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The response of hepatitis C virus and TT virus to high dose and long duration interferon-alpha therapy in naïve chronic hepatitis C patients

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Abstract

To investigate responses of hepatitis C virus (HCV) and TT virus (TTV) to high dose and long duration interferon-alpha (IFN-alpha) therapy (540 million units in 36 weeks) and factors associated with the viral clearance, sera of 165 Taiwanese naïve chronic hepatitis C patients were tested for alanine aminotransferase, HCV RNA levels, HCV genotypes and TTV DNA. With 41.8% of TTV DNA prevalence, TTV viremia was significantly associated with history of blood transfusion ($P < 0.01$). After IFN therapy, HCV complete response was achieved in 60 (36.4%) patients and significantly associated with lower pretreatment levels of HCV RNA ($P < 0.01$) and HCV genotype non-1b ($P < 0.05$). Fifty-three patients with concurrent TTV infection were evaluated for TTV response. TTV sustained clearance was achieved in 24 (48%) patients and significantly associated with loss of TTV DNA at the end point of treatment. In conclusion, concurrent TTV infection is highly prevalent, related to blood transfusion and independent of HCV infection. After high dose and long duration IFN-alpha therapy, HCV and TTV clearance are achieved among more than one-third and around one-half patients. HCV RNA levels and HCV genotypes are predictors for HCV response and no clinical factors are observed to be associated with TTV clearance.

Keywords: TT virus; Hepatitis C virus; Interferon

1. Introduction

A novel nonenveloped, single-stranded DNA virus was discovered and designated TT virus (TTV) after the initials (T.T.) of the index Japanese patient with posttransfusion hepatitis of

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unknown etiology (Nishizawa et al., 1997; Okamoto et al., 1998). The clinical significance, pathogenicity and hepatopathic effects of TTV infection remains controversial. Although some reports indicated the association between TTV infection and elevated alanine aminotransferase (ALT) levels (Nishizawa et al., 1997; Okamoto et al., 1998), development of chronic liver diseases of unknown etiology (Nishizawa et al., 1997; Tanaka et al., 1998) or even hepatocellular carcinoma (Nakagawa et al., 1999), other investigations demonstrate different results (Lo et al., 1999; Tanaka et al., 1999; Kato et al., 1999].

Hepatitis C virus (HCV) is the second leading cause of chronic hepatitis and hepatocellular carcinoma in Taiwan—a hepatitis B virus (HBV) endemic area (Chuang et al., 1993). Several HCV endemic townships in southern Taiwan were discovered with anti-HCV prevalence rates of 17–58% (Lu et al., 1997; Wang et al., 1999) that were obviously higher than those (0.95–2.6%) of general population (Chen et al., 1991; Chang et al., 1992). Interferon-alpha (IFN-alpha) has been show to be effective and accepted treatment for HCV (Hoofnagle and di Bisceglie, 1997). Regimen with high doses has showed to improve the efficiency of IFN-alpha therapy.

Dual infection of TTV and hepatitis C virus (HCV) is not uncommon. Of HCV infected subjects, 27–36% were TTV viremic in USA (Zein et al., 1999) and Taiwan (Kao et al., 1999). In previous studies, IFN therapy had been reported effective against TTV (Akahane et al., 1999; Hagiwara et al., 1999; Watanabe et al., 2000). Nevertheless, the contribution of high dose and long duration IFN-alpha therapy on TTV clearance still remains unclear.

In the present study, we investigated the effects of high dose and long duration IFN-alpha therapy on HCV and TTV. The associated factors with both TTV and HCV viral clearance and the influence of concurrent TTV infection on clinical characteristics of chronic RCV infection and HCV response to IFN in chronic hepatitis C patients were also studied.

