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Hepatitis C: Basic and clinical studies

Terukatsu ARIMA
Second Department of Internal Medicine, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

Summary: The HCV, a single stranded RNA virus belonging to the family of flavivirus, has been identified as the probable cause of the majority of cases of transfusion-associated NANB hepatitis and community-acquired NANB hepatitis in Japan. The hepatitis virus is present in a least 2% of the blood donor population and is extremely common in high risk groups, such as hemophiliacs and hemodialysis patients. The contribution of HCV infection to sporadic, acute and chronic hepatitis, liver cirrhosis and primary liver cancer has been established. Furthermore anti-HCV in 20% of alcoholic patients with liver injury suggest that HCV may be etiologically associated with liver disease previously attributed to other causes. Therapy of acute and chronic liver disease associated with HCV infection is likely to be undertaken with recombinant IFN alpha in the future to prevent the progression of the disease from acute hepatitis to chronic hepatitis, and from chronic hepatitis to liver cirrhosis or primary liver cancer. However the prevention of HCV infection will be the goal, in addition to screening of donor blood and exclusion to a large degree of positive units likely to decrease the incidence of post-transfusion hepatitis. *Gastroenterol Jpn 1992;27:121–127.*

Key Words: *HCV; non-A, non-B hepatitis*

Introduction

Since 1975, when the first definitive report on non-A, non-B (NANB) hepatitis in transfusion recipients appeared, many important observations about the agents responsible for this infection emerged. However none of the serologic tests or putative virus particles described in reports published before 1988 met simple criteria for a specific association with NANB hepatitis[1].

However knowledge of NANB hepatitis has expanded in the past 2 years following the cloning of the primary causative agent, now designated as the hepatitis C virus (HCV)[2]. For the first time the nature of this virus including its basic genomic organization is known. In addition, serologic assays to detect antibodies to HCV (anti-HCV) and HCV-RNA were developed to clarity the seroepidemiologic surveys and the NANB hepatitis disease spectrum. As we should develop a test to make more precise diagnosis for this disease and a vaccine to prevent this infection, it is timely to review the HCV at this moment.

Cloning of Gene Fragments of Agents Responsible for NANB Hepatitis

Although the source, human with NANB hepatitis or chimpanzee inoculated with NANB hepatitis agents, of serum RNA and the screening method, EIA or RIA for the replica of plaques made by lambda gt11-random-primed cDNA library are different from each other, a Japanese group[3] and an American group[4] independently succeeded in cloning gene fragments from an agent responsible for a large part of the infection.

Arima et al isolated 20 clones having amino acid homology of more than 85% to the sequence originally described by the American group (HCV). They also cloned 29 sequences having less than 85% RNA homology to the HCV and sixty-seven clones having no homology to the amino acid sequences of the HCV. While the genomic structure of HCV is most like those of flavivirus, there is only about 5% RNA homology between the HCV and the flavivirus.

There are ample evidence suggesting the presence

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Address for correspondence: Terukatsu Arima, M.D., Second Department of Internal Medicine, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890, Japan.

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Arima E vs HC-JS(Gan Center)

BASE SEQUENCE(70.7%)

ACGTCGTCTGCTGCTCAATGTCCTATACATGGACAGGTGCCTTGATCACGCCATGCGCTG
**  **          *     *  ** *     *  *   * *  *  *     * ***

CGGAGGAGAGCAAGTTGCCCATCAATCCGTTGAGCAACTCTTTGCTGCGTCACCACACTA
 *      ***     *  *     *  **          ** **    ***   * **

TGGTCTACTCCACAACATCTCGCAGCGCAAGTCTGCGGCAGAAGAAGGTCACCTTTGACA
*   *           *  ** * *  ***    *  **         *         *

AMINO ACID SEQUENCE(83.1%)

VVCCSMSYTWTGALITPCAAEESKLPINPLSNSLLRHHTMVYSTTSRSASLRQKKVTFD
 *      *         **  *           * * **            *

Arima E vs Chiron

BASE SEQUENCE(68.0%)

ATGTCGTGTGCTGCTCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGCGCCG
**  ** *        *     *     *     *  *  *   * *  *  *  * ***

CGGAAGAACAGAAACTGCCCATCAATGCACTAAGCAACTCGTTGCTACGTCACCACAATT
 *  *  **    ** *  *     ** *  *         * ** *  ***   *  **

TGGTGTATTCCACCACCTCACGCAGTGCTTGCCAAAGGCAGAAGAAAGTCACATTTGACAG
*      *     *     ** *       ** **   **      *  *  *      **

AMINO ACID SEQUENCE(79.7%)

