LIVER AND BILIARY DISEASE

Acute hepatitis C virus (HCV) infection in chronic carriers of hepatitis B virus (HBV): the impact of underlying active HBV replication on persistence of HCV infection and antibody responses to HCV

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Background and aims: The aim of this study was to assess whether underlying chronic hepatitis B virus (HBV) infection interferes with persistence of hepatitis C virus (HCV) infection and humoral immune responses to HCV in acute HCV infection.

Methods: Serial sera from 12 patients with acute HCV infection (group A) and 12 hepatitis B surface antigen (HBsAg) carriers with acute HCV infection (seven anti-hepatitis B e antigen (anti-HBe) positive (group B1) and five hepatitis B e antigen (HBeAg) positive (group B2)) were tested for HCV RNA by polymerase chain reaction, and anti-HCV by third generation enzyme immunoassay and confirmatory assay. Serial serum samples from HBsAg carriers were also tested for HBeAg, anti-HBe, and HBV DNA by hybridisation assay.

Results: Persistent HCV viraemia for more than six months was significantly more frequent in groups A (83%) and B1 (86%) than in group B2 (0%). Anti-HCV was detected in 100% and 86% of group A and group B1 one month after onset while only one group B2 patient was transiently anti-HCV positive 1-2 months after onset. Of the latter, three had anti-core 1 less than two months after onset while no patient responded to other HCV antigens. Overall, of six HBsAg carriers with acute self limiting HCV infection, only one had transient anti-HCV and three had transient anti-core 1. HBV DNA became undetectable transiently in four and persistently in one group B2 patient.

Conclusion: The presence of active HBV replication can inhibit the persistence of HCV infection and antibody responses to HCV. Acute HCV infection in HBsAg carriers with active HBV replication usually presents transient HCV viraemia with poor antibody responses to HCV.

Viral interference between hepatitis B virus (HBV) and hepatitis C virus (HCV) has recently become of interest to both clinicians and virologists.^{1,2} Interference of HCV with HBV gene expression and replication has been investigated in many studies³⁻¹⁰: HCV core protein can suppress HBV gene replication and expression in cultured cell lines³; the prevalence of serum hepatitis B e antigen (HBeAg) and HBV DNA is significantly lower in chronic HBV infected patients with concurrent HCV infection than in those without^{4,5}; hepatocyte expression of HBV surface proteins is markedly suppressed in chronic HBV infected patients with concurrent HCV infection⁶; and concurrent HCV infection also can enhance the termination of hepatitis B surface antigen (HBsAg) antigenaemia clinically.¹⁰ In contrast, although it also has been suggested that HBV may suppress replication of HCV,⁶⁻⁸ clinical or experimental data on the interference of HBV with HCV are relatively limited.

Our recent study revealed that HCV accounted for 50-60% of acute non-A, non-B hepatitis and 20% of acute hepatitis superimposed on chronic HBsAg carriers in Taiwan, based on the diagnostic criteria of detecting HCV RNA in acute phase serum specimens.¹¹ An interesting observation was that a substantially low proportion of chronic HBsAg carriers with acute HCV infection were positive for antibodies against HCV (anti-HCV) in acute phase serum samples, using a second generation enzyme immunoassay, compared with non-HBsAg carrier patients (Chu *et al* unpublished observations). These findings suggest that underlying chronic HBV infection may interfere with humoral immune responses to HCV in acute HCV infection. These preliminary data prompted us to study the interference of underlying chronic HBV infection on persistence of HCV infection and antibody responses to HCV antigens in chronic HBsAg carriers with superimposed acute HCV infection.

In this study, serial serum specimens from chronic HBsAg carriers with acute HCV infection were tested for HCV RNA, anti-HCV, and specific humoral immune responses to individual HCV antigens, and the results were correlated with the underlying status of HBV replication. We also compared the results with those of acute HCV infection in non-HBsAg carriers. Moreover, to study the interference of acute HCV superinfection on HBV replication in chronic HBsAg carriers, serial serum samples from these patients were also tested for HBeAg, antibodies against hepatitis B e antigen (anti-HBe), and HBV DNA.