2. Materials and methods

2.1. Subjects

Between March 1993 and June 2000, a total of 165 Taiwanese naive chronic hepatitis C patients, 95 males and 70 females, aged between 18 and 73 years (mean 45.7 ± 12.5 years) were enrolled in the study. To minimize the effects of time-dependent degradation of viral DNA, the serum samples, when collected from patients at the time of their evaluation, were stored at -70 °C before testing. All of the patients were negative for hepatitis B surface antigen (HBsAg) and positive for both HCV antibodies (anti-HCV) and serum HCV RNA. The diagnoses of chronic hepatitis were made histologically by the liver biopsy based on standard criteria. Liver cirrhosis was diagnosed histologically or clinico-pathologically. The patients included 39 (23.6%) cases of chronic persistent hepatitis (CPH), 87 (52.8%) chronic active hepatitis (CAH) and 39 (23.6%) liver cirrhosis (LC). Forty-one (24.8%) patients had a history of blood transfusion.

2.2. Laboratory tests

Second-generation anti-HCV and HBsAg were detected with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abbott, North Chicago, IL). ALT levels were measured on a multichannel autoanalyzer with normal upper limit of serum ALT being 25 IU/l.

2.3. Detection of TTV DNA in serum

TTV DNA was detected by nested polymerase chain reaction (PCR) using semi-nested primers targeting the N22 region in the coding region as described previously (Okamoto et al., 1998). Briefly, total DNA was purified from 150 μl serum using the QIAamp Blood Kit (Qiagen Ltd, Hilden, Germany) and eluted in a final volume of 50 μl. Five microliters were added to the first round of PCR with the sense primer NG059 (5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′) and the anti-sense primer NG063 (5′-CTG GCA TTT TAC CAT TTC CAA AGT

T-3′) for 5 min at 95 °C, followed by 35 cycles under amplification condition of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 45 s (additional 7 min for the last cycle). The second round of PCR was performed with the sense primer NG061 (5′-GGC AAC ATG YTR TGG ATA GAC TGG-3′ Y = T or C; R = A or G) and the anti-sense primer NG063 for 25 cycles under the same conditions as the first round of PCR. The amplification products by the first and the second round PCR measured 286 and 271 base pairs (bp). PCR products were analyzed by agarose gel electrophoresis and DNA stained with ethidium bromide.

2.4. Detection/quantification of serum HCV RNA and genotyping

The nested reverse transcription polymerase chain reaction (RT-PCR) to detect serum HCV RNA was performed using 5′-noncoding region specific primers as described previously (Yu et al., 1996). HCV genotypes 1a, 1b, 2a, 2b and 3a were determined by amplification of the core region using genotype-specific primers (Okamoto et al., 1993). Pretreatment serum HCV RNA levels were determined using a branched-DNA assay (Quantiplex HCV RNA 2.0, Bayer, CA) strictly according to manufacturer's instructions. The quantification range was from 0.2 to 120 megaequivalents (Meq) of HCV RNA per ml.

2.5. Detection of GBV-C/HGV RNA and anti-E2 antibody in serum

GBV-C/HGV RNA was detected by nested RT-PCR using primers targeting the 5′ UTR as described previously (Mukaide et al., 1997). The anti-E2 antibody of GBV-C/HGV (anti-E2) was measured by ELISA from Boehringer Mannheim (GmbH, Germany), strictly according to the manufacturer's instruction (Tacke et al., 1997).

2.6. IFN-alpha therapy

Recombinant IFN-alpha 2a ($n = 29$), IFN-alpha 2b ($n = 76$) or lymphoblastoid IFN-alpha n1 ($n = 60$) were given intramuscularly to the all of 165 patients after they had given their informed consents. The high dose and long duration regimen of therapy was used with 6 million units (MU) of IFN-alpha thrice a week for 24 weeks followed by 3 MU thrice a week for 12 weeks (540 MU for 36 weeks). The presence of HCV RNA in the serum was assessed every 3 months since beginning of therapy to 6 months after the cessation of therapy. HCV complete responders (CR) for IFN-alpha were defined as patients showing normal ALT levels and clearance of serum HCV RNA by nested RT-PCR at 6 months after the cessation of therapy. All other patients were classified as HCV non-responders (NR). For evaluating the effects of IFN-alpha therapy on TTV, serum TTV DNA was followed at the end point of the therapy (E/T) and 6 months after the cessation of therapy for 53 patients with concurrent TTV infection. TTV clearance was defined as the disappearance of serum TTV DNA by nested PCR. We defined the sustained clearance of TTV as TTV clearance at 6 months after cessation of IFN-alpha therapy.

