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Low-Level Viremia and Intracellular Expression of Hepatitis B Surface Antigen (HBsAg) in HBsAg Carriers with Concurrent Hepatitis C Virus Infection

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Assays of hepatitis B virus (HBV) replication and antigen expression in HBV surface antigen (HBsAg) carriers with concurrent hepatitis C or D virus (HCV or HDV) infection revealed that HCV and HDV can suppress HBV replication but that HCV also substantially suppresses HBV surface protein expression. HBsAg carriers with concurrent HCV infection thus have low-level viremia and intracellular HBsAg.

In chronic hepatitis B virus (HBV) infection, levels of HBV surface antigen (HBsAg) in serum and in the liver bear an inverse relation to each other. During the highly replicative phase, there are high serum titers of HBsAg but low tissue levels of HBsAg. During the low-replicative phase, titers of HBsAg in serum decrease and this decrease is accompanied by increased levels of HBsAg in tissue (1, 2, 9, 13). Notably, patients with fibrosing cholestatic hepatitis have high-level viremia, high titers of HBsAg in serum, and high-level intracellular HBsAg (10). Immunosuppressive therapy probably plays an important role in the evolution of fibrosing cholestatic hepatitis (11, 18). Recently, we have noted that some HBsAg carriers had low-level viremia, low titers of HBsAg in serum, and low-level intracellular HBsAg, and many of them were found to have concurrent hepatitis C virus (HCV) infection (6a). These preliminary observations prompted us to study the effect of concurrent HCV infection on HBV replication and antigen expression in HBsAg carriers.

Patients. Between 1977 and 1993, 992 consecutive patients with chronic hepatitis B were studied at our unit. Of these, 11% were positive for the antibody to hepatitis D virus (HDV; anti-HDV) and 8% were positive for antibodies to HCV (anti-HCV) (20). Three groups of patients, matched for age and sex, who had available serum specimens drawn on the day of liver biopsy were randomly selected for study. In one group, patients had concurrent HBV and HCV infections (n = 29). All patients had been HBsAg and anti-HCV positive for at least 12 months. All had HCV RNA detectable by PCR. Genotyping of HCV revealed 1b in 19, 2a in 6, 2b in 3, and 1b plus 2a in 1. The duration and relative timing of the two viral exposures were unknown. Seven patients had a history of blood transfusion 3 to 23 years ago. In the second group, patients had concurrent HBV and HDV infections (n = 35). All patients had been HBsAg and anti-HDV positive for at least 12 months. All had HDV antigen in liver tissue detectable by direct immunofluorescence (3). In the third group, patients had chronic HBV infection alone (n = 42). All patients were anti-HCV and anti-HDV negative and had no detectable HCV RNA in serum or HDV antigen in liver tissue. All patients denied any history of homosexual behavior or intravenous drug abuse. None had ever received antiviral or immunosuppressive therapy. Their clinical and laboratory data are summarized in Table 1.

Laboratory methods. HBsAg, HBV e antigen (HBeAg), anti-HBe, and anti-HDV were assayed with radioimmunoassay kits (Abbott Laboratories). Anti-HCV was assayed by second-generation enzyme immunoassay (HCV-EIA; Upstate Biotechnology Inc.). HBV DNA was assayed by spot hybridization with ^{32}P-labeled cloned HBV DNA. The detection sensitivity was 0.5 pg/50 μl. Levels of HBV DNA in serum were semiquantitatively scored on a 1^+-to-4^+ scale corresponding to ≤500, 501 to 1,000, 1,001 to 2,000, and >2,000 pg/ml, respectively (2). Levels of HBV DNA in serum were also assayed by PCR (22) when spot hybridization results were negative. Levels of HCV RNA were assayed by reverse transcription-PCR (4). HCV genotypes were analyzed by genotype-specific probe-based assay of the 5′-untranslated region (LiPA; Innogenetics, Ghent, Belgium). Pre-S1, pre-S2, and HBsAg proteins were assayed by enzyme immunoassay using pre-S1-, pre-S2-, and HBsAg-specific monoclonal antibodies (Institute of Immunology, Tokyo,

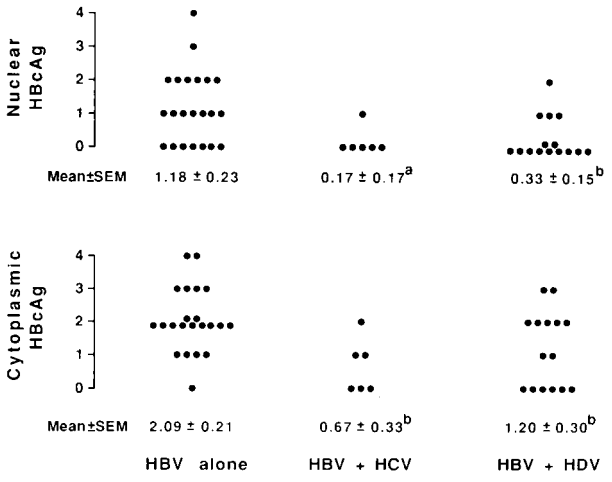

FIG. 1. The degree of intracellular nuclear and cytoplasmic expression of HBcAg in the HBsAg- and HBeAg-positive patients with HBV infection alone, concurrent HCV infection (HBV + HCV), and concurrent HDV infection (HBV + HDV). The expression of HBcAg in the liver was semiquantitatively scored on a 0-to-4 scale corresponding to positivity in 0%, 1 to 10%, 11 to 25%, 26 to 50%, and >50% of hepatocytes examined, respectively. The superscripts a and b indicate that $P < 0.05$ and $P < 0.01$, respectively, versus HBV alone. SEM, standard error of the mean.