VVCCSMSYSWTGALVTPCAAEEQKLPINALSNSLLRHHNLVYSTTSRSACQRQKKVTFD
 *            *   **  *     *     * *  *         ** *

Gan Center vs Chiron
BASE SEQUENCE(76.8%)
AMINO ACID SEQUENCE(88.1%)
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Fig. 1
A sequence, Arima E, having a low homology to HCV. Points of the disagreement of sequences of RNA and amino acid are indicated by asterisks. The sequence of Arima E is compared with those of Chiron Corporation and HC-JS.

of many mutants in HCV. Among such we have cloned by immunoscreening is clone E with 71% RNA and 83.1% amino acid homology to the Japanese HC-JS strain, and 68% RNA and 79.7% amino acid homology to the Chiron strain (**Fig. 1**). Surprisingly HC-JS has 76.8 RNA and 79.7% amino acid homology to Chiron strain as well.

As the entire sequence of HCV genome was determined by utilizing overlapping sequences, the sequence can be a mosaic consisting of gene fragments derived from many mutants. The genome is a single-stranded, positive-sense RNA consisting of approximately 9000 bases having one open reading frame. The genome consists of a structural region at the 5'-end and 4-5 nonstructural components at the 3'-end[2]. A microfiltration study revealed that the HCV appears to be a 30-60 nm in diameter. As the infectivity of the virus to chimpanzee was destroyed by treating with chloroform it is considered to be enveloped by lipids. The HCV has structural and genomic similarities to the arthropod-borne flavi-and pestivirus though there is only a small degree of RNA homology with any of the known viruses in this family[2]. It is currently classified as flavi-like. The actual virus particle has not yet been identified and the agent has not been cultured.

Prospects for Vaccine Development

Arima et al isolated cDNA clones covering approximately half of the HCV genome by the immunoscreening method. The sites where the epitopes reside

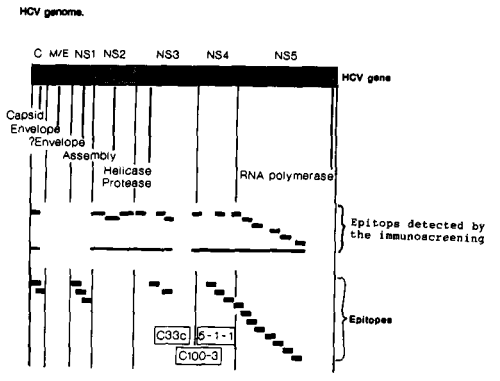

Fig. 2 Epitopes on HCV genome detected by the immunoscreening. The epitopes are indicated by bars. Epitopes detected by immunoscreening are shown at the top of this figure. Two method, the immunoscreening and theoretical calculation method, give similar results. It should be stressed that there is no epitope in the region of M/E where the neutralizing antigen is considered to be located.

are shown as bars in the upper part of **Figure 2** while epitopes predicted from the sequence described by the Chiron Corporation are shown in the lower part of **Figure 2**. Strikingly they agree with each other very well except for discrepancies in NS1 and NS2. However it is very important that there is no epitope in the M/E region where the neutralizing antigen of this virus must be located. In addition, as mentioned before in this issue there must be many mutants of this virus which can escape from neutralizing antibodies raised by a vaccine derived from one such mutant of HCV. Five clones derived from the same region having 500 bases in the NS5 domain isolated by the immunoscreening and 3 sequences obtained by extension of the HCV sequences showed similar replacements of nucleotides at the points of the HCV genome whether they prepared by the immunoscreening or the extension method from a known sequence of the HCV as far as using RNA from the same source. From our study it can be concluded that by the extension method we can clone mutants having replacement of RNA less than 25% and that for the isolation of mutants having a replacement more than 25% in terms of RNA the immunoscreening should be conducted as indicated above. At this moment we don't have sufficient information regarding mutation on the neutralizing antigens which is predicted to be located in the M/E region of the HCV genome. Therefore it will take a long time to develop an effective vaccine to prevent HCV infection.

Spectrum of NANB Hepatitis

Two test kits for the detection of HCV infection designated as Arima-14 and C-100-3 were developed from the sequences cloned by the 2 groups of which one is commercially available and the other one is under application for approval by the Japanese Government[5]. Using these tests it became apparent that approximately 90% of the transfusion-associated hepatitis was related to the NANB hepatitis agent. Prospective analysis of stored sera showed that 9 (64.3%) out of 14 donors who provoked hepatitis in recipients were Arima-14 antibody-positive while C-100 was positive in these 4 donors who were also positive for Arima-14. Seven of the 14 recipients and six corresponding donors were positive for Arima-14 while six of the 14 recipients and corresponding 4 donors were positive for C-100. These results suggest a close correlation between donors blood positive for HCV-antibodies and transfusion-associated hepatitis.