2.7. Statistical analyses

Descriptive statistics such as means and proportions were calculated: Frequency was compared between groups using the Chi-squared-test (χ^2-test) or Fisher's exact test, and group means were compared using the t-test. The presence of a statistical significance was inferred when P was less than 0.05. Serum HCV RNA levels were expressed as the mean $\pm$ standard deviation (S.D.) after logarithmic transformation of original values. For the purpose of analyzing the data with suitable statistical methods, we assigned a nominal value of 0.1 Meq/ml to samples that were negative by the branched DNA assay but positive for HCV RNA by nested RT-PCR. Stepwise logistic regression method was used to analyze the study data. Odds ratios (ORs) and their associated 95% confidence intervals (CIs) were used to quantify the magnitude of their associations.

3. Results

3.1. TTV viremia, GBV-C/HGV viremia and GBV-C/HGV exposure in chronic hepatitis C patients

Of 165 naïve chronic hepatitis C patients with positive HCV RNA, the pretreatment HCV RNA level was less than 0.2 Meq/ml in 35 patients. The mean pretreatment HCV RNA levels was 3.19 ± 5.07 (ranging from 0.1 to 32) Meq/ml. The genotype distribution was as follows: 1b in 76 patients, 2a in 64 patients, 2b in 13 patients and mixed in 12 patients. There was 46.1% of HCV isolates deduced to genotype 1b. Sixty-nine patients were positive for TTV DNA showing an overall prevalence of 41.8%. Twenty-eight (17.0%) and 30 (18.2%) patients were positive for GBV-C/HGV RNA and anti-E2. The prevalence of GBV-C/HGV exposure, defined as positive for serum GBV-C/HGV RNA and/or anti-E2, was 33.9%. The clinical characteristics between individuals with and without TTV DNA were analyzed and are shown in Table 1. The positive rate of TTV DNA was significantly higher in the patients with history of blood transfusion than those who were never transfused (24/41, 58.5 vs. 45/124, 36.3%; $P < 0.01$). No other clinical and virological factor was related to positive TTV DNA.

Table 1
Comparison of clinical characteristics between individuals with and without TT virus viremia in chronic hepatitis C patients

	Number	Number of patients with TTV DNA (%)		P
		Positive ($n = 69$)	Negative ($n = 96$)	
Sex				NS
Male	95	40 (42.9)	56 (57.1)	
Female	70	30 (41.1)	40 (58.9)	
Age (year, mean ± S.D.)[a]		46.3 ± 11.7	45.2 ± 13.0	NS
ALT (IU/l, mean ± S.D.)[b]		88.4 ± 68.4	94.5 ± 81.8	NS
Normal (≤25 IU/l)	15	9 (60)	6 (40)	NS
Abnormal (>25 IU/l)	150	60 (40)	90 (60)	
GBV-C/HGV RNA[c]				NS
Positive	28	11 (39.3)	17 (60.7)	
Negative	137	58 (42.3)	79 (57.7)	
GBV-C/HGV exposure[d]				NS
Positive	56	28 (50)	28 (50)	
Negative	109	41 (37.6)	68 (62.4)	
HCV RNA level (log Meq/ml, mean ± S.D.)[e]		5.97 ± 0.68	6.08 ± 0.73	NS
HCV genotype				
1b	76	36 (43.4)	40 (56.6)	NS
Non-1b	89	33 (37.1)	56 (62.9)	
Histopathology				NS
Chronic active hepatitis	86	36 (41.9)	50 (58.1)	
Chronic persistent hepatitis	38	16 (42.1)	22 (57.9)	
Liver cirrhosis	41	17 (41.5)	24 (58.5)	
Blood transfusion				<0.05
Positive	41	24 (58.5)	17 (41.5)	
Negative	124	45 (36.3)	79 (63.7)	