MATERIALS AND METHODS

Patients

Twelve patients with acute HCV infection (group A) and 12 chronic HBsAg carriers with superimposed acute HCV infection (group B), who had serial serum specimens taken less than one month, 1-2 months, and more than six months after the onset

Abbreviations: ALT, alanine aminotransferase; anti-HBc, antibodies against hepatitis B core antigen; anti-HBe, antibodies against hepatitis B e antigen; anti-HCV, antibodies against hepatitis C virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HEV, hepatitis E virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; IgM, immunoglobulin class M; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.

Table 1 Clinical and laboratory characteristics of the study patients

Characteristic	Acute hepatitis C (n=12)	Acute hepatitis C in HBsAg carriers (n=12)	p Value
Age (y)	52.8 (4.4)	46.7 (2.5)	NS
Sex (M:F)	8:4	9:3	NS
Albumin (g/dl)	3.8 (0.2)	3.4 (0.2)	NS
AST (U/l)	836 (214)	1139 (135)	NS
ALT (U/l)	1192 (185)	1552 (210)	NS
Bilirubin (mg/dl)	9.9 (2.4)	18.0 (3.3)	0.05
PT (s, prolonged)	0.4 (0.2)	6.3 (2.8)	0.03
HCV genotype			NS
1b	11	9	
1b+2b	1	2	
Other	0	1	
HCV RNA titre			NS
<10 ⁵ copies/ml	7	9	
10 ⁵ –10 ⁷ copies/ml	4	3	
>10 ⁷ copies/ml	1	0	

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen.

Data are expressed as number of patients or mean (SEM) where appropriate.

NS, non-significant ($p>0.05$).

of illness available for investigation, were randomly enrolled in the study. All were in good health. All chronic HBsAg carriers were asymptomatic before the episode of acute HCV infection, and none had ever received liver biopsy. No patient admitted intravenous drug abuse or homosexual activity. They presented with symptoms of overt acute hepatitis, and serum alanine aminotransferase (ALT) levels were at least 10 times the upper normal value. All denied a history of blood transfusion, operation, dental procedures, acupuncture, or tattooing within the six months before the onset of acute hepatitis. Drug and alcohol were excluded as likely causes. All patients had serum samples taken less than one month after the onset of illness available for serodiagnosis of acute viral hepatitis. Group A patients were positive for HCV RNA but negative for HBsAg. Group B patients were positive for HCV RNA and HBsAg. Both group A and group B patients were negative for immunoglobulin class M (IgM) antibodies against hepatitis B core antigen (IgM anti-HBc), IgM antibody against hepatitis A virus (IgM anti-HAV), IgM antibody against hepatitis D virus (IgM anti-HDV), IgM antibody against hepatitis E virus (IgM anti-HEV), IgM antibody against cytomegalovirus (IgM anti-CMV), and IgM antibody against Epstein-Barr virus capsid antigen (IgM anti-EBV). The clinical and virological features of the patients are listed in table 1. Among group B patients, seven were anti-HBe positive (group B1) while the other five were HBeAg positive (group B2).

Serological and virological tests

HBsAg, IgM anti-HBc, IgM anti-HAV, HBeAg, and anti-HBe were assayed using radioimmunoassay kits (Ausria II, Corab-M, HAVAB-IgM, and HBe-RIA; Abbott Laboratories, North Chicago, Illinois, USA). IgM anti-HDV was assayed by radioimmunoassay (Deltassay IgM; Cambridge Biotech, Dublin, Ireland). IgM anti-HEV was detected using an enzyme linked immunosorbent assay (Genelabs, Inc., Redwood City, California, USA). IgM anti-CMV was assayed by enzyme immunoassay (CMV IgM; Merck, Darmstadt, Germany). IgM anti-EBV was assayed by indirect immunofluorescence (EBV IgM; Gull, Saltlake, Utah, USA). Anti-HCV was assayed by a third generation enzyme immunoassay (Abbott Laboratories). HBV DNA was assayed by sandwich molecular hybridisation assays using a Digene Hybrid Capture System (Digene Diagnostics, Inc., Beltsville, Maryland, USA). Sensitivity for detection was 0.5 pg/ml. HCV RNA was detected by inhouse polymerase chain reaction (PCR), as described previously,¹² and was reconfirmed by a combined reverse transcription-polymerase chain reaction (RT-PCR) assay (Amplicor HCV

