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SIGNIFICANCE OF HEPATITIS C VIRUS INFECTION IN JAPANESE PATIENTS EXHIBITING AUTOIMMUNE-HEPATITIS-LIKE CLINICAL MANIFESTATIONS

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To assess the significance of hepatitis C virus (HCV) infection in Japanese patients having clinical manifestations compatible with autoimmune hepatitis (AIH), we retrospectively examined both antibody to HCV (anti-HCV) and HCV-RNA in 27 previously diagnosed AIH patients. Based on these HCV-related markers, the patients were divided into three groups: 1) patients negative for both anti-HCV and HCV-RNA (group 1, n=8, 30%), 2) patients positive for anti-HCV but not HCV-RNA (group 2, n=9, 33%), and 3) patients positive for both anti-HCV and HCV-RNA (group 3, n=10, 37%). The patients in group 3 had higher levels of anti-HCV and lower levels of both serum immunoglobulins and anti-nuclear antibody than those in the other two groups. These findings, suggest that the autoimmune-hepatitis-like clinical manifestations in group 3 had been caused by HCV infection (C-AIH). Both the prevalence of each genomic subtype and HCV-RNA levels were almost identical in the C-AIH and non-autoimmune type HCV-induced chronic hepatitis patients, suggesting that the aberrant immune response induced by HCV infection may be related to genetic factors of the infected hosts rather than to the virus itself.

Introduction

Autoimmune hepatitis (AIH) is a severe, progressive liver disease characterized by marked mononuclear cell infiltration of the liver associated with piecemeal, parenchymal liver cell injury[1]. The concept of an autoimmunity for chronic liver disease was first supported by the discovery of hypergammaglobulinemia and several autoantibodies in patients' sera, and later by their response to treatment with immunosuppressive drugs such as corticosteroids and azathioprine[2][3]. Measles virus was reported as a possibly cause of AIH[4], the same as infection by Epstein-Barr virus in Sjögren's syndrome[5]. However, the real trigger of this disease has not been clearly identified.

A cloned cDNA derived from the genome of a non-A, non-B hepatitis virus carrier was recently isolated and designated as hepatitis C virus (HCV)[6][7]. Since then, several studies using the assay system for antibody to HCV (anti-HCV) have indicated[8]~[10] that some AIH patients are positive for anti-HCV. However, the prevalence of anti-HCV varies considerably in different countries, and thus geographic factors may also be involved. Therefore, in the present study, we attempted to investigate the clinical manifestations of Japanese AIH patients in conjunction with the presence of either anti-HCV or HCV-RNA.

Materials and Methods

Patients

Before the assay for anti-HCV had become well established, 27 female patients were diagnosed with AIH according to classical diagnostic criteria[2], such as elevated serum gammaglobulin (>2.0 g/dl) and immunoglobulin G (IgG) (>2,000 mg/dl) and the presence of anti-nuclear antibody (ANA) in two hospitals, the Institute of Gastroenterology of Tokyo Women's Medical College and the National Yokohama Hospital. The histological findings of liver specimens from these patients were identical to the findings in either chronic active hepatitis or early liver cirrhosis (Table 1). To compare the genomic subtypes and quantity of HCV-RNA in these patients, we obtained sera from 57 other randomly selected HCV-RNA-positive female patients with chronic hepatitis (CH) without AIH-like clinical manifestations. As in a previous report[11], all the patients studied here were also negative for anti-liver-kidney microsome antibody type 1 (LKM-1 antibody) (data not shown). The sera obtained from all these patients were stored