[a] Mean ± S.D., mean ± standard deviation.
[b] ALT, alanine aminotransferase.
[c] GBV-C/HGV, GB Virus C/Hepatitis G virus.
[d] GBV-C/HGV exposure, positive for GBV-C/HGV RNA and/or anti-E2 antibodies.
[e] Meq/ml, megaequivalents per ml.

Table 2
Comparison of clinical and virological features between complete- and non-responders of chronic hepatitis C patients after interferon therapy

	Number	Number of HCV response to interferon therapy (%)		*P*
		Complete-response ($N = 60$)	Non-response ($N = 105$)	
Sex				NS
Male	95	35 (36.8)	60 (63.1)	
Female	70	25 (35.7)	45 (64.3)	
Age (year, mean ± S.D.)		44.8 ± 14.8	46.2 ± 10.9	NS
ALT (IU/l, mean ± S.D.)		107.3 ± 87.9	83.1 ± 61.6	0.049
IFN-alpha preparation				NS
IFN-alpha 2b	76	24 (31.6)	52 (68.4)	
IFN-alpha 2a	29	8 (27.6)	21 (72.4)	
IFN-alpha n1	60	28 (46.7)	32 (53.3)	
History of transfusion				NS
Positive	41	10 (24.4)	31 (75.6)	
Negative	124	50 (40.3)	74 (59.7)	
Liver cirrhosis				NS
Positive	39	10 (25.6)	29 (74.4)	
Negative	126	50 (39.7)	76 (60.3)	
HCV RNA levels (log Meq/ml, mean ± S.D.)		5.77 ± 0.66	6.14 ± 0.71	<0.01
HCV genotype				
1b	76	21 (27.6)	55 (72.4)	<0.05
Non-1b	89	39 (43.8)	50 (56.2)	
GBV-C/HGV RNA				NS
Positive	28	8 (28.6)	20 (71.4)	
Negative	137	52 (38.0)	85 (62.0)	
GBV-C/HGV exposure				NS
Positive	56	21 (37.5)	35 (62.5)	
Negative	109	39 (35.8)	70 (64.2)	
TTV DNA				NS
Positive	69	22 (31.9)	47 (68.1)	
Negative	96	38 (39.6)	58 (60.4)	

3.2. Clinical and virological features and HCV response of IFN-alpha therapy

After IFN-alpha therapy with the regimen of 6 MU thrice a week for 24 weeks followed by 3 MU a week for 12 weeks, 60 of the 165 patients (36.4%) were CR. The clinical and virological features of HCV complete and non-responders after IFN-alpha therapy were shown in Table 2. In comparison between these two groups by univariate analysis, the higher rate of CR was significantly related to lower pretreatment levels of HCV RNA ($P < 0.01$) and HCV genotype non-1b ($P < 0.05$). The mean ALT levels were higher among HCV CR than NR with borderline significance of difference statistically ($P = 0.049$). Other clinical and virological factor was not related to HCV complete response of IFN-alpha therapy. Based on multivariate regression analyses, the significant factors associated with HCV complete response after IFN-alpha therapy were lower pretreatment HCV RNA levels and HCV genotype non-1b with the OR and 95% CI of these factors summarized in Table 3.

