Hepatitis C virus infection can mimic type 1 (antinuclear antibody positive) autoimmune chronic active hepatitis

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Abstract

Hepatitis C virus (HCV) has been shown to induce anti-liver-kidney microsomal-1 (LKM1) antibody positive chronic active hepatitis, simulating type 2 autoimmune chronic active hepatitis. The cases of five patients presenting with features of type 1 (antinuclear antibody positive) autoimmune chronic active hepatitis and extrahepatic autoimmune manifestations, in whom immunosuppressive treatment had no effect on liver disease are presented. In these patients, HCV infection could be shown by the presence in serum of anti-HCV antibodies and HCV-RNA detected by polymerase chain reaction. These cases suggest the following: (a) chronic HCV infection can mimic type 1, as well as type 2, autoimmune chronic active hepatitis; (b) HCV infection might be systematically sought in patients presenting with features of type 1 autoimmune chronic active hepatitis, with special care in patients who are unresponsive to immunosuppressive treatment.

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It has recently been suggested that hepatitis C virus (HCV) may induce various autoimmune abnormalities.¹⁻³ Among them, type 2 – that is anti-liver-kidney microsomal-1 antibody positive – autoimmune chronic active hepatitis has been shown to be related to chronic HCV infection in more than 50% of the cases in adult patients.⁴⁻⁶ The purpose of this study is to report the cases of five patients presenting as type 1 – that is antinuclear/antismooth muscle antibody positive – autoimmune chronic active hepatitis, associated with extrahepatic autoimmune manifestations, in whom HCV infection was implicated once tests for anti-HCV antibodies became available.

Case reports

LIVER ABNORMALITIES

Four women and one man (mean(SD) age 58.0(7.9) years, range 49–70) seen for the first time in the Liver Disease Unit of our hospital between November 1981 and October 1990, presented with chronic active hepatitis and extrahepatic immunological manifestations. Serum biochemical results for all patients are

shown in Table I. None of the patients were jaundiced. Liver biopsy specimens showed chronic active hepatitis in five cases, with cirrhosis in three of them. Three patients had mild to marked steatosis and portal lymphoid infiltrates were seen in one of them (Table II).

AUTOIMMUNE ABNORMALITIES

Serum antinuclear antibodies were present in all the patients, the titres ranging from 1/100 to 1/2560 before treatment. Low titres of anti-smooth muscle antibodies were also found in the five patients. Serum antithyroglobulin and antithyroid microsomal antibodies were detected in one patient, as well as anti-prothrombinase antibodies in one. One patient had a low titre (1/10) of serum anti-mitochondrial antibodies, with the antinuclear antibodies giving an anticentromere antibody pattern (Table III). Extrahepatic autoimmune clinical manifestations were present in all the patients – that is, autoimmune thyroiditis in

TABLE I Serum biochemistry results for all patients

	Mean(SD)	Normal value
Aspartate aminotransferase (IU/l)	182 (142)	(<40)
Alanine aminotransferase (IU/l)	211 (112)	(<45)
Alkaline phosphatase (IU/l)	98 (63)	(<115)
Gamma glutamyl transpeptidase (IU/l)	99 (93)	(<65)
Bilirubin (μmol/l)	14.4 (6.7)	
Gamma globulin (g/l)	18.3 (2.8)	

TABLE II Clinical, histological, and biochemical findings

Patient	Age (y)/sex	AST/ALT (IU/l)	Liver histology
1	59/M	75/56	CAH, mild steatosis
2	59/F	180/291	CAH with cirrhosis
3	70/F	450/320	CAH, portal lymphoid infiltrates, moderate steatosis
4	53/F	150/293	CAH with cirrhosis, marked steatosis
5	49/F	55/93	CAH with cirrhosis

AST=aspartate aminotransferase; ALT=alanine aminotransferase; CAH=chronic active hepatitis.

TABLE III Immunological findings

Patient	Extrahepatic disorders	ANA-ASM (titres)	Associated antibodies
1	Lichen planus	1/320-1/40	–
2	Thrombocytopenic purpura	1/320-1/40	–
3	CREST syndrome	1/2560-1/80	Anticentromere
4	Thrombocytopenic purpura	1/320-1/40	Antimitochondrial
5	Autoimmune thyroiditis	1/100-1/20	Antithyroid microsomal antithyroglobulin

ANA=antinuclear antibodies; ASM=anti-smooth muscle antibodies.

one case, antibody positive thrombocytopenic purpura in two cases, CREST syndrome without histological features of primary biliary cirrhosis in one case, and oral and genital lichen planus in one case.

In these five patients, the association of histologically proved chronic active hepatitis at liver biopsy, serum antinuclear and antismooth muscle antibodies, and extrahepatic autoimmune disorders was consistent with the diagnosis of type 1 autoimmune chronic active hepatitis. Long term immunosuppressive treatment (three months to three years), including corticosteroids and azathioprine and, in one case, cyclosporin, was thus administered to the five patients. In spite of a moderate and transitory decrease in alanine amino transferase (ALT) activities this treatment had no effect on the evolution of liver disease, as assessed by the level of transaminase activities, in any of the patients.

