Acta Medica Okayama

Volume 47, *Issue* 5 1993 *Article* 3

OCTOBER 1993

Autoimmune responses as assessed by hypergammaglobulinemia and the presence of autoantibodies in patients with chronic hepatitis C.

Hirofumi Kawamoto* Kohsaku Sakaguchi† Akinobu Takaki‡
Shin Ogawa** Takao Tsuji††

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

Autoimmune responses as assessed by hypergammaglobulinemia and the presence of autoantibodies in patients with chronic hepatitis C.*

Hirofumi Kawamoto, Kohsaku Sakaguchi, Akinobu Takaki, Shin Ogawa, and Takao Tsuji

Abstract

We investigated autoimmunity, as assessed by hypergammaglobulinemia and the presence of autoantibodies including anti-nuclear antibodies (ANA) and anti-liver membrane antibodies (LMA), in 149 patients with chronic hepatitis C, 55 patients with chronic hepatitis B and 11 patients with autoimmune hepatitis. There was no significant difference in the incidence of these autoantibodies between chronic hepatitis C and chronic hepatitis B. Nine patients with chronic hepatitis C satisfied the serological criteria of autoimmune hepatitis (ANA positive and gammaglobulin or serum IgG greater than 2500 mg/dl), but none of the patients with chronic hepatitis B met the criteria. This suggests that autoimmunity is greater in chronic hepatitis C than in chronic hepatitis B. Of the 9 patients with chronic hepatitis C, all 4 patients tested for human leukocyte antigen (HLA) phenotype had HLA-DR4, which is known to be associated with autoimmune hepatitis in Japanese patients. We believe that hepatitis C virus (HCV) infection enhances the initiation and perpetuation of autoimmunity in susceptible individuals.

KEYWORDS: chronic hepatitis C, autoimmune hepatitis, anti-nuclear antibodyies, anti-liver membrane antibodies HLA

---

*PMID: 8273454 [PubMed - indexed for MEDLINE]

Acta Med Okayama 47 (5) 305-310 (1993)

Autoimmune Responses as Assessed by Hypergammaglobulinemia and the Presence of Autoantibodies in Patients with Chronic Hepatitis C

Hirofumi Kawamoto, Kohsaku Sakaguchi, Akinobu Takaki, Shin Ogawa and Takao Tsuji

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

We investigated autoimmunity, as assessed by hypergammaglobulinemia and the presence of autoantibodies including anti-nuclear antibodies (ANA) and anti-liver membrane antibodies (LMA), in 149 patients with chronic hepatitis C, 55 patients with chronic hepatitis B and 11 patients with autoimmune hepatitis. There was no significant difference in the incidence of these autoantibodies between chronic hepatitis C and chronic hepatitis B. Nine patients with chronic hepatitis C satisfied the serological criteria of autoimmune hepatitis (ANA positive and gammaglobulin or serum IgG greater than 2500 mg/dl), but none of the patients with chronic hepatitis B met the criteria. This suggests that autoimmunity is greater in chronic hepatitis C than in chronic hepatitis B. Of the 9 patients with chronic hepatitis C, all 4 patients tested for human leukocyte antigen (HLA) phenotype had HLA-DR4, which is known to be associated with autoimmune hepatitis in Japanese patients. We believe that hepatitis C virus (HCV) infection enhances the initiation and perpetuation of autoimmunity in susceptible individuals.

Key words : **chronic hepatitis C, autoimmune hepatitis, anti-nuclear antibodies, anti-liver membrane antibodies, HLA**

The development of methods for detecting hepatitis C virus (HCV) (1, 2) has allowed the demonstration of the prevalence of HCV in patients with autoimmune hepatitis (AIH) (3-5), which is serologically characterized by a high level of serum gammaglobulin and the presence of autoantibodies (6, 7). A relatively high prevalence of anti-HCV C100-3 antibodies has been reported in type 1 AIH (positive for anti-nuclear antibodies, ANA) (3, 4) and especially in type 2 AIH (positive for anti-liver-kidney microsome antibodies, LKM) (5). Furthermore, autoimmune mechanisms appear to be involved in chronic hepatitis type C, since antibody cross-reactivity with both cell-nuclear protein and nucleocapsid protein of the virus has been detected in the serum of patients (8). These findings suggest that HCV infection is involved in autoimmune responses.