3.3. Clearance of TTV DNA in chronic hepatitis C patients after IFN-alpha therapy

TTV DNA was followed in 53 chronic hepatitis C patients concomitant with TTV viremia before

IFN-alpha therapy. They were 30 males and 23 females with mean age of 46.8 ± 11.3 years (range: 21–67) and mean ALT level of 88.1 ± 67.5 IU/l (range: 12–313). The mean pretreatment HCV RNA levels was 2.22 ± 3.49 (ranging from 0.1 to 19) Meq/ml. The genotype distribution was as follows: 1b in 26 patients, 2a in 15 patients, 2b in four patients and mixed in eight patients. There was 49.1% of HCV isolates deduced to genotype 1b. Nine (17%) patients were positive for GBV-C/HGV RNA and 22 (41.5%) patients were GBV-C/HGV-exposed. Based on histological or clinico-pathological diagnoses, there were 29, 12 and 12 cases of CAH, CPH and LC, respectively. Sixteen patients (30.1%) had a history of blood transfusion. The clinical characteristics and virological features between individuals with and without TTV DNA sustained clearance after IFN-alpha therapy were analyzed and shown in Table 4. Factors including sex, mean ages, pre-, end- and post-treatment ALT levels, the prevalence of GBV-C/HGV RNA, the rate of GBV-C/HGV exposure, IFN preparation, history of blood transfusion and liver cirrhosis were not related to TTV clearance. Nineteen (35.8%) of the 53 TTV DNA-positive patients were HCV CR after IFN-alpha therapy. No association between TTV sustained clearance and pretreatment HCV RNA levels, distribution of HCV genotypes or HCV response was discovered. At the E/T, TTV DNA was negative in 33 patients (62.3%). When TTV DNA was followed 6 months after the cessation of therapy, 12 of 33 patients (36.4%) with negative TTV DNA at the E/T had reappearance of serum TTV DNA. Of these 12 patients, despite negative TTV DNA at the ET, four cases were HCV NR with abnormal ALT levels. The other eight cases were HCV CR with persistent normal ALT despite reappearance of TTV DNA. Four of 20 patients (20%) with positive TTV DNA at E/T had clearance of TTV DNA 6 months after the cessation of therapy. All of four cases were HCV NR with abnormal ALT levels despite negative TTV DNA 6 months after cessation of therapy. The rate of sustained clearance of TTV DNA after IFN therapy was 47.2% (25/53). As shown in Table 4, the sustained clearance rate of TTV DNA was significantly higher among patients with TTV clearance at the E/T than those who were TTV viremia at the E/T (68.9 vs. 19.1%, $P < 0.001$). No other clinical and virological factor was related to sustained clearance of TTV DNA.

4. Discussion

The prevalence of TTV DNA in previous reports from Taiwan were around 10% (Lo et al., 1999; Kao et al., 1999). TTV viremia rates seems to be higher among chronic hepatitis C patients with reports of 27% (Zein et al., 1999) from Minnesota and 36% (Kao et al., 1999) from northern Taiwan. In our present study, 41.8% of the Taiwanese patients with chronic hepatitis C were concurrent-infected by TTV, which further supported that TTV infection was very common in HCV viremic patients. The clinical implication of TTV infection and etiological importance of TTV in association with liver diseases still remain controversial. When concurrent infection of multiple hepatitis viruses happened, such as the inhibitory action of viral replication in dual HBV and HCV infection, the clinical features of liver disease might be modified (Liaw et al., 1994). TTV infection was also suggested to be more prevalent among patients with advanced liver diseases caused by chronic hepatitis C (Zein et al.,

Table 3
Stepwise logistic regression analysis of factors significantly associated with HCV response after interferon-alpha therapy

Dependent variable	Independent variable	Comparison	Odds ratio (95% C.I.[a])
HCV complete response	HCV RNA level	Per log increase	0.38 (0.22–0.65)
	HCV genotypes	Non-1b vs. 1b	2.4 (1.14–5.01)

[a] C.I., confidence interval.