test; Roche Diagnostic System Inc., Branchburg, New Jersey, USA). The sensitivity of the latter was approximately 10–100 copies/ml. HCV genotypes were analysed using a genotype specific probe based assay (LiPA; Innogenetics, Ghent, Belgium), as reported previously.¹³ Serum titres of HCV RNA were determined using RT-PCR followed by slot/blot hybridisation with a digoxigenin labelled probe, and titres of HCV RNA above 10⁵ copies/ml were quantitatively determined, as reported previously.¹⁴ Specific antibody responses to individual HCV antigens were determined using the INNO-LIA HCV Ab III confirmatory assay (Innogenetics, Ghent, Belgium), which contained synthetic peptides from two non-overlapping regions of HCV core (amino acids 1–32 (core 1) and 31–74 (core 2)), envelope 2/hypervariable region 1 (amino acids 386–409), NS4 (amino acids 1696–1739 and 1916–1944), and NS5A (amino acids 2263–2318), and recombinant subtype 1b NS3 protein (amino acids 1188–1465). A sample was considered positive if one HCV antigen line had a reactivity rating of 2+ or higher or if at least two HCV antigen lines had a minimum reactivity rating of 1+. A sample was considered indeterminate if it was reactive with one antigen with a 1+ or +/- rating with or without other antigen lines presenting a reactivity rating of +/- . A sample was negative when it did not react with any of the HCV antigens.

Statistical analyses

Results were analysed using the χ^2 test with Yates' correction, Fisher's exact test, and the Student's *t* test, as appropriate. A *p* value <0.05 was considered statistically significant.

Table 2 Incidence of persistent infection in acute hepatitis C (group A) and in acute hepatitis C superimposed on chronic HBsAg carriers (group B)

Group	No (%) of patients with HCV viraemia for more than 6 months after onset
Group A (n=12)	10 (83%)*
Group B (n=12)	6 (50%)
Group B1 (n=7)	6 (86%)†
Group B2 (n=5)	0 (0%)* †

Group B1, anti-HBe positive HBsAg carriers; group B2, HBeAg positive HBsAg carriers.

Anti-HBe, antibodies against hepatitis B e antigen; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

* $p=0.006$, † $p=0.015$.

RESULTS

Incidence of persistent HCV infection and clinical course

Incidence of persistent HCV infection

HCV RNA was undetectable 1–2 months after the onset of illness in two, one, and five patients, respectively, in groups A, B1, and B2. Persistent HCV viraemia for more than six months after the onset of illness was significantly more frequent in groups A and B1 than in group B2 (see table 2).

Clinical course

All patients in groups A, B1, and B2 had resolution of clinical symptoms of acute hepatitis within 1–2 months after the onset of illness. Among group A patients, all 10 with persistent HCV viraemia had abnormal ALT levels for more than six months after the onset of illness while the other two had biochemical resolution 2–3 months after the onset of illness. Among group B1 patients, all remained HBsAg positive, anti-HBe positive, and HBV DNA negative for more than six months after the onset of illness. Six patients with persistent HCV viraemia had abnormal ALT levels for more than six months after the onset of illness and the other patient had biochemical resolution 2–3 months after the onset of illness. Among group B2 patients, all remained HBsAg positive for

more than six months after the onset of illness. Figure 1 (A–E) illustrates the sequential changes in ALT levels and serological profiles of HBV and HCV for each group B2 patient. In all, HBV DNA was undetectable, accompanied by decreased P/N ratios (counts per minute/cutoff value) for HBeAg in three patients (case Nos 1, 2, and 3 in fig 1) and anti-HBe seroconversion in two patients (case Nos 4 and 5 in fig 1) within 1–2 months after the onset of illness. Of the three patients with decreased P/N ratios for HBeAg, all had increased P/N ratios for HBeAg associated with reappearance of HBV DNA 3–6 months after the onset of illness. Of the two patients with anti-HBe seroconversion, one had reversion of HBeAg with reappearance of HBV DNA 3–4 months after the onset of illness (case No 4 in fig 1) and the other had sustained anti-HBe seroconversion without detectable HBV DNA for more than six months after the onset of illness (case No 5 in fig 1).