In this study, to investigate autoimmunity induced by HCV infection, we measured the level of gammaglobulin and autoantibodies including anti-liver membrane antibodies (LMA) and anti-nuclear antibodies (ANA) in patients with chronic hepatitis C, and compared them with those in patients with chronic hepatitis B and autoimmune hepatitis. Also, we assessed the immunological background of chronic hepatitis C patients with associated autoimmunity.

* To whom correspondence should addressed.

Subjects and Methods

Patients. One hundred forty-nine patients (91 men and 58 women) with chronic hepatitis C and 55 patients (41 men and 14 women) with chronic hepatitis B, who were admitted to our hospital between October, 1989 and December, 1991, were examined. The results obtained from these patients were compared with those obtained from 11 patients (1 man and 10 women) with autoimmune hepatitis.

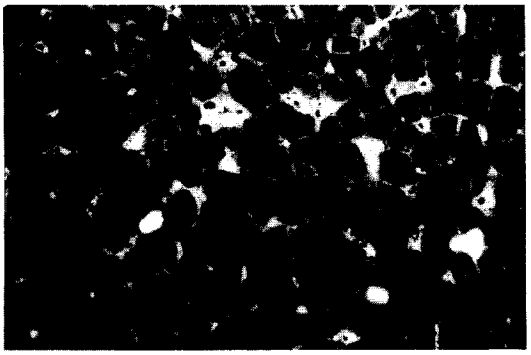

Fig. 1 Typical immunofluorescence pattern of anti-liver membrane antibody (LMA). Linear fluorescence along the hepatocellular membrane is observed.

The patients were diagnosed on the basis of clinical and/or typical findings in liver biopsy specimens according to a review by an international group (9). The 149 patients with chronic hepatitis C included 43 with chronic persistent hepatitis (CPH), 29 with chronic active hepatitis 2A (CAH2A), 55 with chronic active hepatitis 2B (CAH2B), and 22 patients in which liver biopsy was not performed. The 55 patients with chronic hepatitis B included 14 with CPH, 10 with CAH2A, 25 with CAH2B and 6 patients in which liver biopsy was not performed.

Patients with positive anti-HCV antibodies and/or HCV-RNA were diagnosed as having chronic hepatitis C. Patients who were positive for hepatitis B surface antigen (HBsAg) were diagnosed as having chronic hepatitis B. The patients with autoimmune hepatitis satisfied the criteria of autoimmune hepatitis and were confirmed to be negative for HBsAg and anti-HCV antibodies. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglobulin, IgG, IgA, and IgM were measured on admission.

Hepatitis virus markers. Hepatitis B surface antigen was determined by an enzyme-linked immunosorbent assay or radioimmunoassay. Anti-HCV C100-3 antibodies and anti-HCV antibodies detected by the second generation kit were tested by an enzyme-linked immunosorbent assay (Ortho diagnostic system, NJ and Dainabot, Tokyo, respectively). Hepatitis C virus ribonucleic acid (HCV-RNA) in serum was detected by the method of reverse transcriptase-polymerase chain reaction (RT-PCR) using the 5'-non-coding region as a primer (10).

Autoantibodies. ANA was detected by an indirect immunofluorescent method using laryngeal carcinoma cells, HEp-2 as substrate. The titer was established by sequential 2-fold dilution of serum diluted 1:10 until an end-point was reached. Values higher than a titer of 1:20 were regarded as positive.