Table 4
Comparison of clinical characteristics and virological features between chronic hepatitis C patients with and without sustained clearance of TT virus after IFN-alpha therapy

	Number	Number of sustained clearance of TTV (%)		*P*
		Positive ($N = 25$)	Negative ($N = 28$)	
Sex				NS
Male	30	16 (53.3)	14 (46.7)	
Female	23	9 (39.1)	14 (50.9)	
Age (year, mean ± S.D.)		47.5 ± 12.0	46.1 ± 11.1	NS
ALT (IU/l, mean ± S.D.)		83.4 ± 59.9	92.3 ± 74.4	NS
ALT at E/T[a]				NS
Normal	35	18 (51.4)	17 (48.6)	
Abnormal	18	7 (38.9)	11 (61.1)	
ALT response				NS
Complete- responder	32	17 (53.1)	15 (46.8)	
Non-responder	21	8 (38.1)	13 (61.9)	
GBV-C/HGV RNA				NS
Positive	9	4 (44.4)	5 (55.6)	
Negative	44	21 (47.7)	23 (52.3)	
GBV-C/HGV exposure				NS
Positive	22	10 (45.5)	12 (54.5)	
Negative	31	15 (48.4)	16 (51.6)	
IFN-alpha preparation				NS
IFN-alpha 2b	22	10 (45.5)	12 (54.5)	
IFN-alpha 2a	11	5 (45.5)	6 (54.5)	
IFN-alpha n1	20	10 (50)	10 (50)	
HCV RNA level (log Meq/ml, mean ± S.D.)		5.97 ± 0.61	5.87 ± 0.69	NS
HCV genotype				NS
1b	26	14 (53.9)	12 (46.1)	
Non-1b	27	11 (40.7)	16 (59.3)	
Liver cirrhosis				NS
Positive	12	7 (58.3)	5 (41.7)	
Negative	41	18 (43.9)	23 (56/1)	
History of transfusion				NS
Positive	16	7 (43.8)	9 (56.2)	
Negative	37	18 (48.7)	19 (51.3)	
Clearance of TTV at E/T				<0.001
Positive	33	21 (68.9)	12 (31.1)	
Negative	20	4 (19.1)	16 (80.9)	
HCV response				NS
Complete-responder	19	6 (31.6)	13 (68.4)	
Non-responder	34	19 (55.9)	15 (44.1)	

[a] E/T, end point of therapy.

1999). In this study, the demographic feature and clinico-pathological findings of chronic hepatitis C patients did not differ among patients with and without concurrent TTV infection. The results supported that TTV infection was not related to more severe liver disease in chronic hepatitis C patients. Furthermore, by quantification of HCV RNA levels, we identified that TTV viremia was not related to different HCV RNA levels. These results suggested that TTV does not interfere with HCV replication and its hepatopathic effect as HBV does.

In this study, TTV viremia showed significant association with positive history of blood transfu-

sion in HCV viremic patients. As the initial finding of TTV infection in posttransfusion hepatitis, our result indicated the important role of blood transfusion in the transmission route of TTV.

The effects of IFN therapy for chronic hepatitis C with the standard regimen of IFN 3 MU, three times weekly for 24 week was not satisfactory. The sustained viral response (SVR) was achieved in only 10–25% (Garson et al., 1995; Martinot-Peignoux et al., 1998). The significant dose and duration effects were also noted with IFN therapy (Poynard et al., 1996). Induction therapy (with IFN 6 MU daily for the initial 2 weeks then 3 MU thrice a week for 24 weeks) has shown to improve the efficiency of IFN therapy (Layden, 1999). With regimen of 9 MU IFN thrice a week for 24 weeks (total 648 MU), 36% of SVR was reported (Shiratori et al., 1997). With 36.4% of patients being CR in this study, the IFN therapy with higher dose (540 MU) and longer duration (36 weeks) improved the successful rate of IFN therapy than standard regimen.

