Acta Medica Okayama

Volume 49, *Issue* 3 1995 *Article* 4

JUNE 1995

Virological and serological characterization of asymptomatic blood donors positive for anti-hepatitis C virus antibody.

Hideyuki Tsuji* Hiroyuki Shimomura† Masaki Wato‡

Junichi Kondo** Takao Tsuji††

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
††Okayama University,

Virological and serological characterization of asymptomatic blood donors positive for anti-hepatitis C virus antibody.*

Hideyuki Tsuji, Hiroyuki Shimomura, Masaki Wato, Junichi Kondo, and Takao Tsuji

Abstract

To study the virological and serological characteristics of asymptomatic hepatitis C virus (HCV) carriers, 165 blood donors positive for antibody against HCV proteins by the second generation assay, were analyzed for their clinical backgrounds, serological reactivity against antigens derived from HCV by recombinant immunoblot assay, and the amount and genotype of HCV by the polymerase chain reaction. Compared with blood donors having abnormal levels of alanine aminotransferase (ALT), sera from the donors with normal levels of ALT reacted less frequently against NS4 antigens (anti-5-1-1: 34.4% vs. 54.5%, P = 0.0609; anti-c100-3: 34.4% vs. 56.1%, $P < 0.05$). Also the positivity for antibodies against these antigens were more frequent in sera from donors with genotype 1b HCV-RNA than other genotypes (anti-5-1-1: 61.0% vs. 23.5%, $P < 0.01$; anti-c 100-3: 61.0% vs. 26.5%, $P < 0.01$). The prevalence of each genotype in blood donors with normal ALT levels was different from that in patients with advanced liver disease ($P < 0.05$), genotype 1b being less and genotype 2a being more frequent. The number of HCV-RNA copies/0.5 ml in donors with normal ALT was 10(7.9 +/- 1.0) (n = 27) and that in patients with chronic liver disease was 10(7.4 +/- 0.8) (n = 116), the difference being statistically significant ($P < 0.05$). In conclusion, the results of this study suggest that asymptomatic blood donors carrying HCV have the serological and virological characteristics different from the patients with advanced liver disease.

KEYWORDS: hepatitis C virus, blood donor, asymptomatic carrier

---

*PMID: 7545861 [PubMed - indexed for MEDLINE]

ACTA MED OKAYAMA 1995; 49(3): 137-144

Virological and Serological Characterization of Asymptomatic Blood Donors Positive for Anti-Hepatitis C Virus Antibody

Hideyuki TSUJI, Hiroyuki SHIMOMURA*, Masaki WATO, Junichi KONDO and Takao TSUJI

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

To study the virological and serological characteristics of asymptomatic hepatitis C virus (HCV) carriers, 165 blood donors positive for antibody against HCV proteins by the second generation assay, were analyzed for their clinical backgrounds, serological reactivity against antigens derived from HCV by recombinant immunoblot assay, and the amount and genotype of HCV by the polymerase chain reaction. Compared with blood donors having abnormal levels of alanine aminotransferase (ALT), sera from the donors with normal levels of ALT reacted less frequently against NS4 antigens (anti-5-1-1: 34.4% *vs.* 54.5%, $P = 0.0609$; anti-c100-3: 34.4% *vs.* 56.1%, $P < 0.05$). Also the positivity for antibodies against these antigens were more frequent in sera from donors with genotype 1b HCV-RNA than other genotypes (anti-5-1-1: 61.0% *vs.* 23.5%, $P < 0.01$; anti-c100-3: 61.0% *vs.* 26.5%, $P < 0.01$). The prevalence of each genotype in blood donors with normal ALT levels was different from that in patients with advanced liver disease ($P < 0.05$), genotype 1b being less and genotype 2a being more frequent. The number of HCV-RNA copies/0.5 ml in donors with normal ALT was $10^{7.9 \pm 1.0}$ ($n = 27$) and that in patients with chronic liver disease was $10^{7.4 \pm 0.8}$ ($n = 116$), the difference being statistically significant ($P < 0.05$). In conclusion, the results of this study suggest that asymptomatic blood donors carrying HCV have the serological and virological characteristics different from the patients with advanced liver disease.