Anti-liver membrane antibodies (LMA) were also identified in cryostat sections of rat liver by an indirect immunofluorescent method described previously (11). The cold acetone fixed cryostat sections were allowed to react with 1:5 diluted serum for 1h, followed by three washes with phosphate buffered saline (PBS). The rat liver sections were then incubated with fluorescein isothiocyanate (FITC)-labelled anti-human immunoglobulin (DAKO Japan, Tokyo, Japan) for 1h. Following three additional washes with PBS, the sections were mounted in glycerol-carbonate-bicarbonate buffer. The immunofluorescence pattern was assessed by fluorescent microscopy. LMA was judged to be positive when the linear fluorescence was observed along the hepatocyte membrane, as shown in Fig. 1.

HLA phenotype. HLA phenotype was investigated in some patients with autoimmune hepatitis and anti-HCV antibody-positive patients who satisfied the immunological criteria of autoimmune hepatitis. HLA typing of HLA class I and class II was performed by the method of microcytotoxicity using TERASAKI HLA TRAY (One Lambda, CA, USA) (12).

Statistical analysis. Student's t-test was used to determine the significance of differences between means. Differences between incidences were evaluated by χ^2 test.

Results

Liver biopsy was performed in 127 patients out of 149 patients with chronic hepatitis C. Forty-three (33.8 %), 29 (22.8 %), and 55 (43.3 %) patients were histologically diagnosed as having CPH, CAH2A, and CAH2B, respec-

Table 1 Clinical profiles of examined cases

	AIH	CH-C	CH-B
No. of cases	11	149	55
Sex (M:F)	1:10	91:58	41:14
Age (years)	46.5 ± 18.8	52.0 ± 11.3	34.0 ± 11.2
AST (IU/l)	232 ± 173	91 ± 94	132 ± 139
ALT (IU/l)	298 ± 300	129 ± 145	229 ± 251
γ-Glb (g/dl)	3.07 ± 0.70	1.43 ± 0.46[a]	1.25 ± 0.37[b]
IgG (mg/dl)	3901.6 ± 916.6	2171.5 ± 602.9[c]	1945.8 ± 476.0[d]
IgA (mg/dl)	372.8 ± 110.4	308.1 ± 121.5	294.1 ± 115.6
IgM (mg/dl)	359.5 ± 434.8	183.4 ± 131.5	203.8 ± 125.4
IgG and/or γ-Glb ≧ 2.5 g/dl	11 (100 %)	32 (21.5 %)	7 (12.7 %)
ANA	11 (100 %)	34 (22.8 %)	17 (30.9 %)
LMA	8 (72.7 %)	25 (16.8 %)	9 (16.4 %)

AIH: autoimmune hepatitis, CH-C: chronic hepatitis C, CH-B: chronic hepatitis B, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-Glb: gammaglobulin, ANA: anti-nuclear antibody, LMA: anti-liver membrane antibody

$a \sim b$: $p < 0.01$, $c \sim d$: $p < 0.01$

tively, by the presence of typical findings in liver biopsy specimens. Of the 55 patients with chronic hepatitis B, liver biopsy was performed in 49 patients. Fourteen (28.6 %), 10 (20.4 %), and 25 (51.0 %) were also histologically diagnosed as having CPH, CAH2A, and CAH2B, respectively. There were no significant difference in the distribution of histological stages between chronic hepatitis C and chronic hepatitis B.

Clinical features and laboratory data of the patients investigated in this study are listed in Table 1. The mean level of serum gammaglobulin in the patients with chronic hepatitis C was 1.43 ± 0.46 g/dl, which was significantly higher than that in the patients with chronic hepatitis B, 1.25 ± 0.37 g/dl ($p < 0.01$). In contrast, the mean values of AST and ALT in the chronic hepatitis C patients were lower than those in the chronic hepatitis B patients. An increase in gammaglobulin level above 2.5 g/dl or IgG level above 2500 mg/dl was observed in 32 (21.5 %) patients with chronic hepatitis C and 7 (12.7 %) patients with chronic hepatitis B.