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TABLE 1. Clinical and laboratory data of study patients

Antigen and infection status	Mean age (years) ± SEM	Sex ratio (male/female)	Mean level (U/liter) of[a]:		No. of patients with[b]:	
			AST	ALT	CPH	CAH
HBeAg-positive HBsAg carriers with:						
HBV alone (n = 22)	34.0 ± 1.9	17/5	109 ± 19	180 ± 24	10	12
HCV infection (n = 6)	35.3 ± 2.9	4/2	139 ± 20	198 ± 25	1	5
HDV infection (n = 15)	32.4 ± 2.2	15/0	110 ± 22	188 ± 25	4	11
HBeAg-negative HBsAg carriers with:						
HBV alone (n = 20)	39.8 ± 2.2	16/4	30 ± 3[c]	42 ± 4[c]	20[c]	0
HCV infection (n = 23)	43.1 ± 2.2	19/4	129 ± 19	214 ± 28	7	16
HDV infection (n = 20)	37.9 ± 2.2	19/1	99 ± 14	201 ± 21	6	14

[a] AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[b] CPH, chronic persistent hepatitis; CAH, chronic active hepatitis.
[c] $P < 0.001$ versus results for those with concurrent HCV or HCD infection.

Japan) at serial 10-fold dilutions. The highest dilution giving a positive result was designated the titer of the appropriate antigen.

Cryostat sections of liver specimens were examined for HBV core antigen (HBcAg), pre-S1, pre-S2, and HBsAg by indirect immunofluorescence, as described previously (1–3). Paraffin sections of liver specimens were also examined for HBcAg, pre-S1, pre-S2, and HBsAg by the avidin-biotin immunoperoxidase method, as reported before (5).

Statistical analyses were conducted by using the chi-square test with Yates' correction, Student's t test, or the Mann-Whitney rank sum test where appropriate.

Results and discussion. Previous results have shown that HBeAg and HBV DNA are significantly less prevalent in serum in HBsAg carriers with concurrent HCV infection than in those without HCV infection (7, 8, 14, 15, 19), suggesting that HCV, like HDV, might suppress HBV replication. However, the possibility that the HBsAg carriers with concurrent HCV or HDV infection might have been previously anti-HBe-positive, asymptomatic HBsAg carriers with low-level viremia cannot be excluded. All HBeAg-positive patients in this series were found to be HBV DNA positive by PCR, but levels of HBV DNA in serum were found to be significantly lower in those with concurrent HCV (undetectable, 3 patients; 1^+, 2 patients; 2^+, 1 patient; $P < 0.01$) or HDV infection (undetectable, 4 patients; 1^+, 4 patients; 2^+, 5 patients; 3^+, 2 patients; $P < 0.001$) than in those without concurrent HCV or HDV infection (1^+, 7 patients; 2^+, 4 patients; 3^+, 5 patients; 4^+, 6 patients) by spot hybridization. These data provide further evidence suggestive of the suppressive effect of HCV and HDV on HBV replication. In keeping with this postulation, the degree of intracellular expression of HBcAg was significantly lower in HBeAg-positive carriers with concurrent HCV or HDV infection (Fig. 1).

Titers of pre-S1, pre-S2, and HBsAg in serum were significantly lower in HBeAg-positive patients with concurrent HCV or HDV infection (Table 2), most probably as a result of a decreased level of viral replication. Levels of HBV surface proteins in tissue showed little or no difference in HBeAg-positive patients with HDV infection but were modestly lower in those with HCV infection compared to those without HCV or HDV infection (Fig. 2). These findings might suggest that in the HBeAg-positive phase of chronic HBV infection HCV not only suppresses HBV replication but also tends to suppress the expression of HBV surface proteins.