Key words: hepatitis C virus, blood donor, asymptomatic carrier

Before the identification of hepatitis C virus (HCV) genome and introduction of the test for anti-HCV antibody for the diagnosis of liver diseases or screening donated blood (1, 2), post-transfusion non-A, non-B hepatitis (NANBH) posed a significant health risk worldwide to those receiving blood transfusions. In Japan, 4.9 % of recipients of 1- to 10-unit transfusions, and 16.3 % of those who received 11- to 20-unit transfusions, suffered from post-transfusion NANBH despite the use of markers such as high serum alanine aminotransferase (ALT) levels when screening donors (3). Since the Japanese Red Cross started screening donated blood for antibody against HCV c100-3 protein in November 1989, the incidence of post-transfusion NANBH declined markedly (3). Furthermore, introduction of the second-generation anti-HCV assay for screening reduced the incidence even more. It is reported that the prevalence rate of anti-HCV was 1 % in Japan (4). In some Japanese populations anti-HCV-positive carriers have been reported without overt health problems. Before the best strategy to manage such carriers can be determined, it is necessary to understand the virological and pathophysiological significance of such carriers.

The natural course of hepatitis C has been reported, but it remains unknown whether persistent HCV infection necessarily leads to clinical symptoms or signs of hepatitis. In the case of hepatitis B, the so-called healthy carrier of hepatitis B virus (HBV) has been considered as a stage of immune tolerance against HBV life-long, or before clearance of HBV by immunological mechanisms in some patients. It is not known whether there is such a carrier state for HCV.

In this study, we investigated the serological and virological markers of HCV on blood donors with anti-HCV in an attempt to characterize the state of

* To whom correspondence should be addressed.

asymptomatic HCV carrier in comparison with the patients with chronic liver disease C.

Subjects and Methods

Patients. One hundred and sixty-five anti-HCV-positive blood donors (82 men and 83 women; mean ± SD of 49 ± 10 years of age, range 22-67 years) who visited Okayama University Hospital and affiliated hospitals in Okayama Prefecture were examined. They were tested positive for anti-HCV after a screening test using the second generation antibody detection system (5) by the Japanese Red Cross Okayama Blood Center and had been encouraged to consult with a doctor in a nearby hepatology clinic. Their histories were taken and serum liver function tests, serological tests and abdominal ultrasonographic examinations were performed. These 165 donors who showed following findings on ultrasonography (US) were classified as having "liver injury": dull or rounded edge, uneven surface, and rough internal echo of the liver. Out of 165, 110 donors were followed up more than five times for more than 6 months. At the first visit, 79% (131 cases) of them had normal serum ALT levels (less than 38 IU/l). Forty-seven donors whose ALT levels remained within the normal range during the follow-up period were classified as group N. In contrast, 74 donors who showed elevated ALT levels at their first visit or during the follow-up period were assigned to group A. Forty-nine patients had undergone liver biopsies under laparoscopy or US-guide after informed consent was obtained, and they were histologically diagnosed (6, 7).

Patients with chronic liver disease C (group D; 220 men and 155 women; mean ± SD of 52 ± 11 years of age, range 13-82 years) were diagnosed at Okayama University Hospital by liver biopsy and in cases of hepatocellular carcinoma (HCC) by angiography, computed tomography (CT), magnetic resonance imaging (MRI) or biopsy under US.

The samples were stored at −20°C until analysis.

Antibodies against HCV. Serum antibodies against four antigens, 5-1-1, c100-3, c33c, and c22-3, derived from HCV were determined by recombinant immunoblot assay (RIBA; Chiron RIBA-II Test, Ortho Diagnostic Systems, Tokyo, Japan).

Detection and quantitation of serum HCV-RNA. To determine the presence of HCV-RNA in serum, the reverse transcriptase-polymerase chain reaction (RT-PCR) using the sequence of the 5' non-coding region was performed as described before (8, 9). Competitive RT-PCR using mutant RNA in which a *Bam*HI site was introduced by *in vitro* mutagenesis was done for quantitation of HCV-RNA in serum as reported previously (10, 11).