Thirty-four (22.8 %) of the 149 patients with chronic hepatitis C and 17 (30.9 %) of the 55 patients with chronic hepatitis B were positive for ANA. Twenty-five (16.8 %) patients with chronic hepatitis C and 9 (16.4 %) patients with chronic hepatitis B were positive for LMA. There were no significant differences in the incidence of ANA and LMA between patients with chronic hepatitis C and those with chronic hepatitis B. An increase in ANA titer above 1:1280 was observed in 4 patients with chronic hepatitis C, whereas all titers were below 320 in the patients with chronic hepatitis B. ANA was detected in all 11 patients with autoimmune hepatitis and LMA was detected in 8 (72.7 %). Also, it is noteworthy that 11 (7.4 %) of the 149 patients with chronic hepatitis C were pesitive for both ANA and LMA. But, none of the 55 patients with chronic hepatitis B reacted positively to ANA or LMA. (Table 2-a, b). The mean values of gammaglobulin and IgG in chronic hepatitis C patients positive for both ANA and LMA were higher than those in any other group, whereas there was no significant difference in the level of AST and ALT among these patient groups.

Table 2-a Clinical profiles of patients with chronic hepatitis type C classified by presence or absence of ANA and LMA

	LMA (+) ANA (+)	LMA (+) ANA (−)	LMA (−) ANA (+)	LMA (−) ANA (−)
No. of cases	11 (7.4 %)	14 (9.4 %)	23 (15.4 %)	101 (67.7 %)
Sex (M:F)	4:7	9:5	9:14	69:32
Age	57.5 ± 14.0	55.1 ± 10.2	51.1 ± 11.5	51.1 ± 11.1
AST (IU/l)	87 ± 63	78 ± 45	88 ± 56	93 ± 109
ALT (IU/l)	84 ± 65	114 ± 86	122 ± 96	137 ± 166
γ-Glb (g/dl)	2.08 ± 0.80	1.40 ± 0.41	1.43 ± 0.44	1.37 ± 0.36
IgG (mg/dl)	3139.7 ± 1052.1[a]	2011.8 ± 570.1[b]	2109.0 ± 491.3[c]	2111.1 ± 485.5[d]
IgA (mg/dl)	348.0 ± 176.2	313.6 ± 106.4	237.8 ± 92.3	320.2 ± 119.0
IgM (mg/dl)	348.0 ± 304.8	183.2 ± 135.2	230.2 ± 136.5	155.1 ± 78.9

$a \sim b$: $p < 0.01$, $a \sim c$: $p < 0.01$, $a \sim d$: $p < 0.01$. LMA, ANA, AST: See Table 1.

Table 2-b Clinical profiles of patients with chronic hepatitis type B classified by presence or absence of ANA and LMA

	LMA (+) ANA (+)	LMA (+) ANA (−)	LMA (−) ANA (+)	LMA (−) ANA (−)
No. of cases	0 (0.0 %)	9 (16.4 %)	17 (30.9 %)	29 (52.7 %)
Sex (M:F)	—	6:3	10:7	25:4
Age	—	34.7 ± 14.8	29.3 ± 7.4	36.8 ± 11.3
AST (IU/l)	—	167 ± 100	127 ± 114	124 ± 165
ALT (IU/l)	—	248 ± 181	243 ± 226	216 ± 289
γ-Glb (g/dl)	—	1.26 ± 0.39	1.19 ± 0.35	1.29 ± 0.39
IgG (mg/dl)	—	2000.3 ± 388.9	1826.9 ± 385.2	2010.0 ± 562.4
IgA (mg/dl)	—	371.8 ± 125.9	250.8 ± 104.2	293.8 ± 107.0
IgM (mg/dl)	—	252.9 ± 118.4	158.2 ± 58.1	217.0 ± 154.6

LAM, ANA, AST, ALT: See Table 1.

Furthermore, 9 of the 149 patients with chronic hepatitis C satisfied the serological criteria for the diagnosis of autoimmune hepatitis (serum gammaglobulin and/or IgG level ≧ 2.5 g/dl or more, and the presence of autoantibodies including ANA). But, none of the 55 patients with chronic hepatitis B met the criteria (Table 3). HCV-RNA was detected in all 9 patients. The great majority of patients with chronic hepatitis C or chronic hepatitis B show a selflimited response or no autoimmune response, but some patients with chronic hepatitis C may exhibit autoimmunity similar to autoimmune hepatitis.