While the incidence of transfusion-associated hepatitis has progressively decreased from 20% to under 10%, the proportion of cases related to the NANB hepatitis agent has remained relatively constant at 90-95% throughout the world.

Approximately 90% of hemophiliacs and 30% of hemodialysis patients were positive for either of the antibody tests. The major form of parenteral transmission is that among intravenous drug addicts and here again, the test with C-100 revealed a prevalence of 60-90%[2].

By a CDC study done in sentinel counties in four areas of the United States, it was revealed that approximately 50% of community-acquired cases had no known parenteral exposure[2]. It also became evident that a very high proportion of community-acquired cases are HCV-related and that the proportion of anti-HCV positive cases is the same whether their source of exposure is parenteral or unknown (**Table 1**). It has also been demonstrated that HCV has a close relation to primary liver cancer (**Table 1**).

Histological patterns of alcohol-induced liver disease are usually classified into fatty liver, alcoholic hepatitis and chronic active hepatitis, and liver cirrhosis. Serum samples were sampled from 114 con-

Table 1 Prevalence of anti-HCV in NANB liver disease (%)

Disease	Blood transfusion	Number of patients	Arima-14 antibody	C-100 antibody
Chronic hepatitis	+	43	32 (74.4)	33 (76.7)
	–	138	103 (74.6)	89 (64.5)
Liver cirrhosis	+	21	9 (42.9)	14 (66.7)
	–	67	41 (61.2)	52 (78.8)
Liver cancer	+	11	7 (63.6)	11 (100)
	–	13	7 (53.8)	7 (53.8)

Table 2 Prevalence of anti-HCV in chronic liver disease in C-district (%)

		Arima-14		Total
		(+)	(–)	
C-100	(+)	9 (11.0)	12 (12.2)	21 (25.6)
	(–)	7 (8.5)	54 (65.9)	
Total	1	6 (19.5)		82 (100)

secutive patients with chronic liver disease with alcohol abuse. All subjects were HBsAg-negative. 23 (20.2%) and 22 (19.3%) patients were Arima-14 and C-100 positive respectively. The result suggests that HCV infection is responsible for some case of alcoholic hepatitis.

Evidence for Multiple NANB Hepatitis Agents

A second type of NANB hepatitis agent distinct from HCV has been suggested by observations of different incubation periods, multiple episodes of hepatitis and cross-challenge studies in chimpanzee[2]. Although only 80% of chronic NANB hepatitis cases and 50% of acute cases are anti-HCV positive suggesting the presence of a second NANB hepatitis agent, the cause for the negative result might be derived from incorrect diagnosis, inadequate sampling for anti-HCV testing, and/or relative insensitivity of the first generation tests currently available. As these tests are improved, as additional epitopes are used in the capture assays, and as viral RNA in serum is detected by PCR, it is anticipated that the number of NANB hepatitis cases which are anti-HCV negative will considerably diminish and it is probable that most, if not all, cases will prove to be HCV related[2].

However there is evidence suggesting the presence of a new type of NANB hepatitis agent. In C-district, approximately one million in population, only 19.5% and 25.6% of 82 chronic non-B hepatitis cases were positive for Arima-14 and C-100 respectively. Of these 11.0% were positive for both tests and 65.9% were negative for the tests (**Table 2**). In addition, cases positive for PCR in this district showed very weak signals compared to controls, which suggests a weak affinity of HCV-RNA tested to primers having sequences of the HCV-RNA originally described by the Chiron Corporation.

Apart from the new NANB hepatitis virus, there is also evidence suggesting the presence of highly transformed HCV having an amino acid homology of less than 85% to the HCV. A clone, F1-3 having 65% homology to HCV-RNA and 74% homology to the amino acid sequence, was isolated from serum pooled from paid donors with elevated ALT but negative for HBsAg, Arima-14, C-100 and PCR (non-B, non-C: NBNC). The antibody testing made from the sequence was positive for 10 (55.5%) of 18 chronic NBNC cases negatie for Arima-14, C-100 and PCR. The test was also positive for half of the 8 chronic hepatitis cases positive for C-100 and Arima-14. The result suggests that F1-3 derived from a mutant with low homology to HCV and was supplementary to C-100 and Arima-14 in testing of anti-HCV.