Detection of anti-HCV

The frequency of the anti-HCV response of the study patients is summarised in table 3. Anti-HCV was detected at a similar frequency in groups A and B1 but much less frequently in group B2 using a third generation enzyme immunoassay. All two group A patients with acute self limiting HCV infection

Figure 1 Follow up of alanine aminotransferase (ALT) levels and serological markers of hepatitis B virus (HBV) and hepatitis C virus (HCV) in five hepatitis B e antigen (HBeAg) positive chronic hepatitis B surface antigen (HBsAg) carriers with superimposed acute HCV. Data are expressed as "p" (positive results) or "n" (negative results) for HBeAg, antibodies against hepatitis B core antigen (anti-HBe), HCV RNA, anti-HCV, and antibodies against core 1 of hepatitis C virus (anti-core 1). Data in parentheses for the positive results of HBeAg indicate the P/N ratios (counts per minute/cutoff value), and for positive results of HCV RNA serum titres (copies/ml). Serum levels of HBV DNA are expressed as pg/ml, and HBV DNA levels <0.5 pg/ml indicate undetectable levels. The upper normal limit for ALT was 36 U/l.

Table 3 Anti-HCV response in acute hepatitis C (group A) and in acute hepatitis C superimposed on chronic HBsAg carriers (group B)

Patients	Time after onset of illness		
	<1 month	1–2 months	>6 months
Group A (n=12)	10 (10)	12 (12)	12 (12)
Group B (n=12)	4 (4)*	7 (7)	6 (6)
Group B1 (n=7)	4 (4)	6 (6)	6 (6)
Group B2 (n=5)	0 (0)†	1 (1)*	0 (0)†

Group B1, anti-HBe positive HBsAg carriers; group B2, HBeAg positive HBsAg carriers.

Data are expressed as number of patients positive for anti-HCV by third generation enzyme immunoassay (number of patients positive for anti-HCV by third generation confirmatory assays).

‡Two patients were reactive for anti-core 1 with a rating of 1+ which were read as indeterminate by third generation confirmatory assays. Anti-HBe, antibodies against hepatitis B e antigen; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen.

*p<0.05 versus group A; †p<0.05 versus group B1.

had anti-HCV detectable less than one month, 1–2 months, and more than six months after the onset of illness. Of the six group B patients with acute non-resolving HCV infection, anti-HCV was detectable in four, six, and six, respectively, less than one month, 1–2 months, and more than six months after the onset of illness. In contrast, only one of six patients in group B with acute self limiting HCV infection was transiently anti-HCV positive 1–2 months after the onset of illness.

All serum specimens that were anti-HCV positive by third generation enzyme immunoassay were also positive by third generation confirmatory assays. Two serum samples less than one month after the onset of illness in group B2 patients (case Nos 1 and 4 in fig 1) were negative for anti-HCV by third generation enzyme immunoassay but were reactive to core 1, with a rating of 1+, and were indeterminate by third generation confirmatory assays (see table 3). Of the five group B2 patients, only three were reactive to core 1 during the acute phase of HCV infection (case Nos 1, 2, and 4 in fig 1) and no patient responded to other HCV antigens. Overall, of the six chronic HBsAg carriers with self limiting acute HCV infection, only three had transient antibody responses to core 1 solely during the acute phase of infection.

DISCUSSION

Ten (83%) of 12 patients diagnosed as having acute HCV infection in this series were anti-HCV positive, as assessed by initial acute phase serum samples. The presence of anti-HCV in acute phase serum samples in these patients cannot exclude the possibility of a relapse of chronic HCV infection. However, this value is similar to those in previous reports in which 65–89% of patients with presumed acute HCV infection had anti-HCV detectable in initial serum specimens using second or third generation enzyme immunoassays.^{15–19}

Serum levels of bilirubin and prothrombin time were significantly higher in chronic HBsAg carriers with acute HCV infection than in non-HBsAg carriers (see table 1). Asymptomatic HBsAg carriers are known to harbour various liver lesions that may aggravate the severity of acute hepatitis. On the other hand, it may also be speculated that hepatocytes harbouring HBV might be unusually susceptible to injury by a new virus or may be limited in the ability to regenerate.