In regard to clinical factors related to the response of IFN therapy for chronic hepatitis C, the most significantly important predictors for SVR in our study with high dose and long duration regimen were pretreatment HCV RNA levels and HCV genotypes, such as reports in previous studies with 3–6 MU thrice a week for 24 weeks (Garson et al., 1995; Martinot-Peignoux et al., 1998). The histologic activity and pretreatment serum transaminase levels was suggested to be correlated with the second-phase viral decrease with IFN-alpha therapy (Layden, 1999). Chronic hepatitis C patients with cirrhosis might have longer duration of infection and higher complexity of quasispecies and show more resistant to IFN therapy. Nevertheless, no association between presence of liver cirrhosis and treatment responses was observed in this study. It is suggested that such a high dose and long duration regimen of IFN therapy may diminish the unfavorable therapeutic effects resulted from cirrhosis. The response of HCV to IFN-alpha treatment was not affected when the concurrent infection of TTV or GBV-C/HGV as well as exposure of GBV-C/HGV occurred. The results deny the necessity for evaluation of status of TTV or GBV-C/HGV infection when chronic hepatitis C patients prepare receiving IFN-alpha therapy.

After IFN-alpha administration with regimen of 6 MU thrice a week for 24 weeks followed by 3 MU thrice a week for 12 weeks, 24 of 50 (48%) concurrent TTV-infected patients achieved complete clearance of TTV DNA 6 months after the cessation of therapy. With 7–8% TTV spontaneous clearance rate annually reported in previous reports (Oguchi et al., 1999), our results indicated that IFN-alpha has a potential antiviral effect on TTV. In previous studies, IFN therapy was effective against TTV with eradication rate of 45–55% (Akahane et al., 1999; Hagiwara et al., 1999; Watanabe et al., 2000). Even with the higher dose and longer duration (540 MU for 36 weeks) regimen than other reports, the eradication rate of TTV (48%) in our study obtained similar results. Transient disappearance of TTV viremia during IFN-alpha therapy was observed in the present and previous reports, demonstrating the direct antiviral effects of IFN-alpha on the suppression of TTV. Nevertheless, delayed TTV clearance (TTV DNA positive at E/T and negative after cessation of therapy) that had not been reported previously occurred in four patients. Delay complete virological response was observed in chronic hepatitis B patients after the end of therapy with Thymosin alpha1 (Chien et al., 1998) or IFN (Lin et al., 1999) that revealed immune modulation effects (Main et al., 1998). Since TTV is a DNA virus as HBV, delay clearance of TTV after IFN therapy may indicate that immune modulation plays an important role. The findings in the present study implied that both antiviral effects and immunomodulatory actions of IFN-alpha are important on the eradication of TTV. Further studies are needed to investigate and clarify the actual mechanism of responsiveness of TTV to IFN-alpha.

In evaluating the clinical characteristics and virological features related to clearance of TTV after IFN-alpha therapy, the viral clearance at the E/T was the only important factor associated with clearance of TTV viremia. Neither the pretreatment ALT levels nor the histopathology was predictors for TTV clearance. Besides, there was no correlation between response of HCV and TTV.

All the results may indicate the difference of virologic kinetics and mechanism of IFN effects between TTV and HCV that influence the response and resistance to IFN-alpha. The ALT levels at the E/T or 6 months after cessation of treatment were not related to TTV but HCV viremia also denied the hepatopathic effects of TTV infection.

In conclusion, concurrent TTV infection is highly prevalent and related to blood transfusion among Taiwanese chronic hepatitis C patients. TTV infection stands independent of HCV infection without influence on liver disease caused by HCV infection and HCV replication based on quantification of HCV RNA levels. With high dose and long duration regimen of IFN-alpha therapy (6 MU thrice a week for 24 weeks followed by 3 MU thrice a week for 12 weeks), more than one-third naïve patients are HCV complete responders. With such a regimen, both lower pretreatment HCV RNA levels and HCV genotype non-1b are the most important predictors for favorable response and liver cirrhosis does not indicate poor response. TTV is sensitive to high dose IFN and can be eradicated effectively in around one-half patients with TTV viremia. Both antiviral effects and immunomodulatory actions of IFN-alpha seems important on the eradication of TTV. Except viral clearance at the end of therapy, no clinical or virological factors were observed to be significantly associated with TTV clearance. The ALT levels were mainly correlated with the presence of HCV RNA regardless of the effect of IFN alpha on TTV replication.

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