Genotyping of HCV. The genotype of HCV was determined by RT-PCR using mixed type-specific primers against the NS5 region according to the method of Enomoto (12) as modified by Chayama (13). Type-specific primer set No.12 (13) was used in this examination. In brief, RNA was extracted from 200 μl of serum by the acid-guanidinium-phenol-chloroform method (14) and the final product was dissolved in 5 μl of water. It was reverse-transcribed in a solution containing 1 × PCR buffer [50 mM of KCl, 10 mM Tris-HCl (pH 9.0), 0.1% of Triton X-100, (Promega Biotec, Madison, WI, USA)], 3.0 mM of $MgCl_2$, 0.1 mM dNTPs, 1 mM of dithiothreitol, 4 units of ribonuclease inhibitor, 0.5 μM of anti-sense primer, and 2.5 units of Rous-associated virus 2 reverse transcriptase (Takara, Ohtsu, Japan). After pre-amplification heating at 70°C, reverse transcriptase products were sequentially supplemented with PCR mixture containing 1 × PCR buffer, 0.1 mM of dNTPs, 10% dimethyl sulfoxide (DMSO), 1 mM type-specific sense primers No. 12 described by Chayama, and 0.5 units of *Taq* DNA polymerase (Takara), and amplified for 40 cycles in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT, USA). The genotype was determined with the length of PCR products stained with ethidium bromide after agarose gel electrophoresis. Oligonucleotides used as primers were synthesized on a DNA synthesizer (Applied Biosystems Japan, Tokyo, Japan).

Genotypes of HCV were described according to the system for the nomenclature of HCV genotypes proposed by Simmonds *et al.* (15).

Statistical analyses. The chi-square test, two-tailed Student's *t*-test, and the Kruskal-Wallis test were used for statistical analysis and $P < 0.05$ was considered to be statistically significant.

Results

We studied 165 cases, including 110 donors who were followed up more than five times for more than 6 months. Of these, 131 (79%) had normal ALT levels at first visit. Eighty-seven donors with normal ALT levels

Table 1 Clinical backgrounds of anti-HCV positive blood donors

	Blood transfusion*	Family history*	Alcohol*	ZTT (KU)	K_{ICG}	ANA*	RF*
Group N[a] (47)	17.0% (47)	32.6% (46)	17.0% (47)	14.3 ± 4.8 (47)	0.15 ± 0.03 (32)	45.5% (22)	65.0% (20)
Group A[b] (74)	24.3% (70)	25.7% (70)	20.3% (69)	13.8 ± 4.4 (72)	0.15 ± 0.03 (44)	22.9% (35)	76.2% (21)

*Percentage of positive cases or cases with blood transfusion, family history or alcohol intake. Donors with ALT levels [a]within and [b]without normal ranges. Numbers in parenthesis indicate the numbers of cases studied. Abbreviations used in this table: ANA, anti-nuclear antibody; RF, rheumatoid factor.

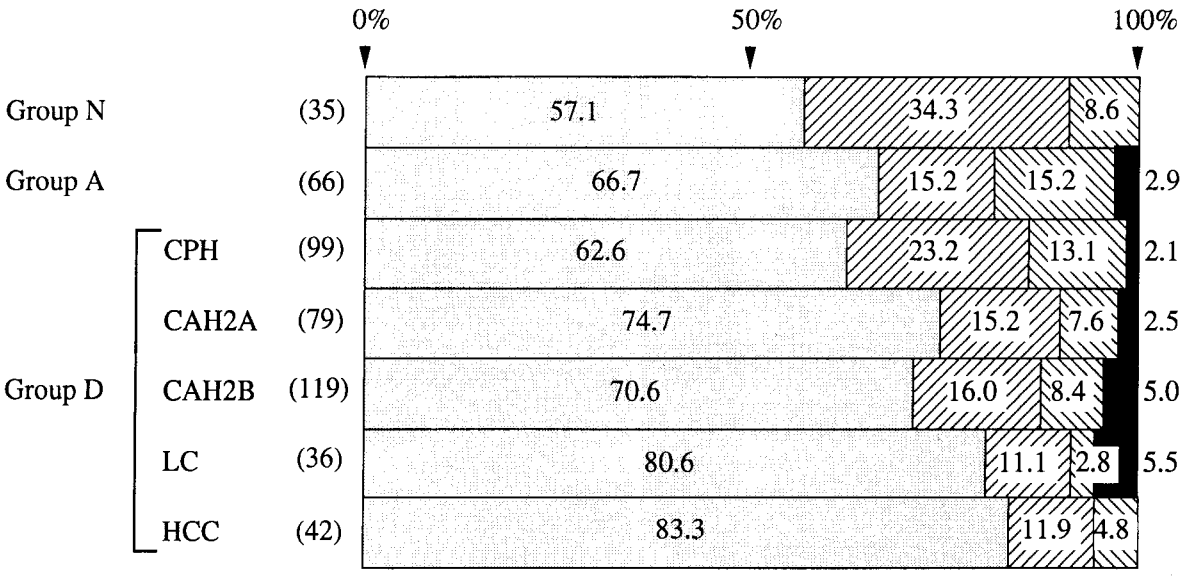

Fig 1 Prevalence rate of HCV genotype and clinical stages of chronic liver disease C. Genotyping of HCV-RNA was done using NS5 region as described in Subjects and Methods. Group N, donors with sustained normal ALT at every visits more than five times for 6 months; Group A, donors showed abnormal ALT level at their first visit or during follow-up period; Group D, patients of chronic liver disease C. □ = type 1b; ▨ = type 2a; ▧ = type 2b; ■ = mixed type.