All HBeAg-negative patients in this series were found to have no detectable serum HBV DNA by spot hybridization or liver HBcAg by immunohistochemistry, though 80 to 90% of

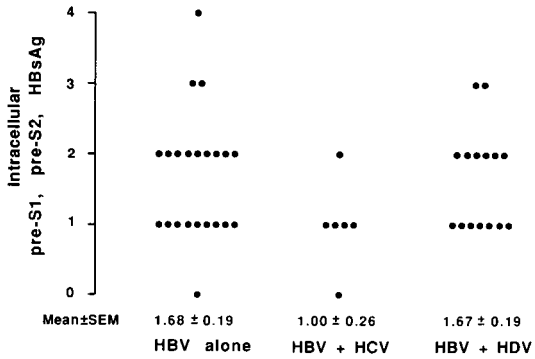

FIG. 2. The degree of intracellular expression of pre-S1, pre-S2, and HBsAg in HBsAg- and HBeAg-positive patients with HBV infection alone, concurrent HCV infection (HBV + HCV), and concurrent HDV infection (HBV + HDV). The expression of pre-S1, pre-S2, and HBsAg in the liver was semiquantitatively scored on a 0-to-4 scale corresponding to positivity in 0%, 1 to 10%, 11 to 25%, 26 to 50%, and >50% of hepatocytes examined. For concurrent HBV and HCV infections versus HBV infection, either alone or with concurrent HDV infection, $0.05 < P < 0.1$; for concurrent HBV and HDV infections versus HBV infection alone, $P > 0.3$. SEM, standard error of the mean.

TABLE 2. Titers of pre-S1, pre-S2, and HBsAg in serum in HBsAg carriers with HBV alone or with concurrent HCV or HDV infection

Patient status[d]	Log mean serum titers ± SEM of:		
	pre-S1	pre-S2	HBsAg
HBeAg-positive patients with:			
HBV alone (n = 22)	3.18 ± 0.23	3.41 ± 0.19	5.14 ± 0.17
HBV + HCV (n = 6)	1.50 ± 0.22[a]	1.67 ± 0.21[a]	4.33 ± 0.34[b]
HBV + HDV (n = 15)	1.87 ± 0.34[a]	2.06 ± 0.32[a]	4.53 ± 0.25[b]
HBeAg-negative patients with:			
HBV alone (n = 20)	0.75 ± 0.18[a]	0.95 ± 0.18[a]	3.60 ± 0.17[a]
HBV + HCV (n = 23)	0.39 ± 0.13[c]	0.52 ± 0.13[c]	3.13 ± 0.19[c]
HBV + HDV (n = 20)	0.70 ± 0.18	0.90 ± 0.19	3.50 ± 0.18

[a] $P < 0.001$ versus HBeAg-positive patients with HBV alone.
[b] $P < 0.05$ versus HBeAg-positive patients with HBV alone.
[c] $0.05 < P < 0.1$ versus HBeAg-negative patients with HBV alone.
[d] HBV + HCV, concurrent HBV and HCV infections; HBV + HDV, concurrent HBV and HDV infections.

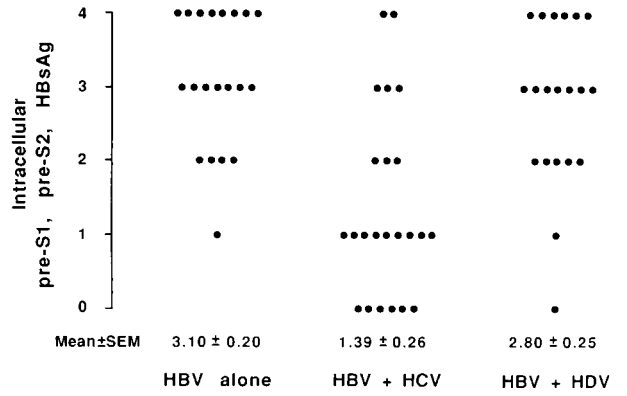

FIG. 3. The degree of intracellular expression of pre-S1, pre-S2, and HBsAg in HBsAg-positive and HBeAg-negative patients with HBV infection alone, concurrent HCV infection (HBV + HCV), and concurrent HDV infection (HBV + HDV). The expression of pre-S1, pre-S2, and HBsAg in the liver was semiquantitatively scored on a 0-to-4 scale corresponding to positivity in 0%, 1 to 10%, 11 to 25%, 26 to 50%, and >50% of hepatocytes examined. For concurrent HBV and HCV infections versus HBV infection, either alone or with concurrent HDV infection, $P < 0.001$; for concurrent HDV and HBV infections versus HBV infection alone, $P > 0.5$. SEM, standard error of the mean.

them were HBV DNA positive by PCR. However, levels of HBV surface proteins in tissue were markedly decreased ($P < 0.001$) and titers of HBV surface proteins in serum were modestly decreased ($P < 0.1$) in patients with concurrent HCV infection (Table 2 and Fig. 3). These changes are not observed in patients with concurrent HDV infection and thus do not appear to be secondary to the increased inflammatory activity in the liver. It has been shown that acute HDV infection can transiently suppress the expression of HBV (6, 12, 16). The present findings that chronic HDV infection had little or no effect on the expression of HBV surface proteins might be compatible with the biologic characteristics of HDV, which requires the helper function of HBsAg (17). The current data thus demonstrate that both HCV and HDV can suppress the replication of HBV but that, unlike HDV, HCV also can substantially suppress the expression of HBV surface proteins. These findings seem to be in accordance with the in vitro observation that HCV core protein can suppress HBV gene expression and replication (21) as well as the clinical observation that concurrent HCV infection can enhance the termination of the HBsAg carrier state in chronic HBsAg carriers (20).

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