To clarify the immunogenetical background, 4 patients with chronic hepatitis C, who also satisfied the serological criteria of autoimmune hepatitis, and 7 of the 11 patients with autoimmune hepatitis were studied for HLA association. All 4 patients with chronic hepatitis C with associated autoimmunity phenomena and 6 of the 7 patients with autoimmune hepatitis had HLA-DR4 (Table 4), which is known to be strongly associated with autoimmune hepatitis in Japanese patients (13).

Table 3 Patients with positive ANA and IgG ≧ 2500 mg/dl

No.	Age	Sex	Diagnosis	ANA	LMA	IgG (mg/dl)	Histology
1.	49	F	CH-C	1280	+	4422	CAH2B
2.	59	F	CH-C	80	+	4738	CAH2B
3.	48	F	CH-C	80	+	3614	CAH2A
4.	57	M	CH-C	20	+	3854	CAH2B
5.	68	M	CH-C	20	+	2552	CAH2A
6.	53	F	CH-C	2560	−	3335	CAH2B
7.	50	F	CH-C	1280	−	2581	CAH2B
8.	48	M	CH-C	160	−	3238	CAH2B
9.	52	M	CH-C	40	−	2577	CAH2B

ANA, LMA, CH-C: See Table 1.

Discussion

Several studies investigating the prevalence of anti-HCV antibodies in patients with autoimmune hepatitis have demonstrated a relatively high prevalence of anti-HCV antibodies in patients with autoimmune hepatitis type 1 (3, 4) and autoimmune hepatitis type 2 (5). In contrast, few studies have been performed to analyze the autoimmune response in patients with chronic hepatitis C confirmed by the presence of anti-HCV antibodies and/or HCV-RNA. In this study, we demonstrated that the autoimmune responses, as judged from a high level of gammaglobulin and the presence of autoantibodies including ANA and LMA, may be more marked in patients with chronic hepatitis C than in patients with chronic hepatitis B. The population of chronic hepatitis C patients appears to include a subset of patients with accompanying autoimmune phenomena similar to those observed in patients with autoimmune hepatitis.

Anti-nuclear antibodies are the most characteristic marker for the diagnosis of autoimmune hepatitis (6, 7). Anti-liver membrane antibodies have also been found to be associated with autoimmune hepatitis (11, 14). These autoantibodies are employed in the serodiagnosis of autoimmune hepatitis (15). In our patients with autoimmune hepatitis, ANA was detected in all 11, and both ANA and LMA were simultaneously detected in 8 (72.7

Table 4 HLA typing of autoimmune hepatitis patients and chronic hepatitis C patients who satisfied the serological criteria of autoimmune hepatitis

No.	Age	Sex	Diagnosis	ANA	LMA	γ-Glb	IgG	HLA-A	HLA-B	HLA-C	HLA-DR
1	19	F	AIH	2560	+	2.1	2968	A24, —	BW52, BW61	CW3, —	DR2, DR4
2	58	M	AIH	640	−	3.0	3850	A26, —	BW61, BW62	CW3, —	DR4, DR9
3	42	F	AIH	160	+	3.1	3175	A2, A24	BW46, BW54	CW1, —	DR4, DRW8
4	62	F	AIH	1280	+	2.7	3940	A2, AW33	BW52, B44	—, —	DR2, DRW6
5	53	F	AIH	160	+	4.6	6050	A24, —	BW52, BW54	CW8, —	DR2, DR4
6	41	F	AIH	1280	−	3.2	4196	A24, A31	B51, BW61	CW3, —	DR2, DRW8
7	53	F	AIH	1280	−	2.6	—	A2, A24	BW52, BW55	CW1, —	DR2, DR4
8	53	F	CH-C	2560	−	2.6	3335	A2, A24	B35, BW52	CW3, —	DR2, DR4
9	49	F	CH-C	1280	+	3.5	4422	A2, A24	BW54, BW61	CW1, CW8	DR4, —
10	59	F	CH-C	80	+	3.4	4738	A24, A31	BW46, BW54	CW1, —	DR4, DRW8
11	48	M	CH-C	160	−	2.3	3238	A2, A24	B51, BW59	CW1, —	DR4, DRW6

HLA: human leukocyte antigen. Other abbreviations: See Table1.