Table 1 Laboratory data of studied patients

	Anti-HCV		γ-glo (g/dl)	IgG (mg/dl)	ANA	ADNA (OD value)	Histological findings
	1st (OD value)	2nd (Cut-off index)					
Group 1	α-HCV (−), HCV-RNA (−)						
Case 1	−	−	3.1	3,235	80	0.193	CAH
Case 2	−	−	4.4		160	0.249	CAH
Case 3	−	−	4.0	4,498	4,000	0.373	CH
Case 4	−	−	6.2	5,680	20,000	0.210	LC
Case 5	−	−	2.1	2,532		0.329	CH
Case 6	−	−	3.0	3,112	80	0.152	CAH
Case 7	−	−	3.1			0.224	CH
Case 8	−	−	3.4	4,420	80	0.106	CAH
Group 2	α-HCV (+), HCV-RNA (−)						
Case 1	+1.037	−	4.2	4,730	160	0.505	LC
Case 2	+0.488	−	2.3	2,955	160	0.345	CAH
Case 3	+1.250	−	3.6	8,000		0.300	CAH
Case 4	+2.047	−	4.7	6,000	640	1.519	CAH
Case 5	+0.510	−	2.7	2,878		0.103	CAH
Case 6	+0.488	−	3.1	3,336	1,280	0.345	CAH
Case 7	+3.000	−	4.3	4,356	640	0.405	LC
Case 8	+1.984	+1.46	5.2	6,100	640	1.904	
Case 9	+1.367	+2.73	3.6	2,843	40	0.599	LC
Group 3	α-HCV (+), HCV-RNA (+)						
Case 1	−	+>4.00	2.2	2,594	40	0.549	CAH
Case 2	−	+ 3.97	2.1			0.636	LC
Case 3	+0.751	+>4.00	2.9	3,950	320	0.865	CAH
Case 4	+3.000	+>4.00	2.5	1,974	1,280	0.165	CH
Case 5	+0.538	+ 2.44	3.4	4,653	160	0.645	CH
Case 6	+3.000	+>4.00	2.7	2,462		0.100	CAH
Case 7	+3.000	+>4.00	2.8	3,625	320		CH
Case 8	+1.810	+>4.00	1.8	2,191	40	0.297	CH
Case 9	+1.874	+>4.00	1.8	2,236	80	0.488	CAH
Case 10	+0.594	+>4.00	2.1		40	0.355	CH

Anti-HCV: antibody to hepatitis C virus, ANA: anti-nuclear antibody, ADNA: anti-DNA antibody, CAH: chronic active hepatitis, CH: chronic hepatitis, LC: liver cirrhosis.

at −20°C until used for assay.

Twenty-two of the previously diagnosed AIH patients were treated either with prednisolone alone or with prednisolone plus azathioprine before testing for HCV-related markers. Prednisolone started at dosages as great as 20~40 mg/day and was gradually reduced by 5 mg every two to four weeks.

Serologic examination

Serum gamma-globulin and IgG concentrations were measured by the radial immunodiffusion method. The autoantibodies were determined by the following methods; ANA by the indirect immunofluorescence test using HepG2 cells and IgG anti-single strand (ss) DNA antibody (ADNA-Ab) by ELISA, as described by Okada et al.[12].

Detection of anti-HCV and HCV-RNA

Two different assays were used to detect anti-HCV: the first generation Abbott anti-HCV Ab ELISA Test (1st-generation assay) and the second generation Abbott anti-HCV Ab EIA Test (2nd-generation assay) (Abbott Co., Ltd., Ill, USA)[13].

HCV-RNA was detected by the nested polymerase chain reaction (PCR) using the previously described method[13]. In brief, serum RNA was extracted from 50 μl of plasma and the final product was dissolved in 12 μl of water. An aliquot of RNA (3 μl) was reverse-transcribed using antisense primer[13]. The single-stranded cDNAs were amplified by PCR using sense and antisense primer pairs from 5′-noncoding HCV sequences of previous isolates. The amplified products were separated by electrophoresis on 2% agarose gel and stained with ethidium bromide.

Genotypic and quantitative analysis of HCV-RNA

Genotypic analysis of HCV-RNA was performed by the method of Kato et al[14]. In brief, three different HCV genotypes (HCV-US, HCV-J, HCV-K2) were identified by primers specific for each genotype[14]. The putative non structural five regions of HCV-J, HCV-US and HCV-K2 were amplified by the nested PCR method described above. Quantitative measurement of HCV-RNA was performed by a new multicyclic RT-PCR method described by Ishiyama et al[15].

Statistical analysis

Statistical analysis was performed using Student's t-test, and $p<0.05$ was considered significant.