In general, although there are intriguing pieces of evidence to suggest at least one additional NANB hepatitis agent, the existing data are equally compatible with a single agent hypothesis[2]

Seroepidemiology

Among 243 transfusion recipients diagnosed as acute NANB hepatitis in a clinical study[5], 34.5% and 35.4% were found to be positive for Arima 14 and C-100 positive respectively. These numbers increased to 74.4% and 76.7% respectively when 43 chronic cases were tested. The discrepancy between acute and chronic cases reflects in part the increased likelihood that acute cases, which are often mild and transient and rarely confirmed by biopsy, are misdiagnosed and do not actually represent infection from a primary hepatotropic virus[2]. In addition, it appears that the level and duration of viremia in acute cases

may be inadequate to induce antibody response to the HCV epitopes currently being measures[2]. It is also expected that detection of HCV in well pedigreed NANB hepatitis cases should then well exceed 90% and possibly approach 100% and that hence, if there is a separate agent, it will account for a very small proportion of NANB hepatitis cases[2].

The onset of hepatitis C is most commonly between 6 and 12 weeks after transfusion exposure and the shortest seroconversion time is reported to be 10 weeks. Seroconversion for anti-HCV was observed in 90% of cases within 6 months[2]. In general there is a 2-4 month interval between disease onset and antibody detection.

The prevalence of anti-Arima-14 and anti-C-100-3 in volunteer donors is low (2.0% and 1.1% of 2476 donors in Kagoshima prefecture, respectively). There is a discrepancy between these two tests and only 0.6% of these donors were positive for both tests. Among 26 donors positive for C-100, 12 were negative for both Arima 14 and RIBA (recombinant immunoblot assay, Chiron Corp., Emeryville, CA). Eighty-six per cent of 14 donors positive for both antibody tests and 26% of 35 donors positive only for Arima-14 were positive for PCR. As the RIBA assay and PCR are being utilized to address the issue of assay specificity, Arima-14 seems to be more specific than C-100, as shown above. The RIBA assay generally confirmed the specificity of the C-100 when testing recipients who developed NANB hepatitis or when testing donors implicated in NANB hepatitis transmission. However, when randomly testing non-implicated donors, only one-third were confirmed by RIBA[2]. At present, there is a considerable problem of non-specificity in donor testing by C-100 and this will be very important in counseling the large number of donors who will be found to be anti-HCV reactive by C-100 test.

Alter[2] reported that 75% of recipients with NANB hepatitis had a RIBA-specific, anti-HCV positive donor. Therefore it is probable that approximately 75% of NANB hepatitis cases could have been prevented by C-100 screening since the RIBA test seems to be specific for the HCV infection. Importantly, however, in 25% of C-100 positive NANB hepatitis cases, HCV was transmitted by donors negative for C-100[2]. This test represents a major advance in blood donor screening but in its present configuration would fail to detect a significant number of HCV carriers. As described before by testing anti-HCV with Arima-14 alone or in combination with these tests, this problem can be improved.

Table 3 HCV-capsid antibody and PCR*

	Number positive			
	AR-142	CP-02	CP-10	CP-9
PCR (+), n=12	12	10	6	5
PCR (–), n=2	2	6	1	1
Number of disagreements	2	8	7	8

*24 sampels were selected from donors blood who were tested for PCR.

To test the reactivity of epitopes in the structural region the following four peptides were synthesized. AR142 derived from an Arima-14 clone; KDRTQ-*QRKTKRSTNRR*RSKNEKKKK, CP-02 derived from a Japanese HCV: MSTNPKPO*QRKTKRNTNRR*-PQDVKFPGGG and CP-10 derived from the same HCV but shorter: PKP*QRKTKRNTNRR*PQDVK. These three peptides are derived from the same region but the sequences adjacent to the region are different. A sequence (CP-9): RRGPRLGVRATRKT-SERSQPRGRRQPIPKVRRPEGR which starts at the 39th of the N-terminal of HCV-amino acid sequence is also included in the test. These sequences were tested with 24 donors of which 12 were PCR-positive (**Table 3**). The peptide, AR 142, a peptide deriving from Arima clone 14, agreed very well with the result tested with PCR. However, the other 3 peptides misjudged approximately one-thirds of the samples. Again peptides derived from the Arima-14 clone seems to be more specific for the testing of anti-HCV in donors blood.