at the first visit were followed-up, of which 40 cases (46 %) subsequently showed elevated ALT levels. Forty-seven patients (54 %) had normal ALT levels for more than 6 months (group N).

The case history and serum ALT, ZTT, K_{ICG}, anti-nuclear antibody (ANA) and rheumatoid factor levels of group N were compared with the 74 donors who showed elevated ALT levels at the first visit or during follow-up (group A) (Table 1). There were no statistically significant differences in the incidence of blood transfusion, family history, the history of drinking (more than 60 g of alcohol intake per day over 10 years), positive rheumatoid factor or ANA, or the level of serum ZTT or K_{ICG} (chi-square test, two-tailed Student's t-test).

The proportions of HCV genotypes are shown in Fig. 1. The proportion of genotype 1b tended to increase and genotype 2a decreased significantly as chronic liver disease C progressed. Group N, for example, had a lower proportion of genotype 1b than group liver cirrhosis (LC) ($P < 0.05$, chi-square test). Group N included a smaller

Table 2 The relationship between RIBA reactivity and genotypes of anti-HCV positive donors in Groups N and A

RIBA reactivity				HCV-genotype[a]						Total
Antigens										
5-1-1	c100-3	c33c	c22-3	1b	2a	2b	Mixed	ND	HCV-RNA(−)	
+	+	+	+	7/24	3/1	0/3	−	0/3	3/0	13/31
+	−	+	+	1/4	−	0/1	−	−	0/1	1/6
−	+	+	+	1/4	0/1	0/1	−	−	1/0	2/6
+	−	−	+	−	−	−	−	−	1/0	1/0
−	−	+	+	8/10	8/7	3/5	0/2	−	0/2	19/26
−	−	−	+	−	1/0	−	−	−	6/1	7/1
Total				17/42	12/9	3/10	0/2	0/3	11/4	43/70

a: Donors with ALT levels within normal range (Group N)/Donors with abnormal ALT levels (Group A). RIBA: RIBA-II test. ND: Not determined.