Results

Prevalence of anti-HCV and HCV-RNA in patients having serological features compatible with AIH

We first examined both anti-HCV and HCV-RNA in the patients with clinical features compatible with AIH. The results (Table 1) show that 8 (30%) of the 27 patients tested were negative for both anti-HCV and HCV-RNA (group 1), 9 (33%) were positive for anti-HCV alone (group 2), and 10 (37%) were positive for both anti-HCV and HCV-RNA (group 3). Seven of the 17 patients having anti-HCV detected by the 1st-generation assay were negative for HCV-RNA. In the contrast, 10 of the 12 patients with anti-HCV detected only by the 2nd-generation assay, were positive for HCV-RNA (C-AIH). The anti-HCV antibody titers in the two patients with anti-HCV detected by the 2 nd-generation assay in the absence of HCV-

Table 2 Immunological parameters of the three patients groups

	Titers of α-HCV	HCV-RNA	γ-glo (g/dl)	IgG (mg/dl)	ANA	ADNA (OD value)
Group 1	-	−	3.66±1.23	3,912±1,160	4,066±7,960	0.230±0.880
Group 2	+	−	3.74±0.95	4,577±1,809	508±430	0.669±0.614
Group 3	+	+	2.43±0.52	2,960±982	285±419	0.456±0.248

*: NS, **: $p<0.05$, ***$p<0.001$.

RNA were lower (1.46 and 2.73 in cut-off-index) than the anti-HCV antibody titers in the group 3 patients.

Comparison of serologic features in AIH and C-AIH

To more precisely analyze the aberrant immune response in C-AIH, we compared the mean titers of serum immunoglobulins, IgG and ANA, which are considered important to the diagnosis of AIH in these three groups. As summarized in Table 2, levels of immunoglobulin and IgG in group 3 were significantly different from those in groups 1 and 2. Although there were no significant differences between their ANA titers, the mean titer in group 3 tended to be lower than in the other two groups. We found no significant differences in any of these studies between groups 1 and 2.

Genotype and quantity of HCV-RNA

With regard to the genomic subtype and the quantity of HCV-RNA in the C-AIH patients and randomly selected HCV-positive chronic liver disease patients who did not exhibit these AIH-like clinical manifestations (referred to as ordinary C-CAH), as shown in Table 3, no US-type HCV-RNA was found in any patients tested in this study; K2-type HCV-RNA was found in 30% of the C-AIH patients and 26% of the ordinary C-CAH patients; a mixed type, J plus K2, was found in none of the C-AIH patients and in only 13% of the ordinary C-CAH patients. Numerous patients in both groups had J-type HCV-RNA (70% in C-AIH and 61% in ordinary C-CAH). Thus, no significant differences were found between the prevalence of the individual subtypes between in these two patient groups.

Table 3 Prevalence of genomic subtypes of HCV-RNA

	AIH-like symptoms (n)	Prevalence (%)			
		US	K2	J	J+K2
41	+ (10)	0 (0)	3 (30)	7 (70)	0 (0)
	− (31)	0 (0)	8 (26)	19 (61)	4 (13)

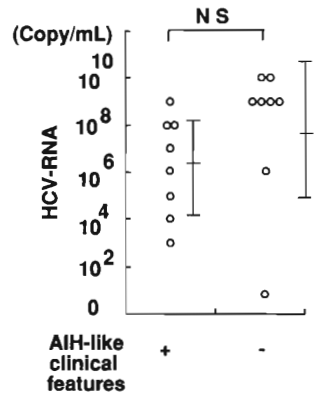

Figure Quantitative comparison of HCV-RNA in HCV-positive autoimmune-type CAH patients and patients with ordinary HCV-induced chronic liver disease.

We then examined the quantity of HCV-RNA in the two groups of HCV-positive patients. As shown in Figure, HCV-RNA levels in the C-AIH patients covered a were ranging, from 10^3 to 10^9, but we again found no significant difference between the quantity of HCV-RNA in patients with C-AIH and ordinary C-CAH.