%).

These autoantibodies have also been found in a proportion of patients with viral hepatitis (11, 16), although the autoantibodies observed in the clinical course of viral hepatitis are generally weaker and more transiently positive than those observed in autoimmune hepatitis (17, 18). The great majority of patients with chronic hepatitis C or chronic hepatitis B who were investigated in this study had a negative or low titer of autoantibodies. Thus there might be differences in autoimmune responses in patients with autoimmune hepatitis and those with viral hepatitis.

However, it is noteworthy that an increase in the titer of ANA above 1280 was observed in 4 patients with chronic hepatitis C, and both ANA and LMA were detected in 11 patients with chronic hepatitis C. Furthermore, patients with chronic hepatitis C demonstrate autoimmune responses, as observed in autoimmune hepatitis, as judged from a high level of serum gammaglobulin and the presence of autoantibodies, in contrast to none of the patients with chronic hepatitis B.

In previous studies on the occurrence of anti-HCV antibodies in autoimmune hepatitis, the non-specific occurrence of pseudo-positive cases accompanied by hypergammaglobulinemia was reported to be frequent (19, 20). However, our 9 chronic hepatitis C patients with autoimmune phenomena were positive for not only anti-HCV antibodies but also HCV-RNA. These findings suggest that the population of chronic hepatitis C patients includes a subset of patients with associated autoimmunity, and that HCV infection may enhance the initiation and perpetuation of autoimmune responses in some patients with chronic hepatitis C.

Recently, it was reported that hepatitis A virus (HAV) infection may be a trigger for ANA-positive type 1 autoimmune hepatitis in susceptible individuals (21). Our chronic hepatitis C patients who satisfied the serological criteria of autoimmune hepatitis had HLA-DR4, which has been reported to be associated with autoimmune hepatitis (17), suggesting that the autoimmunity observed in chronic hepatitis C is associated with HLA-DR4 specificity. The occurrence of autoimmunity in patients with chronic hepatitis C may be related to genetic factors, although the mechanisms by which HCV infection is related to the pathogenesis of AIH are still unknown.

We speculate that HCV infection is related to the mechanism of autoimmune responses in some patients with chronic hepatitis C. Interferon-α, which is generally accepted as being beneficial in the treatment of chronic hepatitis C, has been reported to induce the development of autoantibodies and seriously exacerbate autoimmune hepatitis type 1 and type 2 (22, 23). Unfortunately, at present the optimal treatment strategy for chronic hepatitis C associated with autoimmunity remains to be established. Further study on the mechanisms may provide insight into the pathogenesis of not only autoimmune hepatitis but also other autoimmune diseases in which viral infection has been implicated.

Acknowledgment. We thank Drs. N. Koide, H. Shimomura for their supports and valuable discussions. This work was supported by a Grant-in-Aid for non-A, non-B hepatitis research from the Ministry of Health and Welfare of Japan (1989-1992).

