Ohtaki M, Satoh K, Kanda T, Fujikoshi Y. Local ridge estimate using random coefficient curve models for analyzing repeated measurements. J Jpn Stat Soc 2007;36:177-84.
14. Vonesh EF, Carter RL. Efficient inference for random-coefficient growth curve models with unbalanced data. Biometrics 1987;80:642-50.
15. Katayama K, Kumagai J, Komiya Y, Mizui M, Yugi H, Kishimoto S, Yamanaka R, Tamatsukuri S, Tomoguri T, Miyakawa Y, Tanaka J, Yoshizawa H. Titration of hepatitis C virus in chimpanzees for determining the copy number required for transmission. Intervirology 2004;47:57-64.
16. Tanaka J, Katayama K, Kumagai J, Komiya Y, Yugi H, Kishimoto S, Mizui M, Tomoguri T, Miyakawa Y, Yoshizawa H. Early dynamics of hepatitis C virus in the circulation of chimpanzees with experimental infection. Intervirology 2005;48:120-3.
17. Berninger M, Hammer M, Hoyer B, Gerin JL. An assay for the detection of the DNA genome of hepatitis B virus in serum. J Med Virol 1982;9:57-68.
18. Ulrich PP, Bhat RA, Seto B, Mack D, Sninski. J, Vyas GN. Enzymatic amplification of hepatitis B virus DNA in serum compared with infectivity testing in chimpanzees. J Infect Dis 1989;160:37-43.
19. Prince AM, Stephan W, Brotman B. Beta-propiolactone/ ultraviolet irradiation: a review of its effectiveness for inactivation of viruses in blood derivatives. Rev Infect Dis 1983; 5:92-107.
20. Hsia CC, Purcell RH, Farshid M, Lachenbruch PA, Yu MY. Quantification of hepatitis B virus genomes and infectivity in human serum samples. Transfusion 2006;46:1829-35.
21. Barker LF, Murray R. Relationship of virus dose to incubation time of clinical hepatitis and time of appearance of hepatitis-associated antigen. Am J Med Sci 1972;263:27-33.
22. Sugiyama M, Tanaka Y, Kato T, Orito E, Ito K, Acharya SK, Gish RG, Kramvis A, Shimada T, Izumi N, Kaito M, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. Hepatology 2006;44:915-24. ◘