Using the polymerase chain reaction (PCR), HCV-RNA was detected within 1-2 weeks of exposure in two well pedigreed cases of NANB hepatitis[2]. While it has not been established that the detection of viral RNA equates with infectivity, early comparison of PCR reactivities with infectivity titrations in the chimpanzee suggest that PCR may be a more sensitive indicator of infectivity than the chimpanzee model[2]. Among chronic NANB hepatitis cases. 22 positive for both Arima-14 and C-100, 5 only positive

Table 4 HCV-antibody in chronic non-B hepatitis and PCR

	Arima-14/C-100			
	(+)/(+)	(+)/(−)	(−)/(+)	(−)/(−)*
Number of patients	22	5	7	84
PCR-positive	21 (95.5%)	4 (80.0%)	6 (85.7%)	41 (48.8%)

*Paid donors

for Arima-14 and 7 only positive for C-100, and 84 paid donors with elevated ALT but negative for both anti-HCV tests were tested with PCR (**Table 4**). Although both tests have very high specificity to the chronic HCV infection particulary in cases positive for both tests, there are a significant numbers of PCR-positive cases in paid donors who are negative for both antibody tests.

Transmission Patterns

Parenteral transmission

As mentioned above, more than 74% of transfusion associated NANB hepatitis cases are anti-HCV positive. When 156 cases with hemophilia or hemophilia-related disease were tested, 87.8% were positive for either Arima-14 or C-100 and the majority hade chronic NANB hepatitis. The frequency of anti-HCV among 503 cases of hemodialysis is as high as 23% which increases as the duration of the dialysis increases. The prevalence of anti-HCV among health care workers is not well established, but in community-acquired NANB hepatitis cases, work in the health professions is only rarely established as a route of HCV transmission[2].

Sexual transmission

CDC study on the sexual transmission of HCV disclosed that among 52 patients with non-transfusion-related NANB hepatitis, 12% had more than two sexual partners in the preceding six months as compared with only 1% of age- and sex-matched controls who had that number of partners[2]. This result high suspects the presence of sexual transmission of HCV.

Perinatal transmission

There is very little data relating to perinatal transmission of HCV. There are some reports describing a transient anti-HCV positive in neonates born to mothers positive for anti-HCV, but there is no evidence of active viral replication in the neonates. A report describing mother to infant transmission of HCV showed that 5 of 11 children born to 8 mothers having chronic hepatitis C during their pregnancy, had elevated ALT but only 2 had increased level in more than one sample. All children tested before 6 months of age and were positive for anti-HCV at most up to 7 months of age and then became negative. One child with an elevated level, however, regained anti-HCV positivity at 12 months of age, and a liver biopsy at 21 months of age showed resolving heptitis. Therefore in 1 of 11 children, active anti-HCV production and concomitant liver disease suggested mother to infant transmission of HCV infection[6].

Vector transmission

The similarity of genomic structure of HCV to the arthropod-borne flaviviruses raises the possibility that HCV might also be transmitted by vectors. Although there is no evident experience to indicate such transmission, the fact that 50% of community-acquired NANB hepatitis cases have no identified route of exposure demands that the possibility of vector transmission should be thoroughly investigated[2].

Treatment

Finally, since HCV is most likely a hepatocytopathic virus, it is desirable to have a direct quantitative marker of virus load. It is therefore probable that PCR for HCV-RNA will be necessary for accurate diagnosis of active infection and to document response to treatment[7].

Interferon (IFN) appeared to be the first drug with consistent efficacy against the agents of viral hepatitis, particularly NANB hepatitis. 40-50% of patients will have a complete remission on therapy (3M units thrice weekly for 6 months), although half will relapse when IFn is discontinued. A response can be reinduced with repeated therapy. Although in some patients with chronic hepatitis the IFN-treatment has only a little or not efficacy in reduction of the level of ALT, histological improvements in such patients were frequently observed. This is the first therapeutic

regimen that has shown consistent response. However, the long-term therapeutic efficacy is not known[2].

Prospects for the Future

This is a very exciting new beginning for the 15-year-old field of NANB hepatitis research. Two groups, one in Japan and the other in the United States, are already producing a first generation blood donor screening test that is now also being used for diagnostic purpose. As demonstrated above, more sensitive and accurate tests should follow soon. The above data, indicate that HCV is the predominant agent of blood-transmitted NANB hepatitis. The question remains whether there are other agents to account for the cases devoid of anti-HCV or PCR. As the gene of HCV has been cloned, theoretically, development of a protective vaccine should be a subsequent goal. Although no culture system in which the HCV proliferates, has been developed the development of a recombinant vaccine is realistic as we know several sequences of the HCV genes, pending the elucidation of epitopes which induce neutralizing antibody. However, this will meet with difficulties because of the existence of multiple subtypes or mutants of the HCV. There is also a possibility that the infecting HCV can change the gene structure to escape from the protective antibody produced by the host, since this agent has been confirmed as an RNA virus.

To answer the question concerning the determinant of the continuing chronic characteristics of the HCV infection, the determinant for recovery from the disease should be clarified because approsumately half of the patients with acute NANB hepatitis recover spontaneously from the disease.

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