Eighty three per cent of patients with acute HCV infection in this series had chronic evolution. This value is close to or only slightly higher than that observed in previous studies of patients with transfusion or community acquired acute HCV infection.^{15–17, 20–22} Perhaps the more important finding of the present study is that the incidence of persistent HCV infection

in chronic HBsAg carriers correlated significantly with the underlying status of HBV replication (see table 2). The incidence of persistent HCV infection in chronic HBsAg carriers without active HBV replication was similar to that in non-HBsAg carrier patients. In contrast, acute HCV infection in chronic HBsAg carriers with active HBV replication rarely progressed to chronic infection. These findings suggest that the presence of underlying active replication of HBV can interfere with HCV replication and thereby inhibit the persistence of HCV infection in chronic HBsAg carriers with superimposed acute HCV infection. Because the number of patients enrolled in this series was relatively small, further studies of a larger series of patients are needed to confirm these findings.

Notably, as shown in fig 1, all five HBeAg positive chronic HBsAg carriers with superimposed acute HCV infection had undetectable serum HBV DNA, accompanied by decreased titres for HBeAg or anti-HBe seroconversion, during the acute phase of HCV infection. HBV and HCV seem to inhibit each other's replication at the molecular level: acute HCV infection can suppress underlying HBV replication but the underlying HBV replication may also interfere with HCV replication and inhibit the persistence of HCV infection. In most cases, as shown in case Nos 1–4 of fig 1, suppression of HBV replication by HCV was transient. With resolution of HCV infection, HBV replication can usually resume with reversion or increased titres of serum HBeAg. The mechanism of mutual inhibition between HBV and HCV replication remains unknown. Cellular immune responses have been reported to determine the outcome of HCV infection²³ and it has also been suggested that HBV replication can efficiently be inhibited by T cell derived cytokines.²⁴ It is thus possible that inhibition of HBV replication is mediated by cytokines that are released by intra-hepatic HCV specific T cells. However, as chronic HBsAg carriers with active HBV replication are known to have weak virus specific T cell reactivity but adequate antibody production,²⁵ it is difficult to understand how these patients will have efficient HCV clearance but poor humoral immune response to HCV. Further studies are needed to elucidate whether HBV can directly suppress the replication of HCV, especially in cultured cell lines.

The serological features of acute HCV infection superimposed on chronic HBsAg carriers with or without underlying active HBV replication are somewhat different from those observed in chronic HBsAg carriers with acute HDV superinfection: acute HDV superinfection can transiently or persistently suppress HBV gene expression and replication, as observed in acute HCV superinfection; however, persistent HDV infection occurs in 70% or more of cases, irrespective of the underlying status of HBV replication.²⁶ It seems that replication of HDV, unlike that of HCV, is not suppressed by HBV but instead needs the helper function of HBV.²⁶

The present findings revealed that humoral immune responses to acute HCV infection in chronic HBsAg carriers without active HBV replication were not significantly different from those in non-HBsAg carrier patients, as shown in table 3. On the contrary, acute HCV infection in chronic HBsAg carriers with active HBV replication was usually associated with poor humoral immune responses to HCV. Of the six chronic HBsAg carriers with acute self limiting HCV infection, only one was transiently positive for anti-HCV, and three were transiently reactive to core 1 during the acute phase of infection. Most of these patients were considered to have acute HCV infection solely on the basis of detecting HCV RNA in acute phase serum samples. Previous studies have indicated that testing for anti-HCV of serum specimens from the acute and convalescent phases can actually identify 90% or more of cases of acute HCV infection, irrespective of the source of exposure or the outcome of the disease.^{15–27–29} The present results reveal that testing for anti-HCV also can identify all cases of acute non-resolving HCV infection superimposed on chronic HBsAg carriers, but obviously will miss most cases of

acute self limiting HCV infection superimposed on chronic HBsAg carriers.

In conclusion, the presence of underlying active HBV replication can inhibit the persistence of HCV infection and humoral immune responses to HCV in chronic HBsAg carriers with acute HCV infection. Acute HCV infection superimposed on chronic HBsAg carriers in whom there is active replication of HBV frequently presents only transient HCV viraemia with poor antibody responses to HCV. Detection of anti-HCV is usually insufficient to identify acute HCV infection in these patients.

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