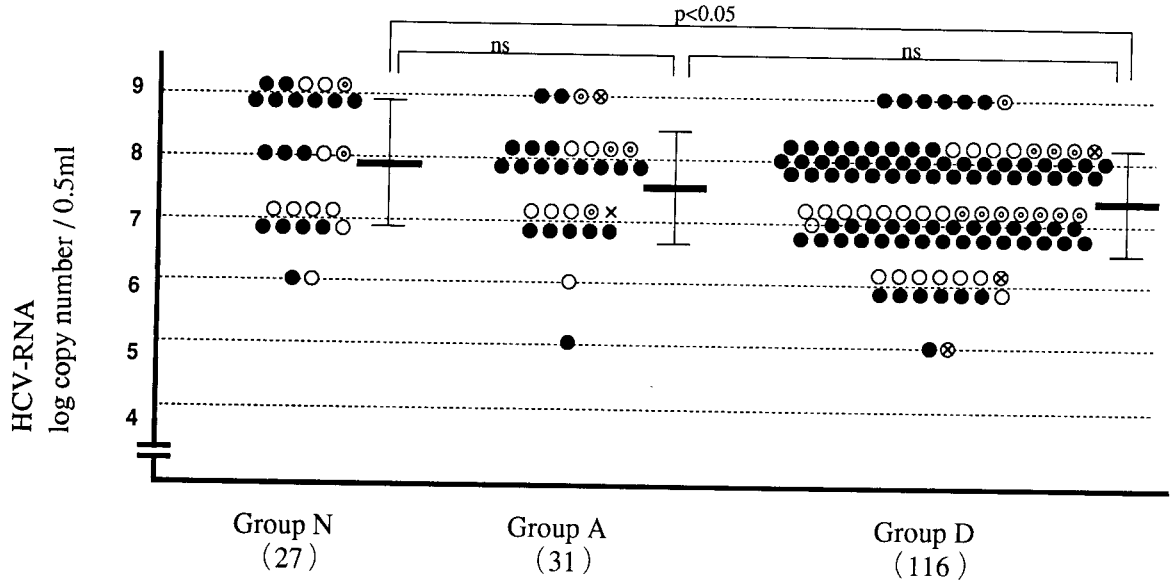

Fig. 2 Comparison of the amounts of HCV-RNA and genotype among three groups.
Group N, group A and group D, see legend to Fig. 1. ● = type 1b; ○ = type 2a; ◎ = type 2b; ⊗ = mixed type; × = not determined. Thick bar indicates the mean of the copy number and thin bar indicates standard deviation of value.

number of genotype 1b and a large number of patients with genotype 2a than group D ($P = 0.06$, chi-square test).

The relationship between the ALT level and HCV genotype, and the seroreactivities against antigens by means of RIBA was studied. As shown in Table 2, anti-c100-3 was positive in a significantly larger number of cases with increased ALT levels than those with normal ALT levels (37/66 *vs.* 11/32, $P < 0.05$) or with genotype 1b than with the other genotypes (36/59 *vs.* 9/34, $P < 0.01$, chi-square test). Anti-5-1-1 showed the same trends as those against c100-3, although the difference in seropositivity was not statistically significant for the ALT level (36/66 for increased *vs.* 11/32 for normal ALT) and significant for genotypes (36/59 for genotype 1b *vs.* 8/34 for other types, $P < 0.01$, chi-square test). In

Table 3 Histological diagnosis of 11 donors in Group N

Case	Age	Sex	Genotype	HCV-RNA titer*	Histology	Ultrasonography	K_{ICG}
1	54	F	2a	6	Liver fibrosis	No abnormal findings	0.17
2	48	F	1b	6	CPH	No abnormal findings	0.11
3	56	F	1b	8	CPH	No abnormal findings	0.15
4	58	F	2a	6	CPH	Liver injury	0.14
5	43	M	ND	Negative	CPH	NT	0.17
6	44	F	ND	Negative	CPH	NT	0.12
7	63	M	NT	NT	CPH	NT	0.15
8	46	F	ND	Negative	CPH	NT	0.13
9	62	F	1b	7	CAH2B	Liver injury	0.18
10	35	F	1b	NT	CAH2B	Liver injury	0.17
11	61	M	2a	7	CAH2B	Liver injury	0.15

*HCV-RNA titer was indicated by logarithm of the copy numbers of HCV-RNA per 0.5 ml of serum. Abbreviations used in this table: F, female; M, male; ND, not determined; NT, not tested; CPH, chronic persistent hepatitis; CAH2B, chronic active hepatitis with severe activity. Group N: See Table 1.

Table 4 Histological diagnosis of 38 donors in Group A

Histology	No. of cases
NSRH	1
CPH	17
CAH2A	16
CAH2B	4

Abbreviations used in this table: NSRH, non-specific reactive hepatitis; CAH2A, chronic active hepatitis with moderate activity and other abbreviations are as indicated in Table 3. Group A: See Table 1.

contrast, all except one of the HCV-RNA-positive donors was reactive to c33c. All sera reacted to c22-3.

The amount of HCV-RNA in serum from blood donors was determined by competitive RT-PCR and compared with that of the patients of group D (Fig. 2). Among circulating HCV-RNA-positive carriers, there was a statistically significant difference between group N ($n = 27$, $10^{7.9 \pm 1.0}$ copies/0.5 ml) and group D ($n = 116$, $10^{7.4 \pm 0.8}$) ($P < 0.05$, Kruskal-Wallis test), but no difference between groups N and A ($n = 31$, $10^{7.6 \pm 0.9}$), or groups A and D. In group N, 11 donors had 10^9 copies/0.5 ml of HCV-RNA.

Forty-nine blood donors had undergone liver biopsies because of their abnormal ultrasonographic findings suggesting liver injury or of low K_{ICG}, and they were histologically diagnosed (Tables 3 and 4). Ten out of 11 cases in group N had chronic hepatitis histologically, 7 cases had chronic persistent hepatitis (CPH) and 3 cases had chronic active hepatitis with severe activity (CAH2B) (Table 3). In group A, 37 of 38 cases were diagnosed as chronic hepatitis either CPH, chronic active hepatitis with moderate activity (CAH2A) or CAH2B. There were no cases of LC or HCC in our study group of blood donors.