HCV INFECTION

The unresponsiveness to treatment, the discovery of HCV as the main agent of non-A, non-B hepatitis,⁷ and the recent observation of its association with autoimmune abnormalities, led us to reconsider the diagnosis and to look for HCV infection. Indeed, sensitive and specific HCV tests (second generation ELISA, Ortho Diagnostic Systems), when available, allowed detection of anti-HCV antibodies, giving high optical densities in the serum of the five patients. This was confirmed by second generation four antigen RIBA (Ortho Diagnostic Systems) in all cases.

In addition, HCV-RNA was sought in serum by a modified cDNA nested polymerase chain reaction procedure, using a highly conserved nucleotide sequence from the 5' untranslated region of the HCV genome. RNA was extracted from 50 µl of plasma and converted into cDNA with 10 pmol of a reverse transcriptase primer HMC2 (5'GGCA-CTCGCAAGCACCCTAT3', nucleotide pos-

ition 296 to 315 in the HCV genome). The cDNA was amplified by *Taq* DNA polymerase for 30 cycles (94°C, 1 min; 55°C, 1 min; 72°C, 2 min) with 5 pmol each of external primers HMC1 (5'CACCATGAATCACTCCCC-TG3', position 28 to 47) and HMC2, followed by 30 cycles (94°C, 1 min; 55°C, 1 min; 72°C, 2 min) with 20 pmol each of internal primers CPC1 (5'TGGCGTTAGTATGAGTGT-CG3', position 86 to 105) and CPC2 (5'GACCCAACACTACTCGGCTA3', position 251 to 270), using the recently described variant technique that has been proved to reduce greatly the risk of contamination.⁸ Furthermore, all polymerase chain reaction procedures were carried out so as to avoid contamination of the samples and carryover of DNA. The specificity of the amplification was assessed by restriction analysis of the polymerase chain reaction products, performed by digesting 10 µl of the PCR mixture with 1 µl of enzymes *Rsa*I, *Mbo*I and *Sma*I. The polymerase chain reaction products and their restriction fragments were analysed by electrophoresis on a 6% polyacrylamide gel with ethidium bromide staining (Fig 1). Using this method, our five patients were shown to be seropositive HCV-RNA.

Discussion

It has been suggested that HCV induces autoimmune abnormalities, such as Sjögren's syndrome,¹ serum anti-GOR autoantibodies that have been found in 80% of patients with chronic hepatitis C,² or anti-liver-kidney microsomal 1 antibody positive (type 2 'autoimmune') chronic active hepatitis.³⁻⁶ Type 2 autoimmune hepatitis seems to follow two distinct patterns. The first one is characterised by the young age of the patients at onset, a female predominance, high titres of anti-liver-kidney microsomal 1 antibodies, a good response to immunosuppressive therapy, and the absence of HCV markers (type 2a). The second pattern is observed in older patients, with a lower female/male ratio, lower anti-liver-kidney microsomal 1 antibody titres, a poor response to immunosuppressive treatment, and an association with HCV infection (type 2b).³ The relation between HCV infection and type 1 autoimmune chronic active hepatitis remains controversial. The high prevalence of anti-HCV antibodies detected by first generation ELISA in patients with type 1 autoimmune hepatitis has been shown to correspond with false-positive results in most cases, often because of hypergamma-globulinaemia.⁹ The presence of antinuclear antibodies has also been reported recently in patients with proved hepatitis C viral chronic active hepatitis; however, no other features suggestive of autoimmune chronic active hepatitis, such as multisystem involvement, were noted in these patients.¹⁰ In this study, therefore, we bring evidence that antinuclear/antismooth muscle antibody-positive chronic active hepatitis associated with extrahepatic autoimmune manifestations (thus mimicking type 1 autoimmune chronic active hepatitis),

Figure Polyacrylamide gel electrophoresis after ethidium bromide staining showing the 185 base pairs (bp) amplified fragment from the 5' non-coding region of the hepatitis C virus (HCV) genome and the restriction fragments obtained after digestion by three different restriction enzymes: these fragments were 114 and 71 bp with enzyme *Mbo*I 107 and 78 bp with enzyme *Rsa*I and 141 and 44 (not seen) bp with enzyme *Sma*I.

can be related to chronic hepatitis C virus infection.

Since approximately 20% of the patients that are considered to have type 1 autoimmune chronic active hepatitis are known to be unresponsive to immunosuppressive treatment,¹¹ it is possible that hepatitis C virus and not immunity is responsible for liver disease in these patients and should therefore be sought extensively in these cases. These findings also raise the problem of the treatment of liver disease, since alpha interferon must be used with caution in subjects such as those reported in this study, because of its possible deleterious effects on extrahepatic autoimmune disorders.

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