Discussion

After the development of the assay for anti-HCV, the antibody was reported to have been demonstrated in AIH patients, 44% of AIH patients in Spain[16] and 86% of type-2 AIH patients in Italy[8]. Based on differences between the prevalence of anti-HCV in AIH in Italy and the UK, it was suspected[9] that certain geographic and/or genetic factors might influence the incidence of HCV-induced autoimmune-type CAH[17]. To clarify whether HCV infection is correlated with the development of AIH in Japanese patients, we investigated the prevalence of anti-HCV detected by either the 1st- or the 2nd-generation assay in association with the presence of HCV-RNA in previously diagnosed Japanese AIH patients. The results of the present study revealed the presence of anti-HCV in 63% of the patients by the 1st-generation assay and in 44.4% by the 2nd-generation assay, which is believed to be a more reliable system for detecting it[13]. Ten of the 12 patients in

whom anti-HCV was detected by the 2nd-generation assay had HCV-RNA in their sera, suggesting that the current HCV infection may have been responsible for the aberrant immune response in these 10 patients (37%) of the previously diagnosed Japanese AIH patients. Hence, HCV-RNA was only detected in group 3 (anti-HCV detected by 2nd generation assay) but not in patients in which anti-HCV was only detected by the 1st generation assay (group 2 in Table 2). The detection of anti-HCV in these patients may have been the result of the presence of nonspecific immunoglobulins, such as superoxide dismutase (SOD)[18]. This prediction was further supported by the fact that HCV-RNA-positive patients (group 3 in Table 1) had lower levels of serum immunoglobulins, IgG and ANA than those in the other two patient groups (Table 2). The present results, including the previous results in which many HCV infected patients were also positive for antibodies to the Gabriel OligoGT Random-hexanucleotide epitope (anti-GOR), which reacts with peptide derived from the host genome[19], suggest that, although the clinical parameters may not be exactly the same as those of pure AIH, in some patients HCV infection can cause signs and symptoms which resemble those of AIH.

To determine whether viral factors might affect the generation of aberrant immune responses in infected hosts, we further examined the genotype and the quantity of HCV-RNA in both anti-HCV positive autoimmune-type CAH (C-AIH) and chronic hepatitis type patients without AIH-like clinical manifestations (ordinary C-CAH). As shown in Figure, no specific deviations in dividual genotype from ordinary C-CAH were found in C-AIH (Table 3). In regard to HCV-RNA genotype, it has been reported[20] that although type-3 HCV (identical to K2 type HCV) was highly detected in LKM-positive patients, no such specific deviation was found in anti-LKM1-antibody negative patients. None of the patients tested in this study were positive for anti-LKM1 antibody (data not shown). All these findings indicate the absence of any specific HCV genotype responsible for the generation of the aberrant immune response in Japanese infected hosts. Furthermore, we also found that the quantity of HCV-RNA was quite similar in these two patient groups (Figure). Consequently, we think that these results indicate that certain infected hosts factors, rather than viral factors, may play some important role(s) in the generation of the aberrant immune response. As reported earlier[21][22], the HLA-DR4 phenotype is frequently detected in Japanese AIH patients. Further studies, including determination of the HLA phenotype, will be required to define patient's genetic factors that may contribute to generating the autoimmune phenomenon as a result of HCV infection.

In conclusion, the results of this study clearly demonstrate that at least 37% of patients with previously diagnosed type-1 Japanese AIH patients possess the evidence of current infection by HCV.

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C型肝炎ウイルス感染による自己免疫性肝炎様臨床症状の解析

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C型肝炎ウイルス（HCV）感染の測定が可能となって以来，自己免疫性肝炎患者（AIH）においてHCV抗体も陽性の症例が多く認められるようになっている．今回我々は27人の抗核抗体陽性，高γ-グロブリン，IgG高値のAIHの診断基準をみたす患者においてELISA法による第1世代と第2世代の抗HCV抗体，およびPCR法によるHCV-RNAを測定し検討した．その結果，抗HCV抗体，HCV-RNAともに陰性8例（30%；1群），抗HCV抗体のみ陽性9例（33%；2群），両者とも陽性10例（37%；3群）であった．3群では他の2群に比べて抗HCV抗体の抗体価が高く，γ-グロブリン値が低い傾向を示した．以上の結果から，過去にAIHと診断された患者の37%はHCVの持続感染による慢性肝炎であることが示唆された．さらにこれらの患者に感染したHCVのウイルス学的な特徴を検討するため，HCV-RNAのサブタイプを測定しAIHを呈さないC型慢性肝炎患者群とで比較検討を加えたが，明らかなウイルス学的な特徴は得られなかった．

以上より，HCV感染により誘導された自己免疫性現象は，感染ウイルスの違いによるものではなく感染宿主側の因子により規定されていると考えられた．