Discussion

Since the HCV genome was identified (1, 2), the diagnosis of hepatitis C has become possible using serological and molecular biological markers. The anti-HCV assay revealed that HCV was a major cause of NANBH. In Japan, the prevalence rate of anti-HCV-positive cases was approximately 1% according to data from blood donors (4). This means that there are some HCV carriers without symptoms or abnormal liver function tests, and whether they have hepatitis or not is an important issue for understanding the pathogenesis and pathophysiology of hepatitis C.

In cases of hepatitis B, histologically normal appearance or minimal changes in the liver tissue were observed in healthy carriers positive for hepatitis B e antigen (HBeAg) (16, 17), and the amount of HBV-DNA and hepatitis B s antigen (HBsAg) in serum were high during the HBeAg-positive stage compared to the anti-HBe-positive stage (16, 18, 19). These results are explained by the host immune tolerance against HBV since the carriers were infected with HBV early in the neonatal period (16). Three phases of the natural history of HBV; the high replicative phase (immune tolerance phase), the low repli-

cative phase (immune clearance phase) and non-replicative phase have been proposed (16). We have proposed that hepatitis C would occur by immunological mechanisms in a manner similar to liver disease B (20–22). In this paper, it was shown that group N might include a distinct entity from patients with chronic liver diseases from the viewpoint that viral genotype and viral amount, in a manner similar to HBV healthy carriers.

When we compared the backgrounds of group N with group A, there were no significant difference between them. As the host's immunological responses are assumed to be related to the pathogenesis of liver disease C, further studies on immunological studies should be done to elucidate the role of factors of the host immune response such as cellular immunity against HCV-infected hepatocytes.

Virological characteristics of genotypes and viral amounts of HCV were not homogeneous either in anti-HCV-positive blood donors and in patients with chronic liver disease. In genotyping study, the prevalence rate of genotype 2a was higher in group N than in group D. We have reported that viral genotype might be one of the factors for the disease progression (23). This result provides further support for the idea that the disease progression might be slower in cases of genotype 2a.

We examined the relation of the ALT level and genotype, and the reactive antigens by means of RIBA. c100-3 antigen was targeted significantly in a large number of cases with increased ALT levels compared with normal or with genotype 1b than with other genotypes. Determination of the anti-5-1-1 showed the same trends as c100-3. As c100-3 antigen was developed to include 5-1-1 antigen (24), this result seemed to be reasonable. In contrast, the reactivities against the antigens c33c and c22-3 were not different between the donors with normal and increased ALT levels, nor among genotypes. There are several reports that antibodies against the products of the NS4 region were more reactive in the cases with increased ALT levels than with normal patients (25), or with type 1b virus than with other types (26, 27). Our results were consistent with these observations.

It was reported that the amount of serum HCV-RNA in the sera increased as the disease advanced (28, 29). In this study, the average amount of serum HCV-RNA in group N was greater than that of group D. We did not compare the amount in each stage of chronic hepatitis in this study. We previously reported that HCV-RNA tended to increase in serum and liver tissue in an advanced disease, but blood donors with normal ALT levels were not included (10). We presumed that the discrepancy between this paper and others might be due to the larger number of samples which were collected from blood donors in this study, including those with large amounts of HCV-RNA such as 10^9 copies/0.5 ml serum. It is suspected that these donors were HCV carriers in an immune tolerance phase, but histological examination could not be performed for ethical reasons. It was reported recently that there was no difference in the amounts of HCV-RNA between normal ALT-positive donors and patients of chronic liver disease C in the study on 41 anti-HCV-positive donors showing normal liver tests (30).

There are several reports on histological characteristics of asymptomatic HCV carriers. Alberti *et al.* reported that there were no normal livers histologically in HCV-RNA-positive cases (31). Other authors reported the existence of histologically normal liver in the case of persistent hepatitis C viremia (30, 32, 33). A laparoscopic study indicated that the degree of liver damage was not the same at different sites in the same liver with chronic liver disease C (34). It was presumed that histological regional differences in the liver specimens exist. This may be the reason for the disagreement regarding the liver histology of asymptomatic HCV carriers. We studied liver histology from 11 cases out of 47 donors with normal ALT for more than 6 months. These 11 cases showed abnormal findings on ultrasonography or ICG examination, and none of them was histologically diagnosed as normal. As we could not examine liver histology from donors with normal liver function and without any abnormal findings of ultrasonography, careful follow-up of these donors would help for the understanding of pathophysiology of such carriers.