References

1. Choo QL, 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**, 359-362.
2. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradely DW and Houghton M: An assay for circulating antibodies to a major etioligic virus of human non-A non-B hepatitis. Science (1989) **244**, 362-364.
3. Esteban JI, Esteban R, Viladomiu L, Lσpez-Talavera JC, González A, Hernández JM, Roget M, Vargas V, Aenescá J, Buti M, and Buardia J: Hepatitis C virus antibodies among risk groups in Spain. Lancet (1989) **2**, 294-297.
4. McFarlane IG, Smith HM, Johnson PJ, Bray GP, Vergani D, Williams R: Hepatitis C virus antibodies in chronic active hepatitis: Pathogenetic factor or false-positive result? Lancet (1990) **335**, 754-757.
5. Lenzi M, Ballardini G, Fusconi M, Cassani F, Selleri L, Volta U, Zauli D, Bianti FB: Type 2 autoimmune hepatitis and hepatitis C virus infection. Lancet (1990) **335**, 258-259.
6. Mackay IR, Taft LI, Cowling DC: Lupoid hepatitis. Lancet (1956) **2**, 1323-1326.
7. Mackay IR: Chronic active hepatides. Front Gastrointest Res(1975) **1**, 147-187.
8. Mishiro S, Hoshi Y, Takeda K, Yoshizawa A, Gotanda T, Takahashi K, Akahane Y, Yoshizawa H, Okamoto H, Tsuda F, Peterson DA, and Muchmore E: Non-A non-B hepatitis specific antibodies directed at host-derived epitope: implication for an autoimmune process. Lancet (1990) **336**, 1400-1403.
9. Review by an international group: Acute and chronic hepatitis revised. Lancet (1977) **2**, 914-919.
10. Morii K, Shimomura H, Nakagawa H, Hasui T, and Tsuji T: Anti-C100-3 antibody status, viral genomic sequences, and clinical features in chronic hepatitis patients with hepatitis C virus RNA in sera. Acta Med Okayama (1992) **46**, 285-293.
11. Tsuji T, Araki K, Naitou K, Inoue J, and Nagashima H: Detection and characterization of antibody to liver cell membrane in sera from patients with chronic active liver diseases. Acta Med Okayama (1979) **33**, 61-66.

12. Terasaki PI, Bernoco D, Park MS, Ozturk G, and Iwaki Y: Microdroplet testing for HLA-A, -B, -C, and -D antigens. Am J Clin Pathol (1978) **69**, 103-120.
13. Seki T, Kiyosawa K, Inoko H, and Ota M: Association of autoimmune hepatitis with HLA-Bw54 and DR4 in Japanese patients. Hepatology (1990) **12**, 1300-1304.
14. Hopf U, Meyer zum Buschenfelde KH, and Arnold W: Detection of a liver-membrane antibody in HBsAg-negative chronic active hepatitis. N Engl J Med (1976) **294**, 578-582.
15. Manns MP: Autoimmune disease of the liver. Clin Lab Med (1992) **12**, 25-40.
16. Tsuji T: Organ-specificity and clinical significance of antibody to insoluble liver cell membrane antigen (anti-LM) in sera from patients with chronic active liver diseases. Jpn J Clin Immun (1985) **7**, 8-17.
17. Tsuji T, Takahashi K, Sawahara M, Matsuura E, and Yamamoto I: Detection and clinical significance of acetone-insoluble liver cell membrane antigen in sera of patients with chronic active liver diseases. Gastroenterol Jpn (1985) **20**, 37-47.
18. Tsuji T: Anti-liver cell membrane antibodies: Detection and clinical significance of anti-LSP and LMA. Kan Tan Sui (1985) **11**, 599-606.
19. Boudart D, Lucas J-C, Muller J-Y, Carrer DL, Planchon B, and Harousseau J-C: False positive hepatitis C virus antibody tests in paraproteinemia (letter to the editor). Lancet (1990) **336**, 63.
20. Ikeda Y, Toda G, Hashimoto N, and Kurokawa K: Antibody to superoxide dismutase, autoimmune hepatitis, and antibody tetes for hepatitis C virus. Lancet (1990) **335**, 1345-1346.
21. Vento S, Garofano T, DiPerri G, Dolci L, Concia E, and Basseti D: Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. Lancet (1991) **337**, 1183-1187.
22. Papo T, Marcellin P, Bernuau J, Durand F, Poynard T, and Benhamou JP: Autoimmune Chronic hepatitis exacerbated by interferon. Ann Intern Med (1992) **116**, 51-53.
23. Vento S, Di Perri G, Garofano T, Cosco L, Concia E, Ferraro T, and Bassetti D: Hazards of interferon therapy for HBV-seronegative chronic hepatitis (Letter). Lancet (1989) **2**, 926.

Received January 4, 1993, accepted February 15, 1993.