In conclusion, we characterized blood donors with positive serum HCV-RNA virologically and serologically, and the results suggest that a certain population with distinct virological characteristics may be present in a group of blood donors with normal ALT level and it may be the group in which host immune system does not react enough like the healthy carrier state of HBV.

Acknowledgments. We thank Drs. Gotaro Yamada, Rieko Miyamoto, Shingo Kinoyama, Hiroshi Matsushima, Hiroshi Ikeda, Masaharu Ando, Mitsuhiko Kawaguchi, Shozo Kiyotoshi, Yasuo Fujita, Yasumasa Sato, Michihiro Jitoku, Hitoshi Harada, Mikio Sato, and Kimio Oka for collecting sera and clinical data. Also we thank Dr. Kozo Fujio for his valuable support and discussions. Also we thank Miss Hiroko Kajitani for excellent technical assistance. This work was partly supported by a grant for Non-A, Non-B

Hepatitis Research from the Ministry of Health and Welfare, and Viral Hepatitis Research Foundation, and a Grant-in-Aid (No. 05670475) for Scientific Research from the Ministry of Education, Science, and Culture.

References

1. Choo Q, Kuo G, Weiner AJ, Overby LR, Bradley DW and Houghton M: Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science (1989) **244**, 362-364.
2. Arima T, Nagashima H, Murakami S, Kaji C, Fujita J, Shimomura H and Tsuji T: Cloning of a cDNA associated with acute and chronic hepatitis C infection generated from patients serum RNA. Gastroenterol Jpn (1989) **24**, 540-544.
3. 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**, 1040-1041.
4. Nishioka K: Hepatitis C virus infection in Japan. Gastroenterol Jpn (1991) **26**, 152-153.
5. Awakihara S, Naoki K, Miyahara M and Sato H: Studies on characterization and information criteria of anti-HCV positive donor group with low titers determined by PHA method. Blood Programme (1994) **17**, 157-164 (in Japanese).
6. Review by an International Group: Morphological criteria in viral hepatitis. Lancet (1971) **1**, 333-337.
7. Review by an International Group: Acute and chronic hepatitis revisited. Lancet (1977) **2**, 914-919.
8. Okamoto H, Okuda S, Sugiyama Y, Tanaka T, Sugai Y, Akahane Y, Machida A, Mishiro S, Yoshizawa H, Miyakawa Y and Mayumi M: 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**, 215-222.
9. Nakagawa H, Shimomura H, Hasui T, Tsuji H and Tsuji T: Detection of negative strand RNA of hepatitis C virus in infected liver and serum. Acta Med Okayama (1993) **47**, 311-316.
10. Nakagawa H, Shimomura H, Hasui T, Tsuji H and Tsuji T: Quantitative detection of hepatitis C virus genome in liver tissue and circulation by competitive reverse transcription-polymerase chain reaction. Dig Dis Sci (1994) **39**, 225-233.
11. Shimomura H, Nakagawa H, Hasui T, Tsuji H and Tsuji T: Quantitation of HCV-RNA. Kan Tan Sui (1992) **25**, 967-974 (in Japanese).
12. Enomoto N, Takada A, Nakao T and Date T: There are two major types of hepatitis C virus in Japan. Biochem Biophys Res Commun (1990) **170**, 1021-1025.
13. Chayama K, Tsubota A, Arase Y, Saitoh S, Koida I, Ikeda K, Matsumoto T, Kobayashi M, Iwasaki S, Koyama S, Morinaga T and Kumada H: Genotypic subtyping of hepatitis C virus. J Gastroenterol Hepatol (1993) **8**, 150-156.
14. Chomczynski P and Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem (1987) **162**, 156-159.
15. Simmonds P, Alberti A, Alter HJ, Bonino F, Bradley DW, Brechot C, Brouwer JT, Chan S-W, Chayama K, Chen D-S, Choo Q-L, Colombo M, Cuypers HTM, Date T, Dusheiko GM, Esteban JI, Fay O, Hadziyannis SJ, Han J, Hatzakis A, Holmes EC, Hotta H, Houghton M, Irvine B, Kohara M, Kolberg JA, Kuo G, Lau JYN, Lelie PN, Maertens G, McOmish F, Miyamura T, Mizokami M, Nomoto A, Prince AM, Reesink HW, Rice C, Roggendorf M, Schalm SW, Shikata T, Shimotohno K, Stuyver L, Trepo C, Weiner A, Yap PL and Urdea MS: A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology (1994) **19**, 1321-4.
16. Chu C-M, Karayiannis P, Fowler MJF, Monjardino J, Liaw Y-F and Thomas HC: Natural history of hepatitis B virus infection in Taiwan: Studies of hepatitis B virus DNA in serum. Hepatology (1985) **5**, 431-434.
17. Chang M-H, Hwang L-Y, Hsu H, Lee C and Beasley RP: Prospective study of asymptomatic HBsAg carrier children infected in the perinatal period: Clinical and liver histologic studies. Hepatology (1988) **8**, 374-377.
18. Chen D-S, Lai M-Y, Lee S-C, Yang P-M, Sheu J-C and Sung J-L: Serum HBsAg, HBeAg, anti-HBe, and hepatitis B viral DNA in asymptomatic carriers in Taiwan. J Med Virol (1986) **19**, 87-94.
19. Berris B, Sampliner RE, Sooknanan R and Feinman SV: Hepatitis B virus DNA in asymptomatic HBsAg carriers: Comparison with HBeAg/Anti-HBe status. J Med Virol (1987) **23**, 233-239.
20. Dudley FJ, Fox RA and Sherlock S: Cellular immunity and hepatitis-associated, Australia antigen liver disease. Lancet (1972) **1**, 723-726.
21. Eddleston ALWF and Williams R: Inadequate antibody response to HBAg or suppressor T-cell defect in development of active chronic hepatitis. Lancet (1974) **2**, 1543-1545.
22. Yamada G and Tsuji T: Immunological mechanism of chronic liver injury in viral hepatitis. Intern Med (1993) **32**, 911-3.
23. Tsuji H, Shimomura H, Hasui T, Wato M, Kondo J, Miyamoto R, Yamada G, Tsuji T, Miyahara M and Sato M: Does type C hepatitis progress differently by its viral genotype? Hepatology (1994) **19**, 1341 (abstract).
24. Kuo G, Choo Q, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL and Alter MJ: An assay for circulating antibodies to a major etiologic virus of human non-A non-B hepatitis. Science (1989) **244**, 362-364.
25. Cuthbert JA: Hepatitis C: Progress and problems: Clin Microbiol Rev (1994) **7**, 505-532.
26. Chan S-W, Simmonds P and McOmish F: Serological responses to infection with three different types of hepatitis C virus. Lancet (1991) **338**, 1391.
27. Alonso C, Qu D, Lamelin JP, DE Sanjose S, Vitvitski L, Li J, Berby F, Lambert V, Cortey ML and Trepo C: Serological responses to different genotypes of hepatitis C virus in France. J Clin Microbiol (1994) **32**, 211-212.
28. Hagiwara H, Hayashi N, Mita E, Naito M, Kasahara A, Fusamoto H and Kamada T: Quantitation of hepatitis C virus RNA in serum of asymptomatic blood donors and patients with type C chronic liver disease. Hepatology (1993) **17**, 545-50.
29. Kato N, Yokosuka O, Hosoda K, Ito Y, Ohto M and Omata M: Quantification of hepatitis C virus by competitive reverse transcription-polymerase chain reaction: Increase of the virus in advanced liver disease. Hepatology (1993) **18**, 16-20.
30. Sakamoto S, Okanoue T, Itoh Y, Takami S, Yasui K, Kagawa K and Kashima K: Virological and clinico-pathological studies of 2nd anti-HCV positive individuals showing normal liver tests. Acta Hepatol Jpn (1994) **35**, 580-586 (in Japanese).
31. Alberti AGM, Chemello L, Cavalleto D, Noventa F, Pontisso P and Ruol A: Hepatitis C viraemia and liver disease in symptom-free individuals with anti-HCV. Lancet (1992) **340**, 697-698.
32. Brillanti S, Foli M, Gaiani S, Masci C, Miglioli M and Barbara L: Persistent hepatitis C viraemia without liver disease. Lancet (1993) **341**, 464-465.
33. McMahon RF, Yates AJ, McLindon J, Babbs C, Love EM and Warnes TW: The histopathological features of asymptomatic hepatitis C virus-antibody positive blood donors. Histopathology (1994) **24**, 517-24.
34. Watanabe T and Kamimura T: Clinicopathological investigation of

chronic hepatitis C: Comparison with chronic hepatitis B. Gastroenterol Endosc (1994) **36**, 847-849 (in Japanese).

Received February 13, 1995; accepted March 30, 1995.