Occult Hepatitis B Virus Infection and Clinical Outcomes of Patients with Chronic Hepatitis C

Jia-Horng Kao,^{1,2*} Pei-Jer Chen,^{1,2,3} Ming-Yang Lai,^{1,2} and Ding-Shinn Chen²

Graduate Institute of Clinical Medicine,¹ Department of Internal Medicine,² and Hepatitis Research Center,³ National Taiwan University College of Medicine and National Taiwan University Hospital, Taipei, Taiwan

Received 16 April 2002/Returned for modification 5 August 2002/Accepted 24 August 2002

Although occult hepatitis B virus (HBV) infections in individuals without detectable hepatitis B surface antigen (HBsAg) may occur and have been reported to be common in patients with chronic hepatitis C, the clinical relevance remains controversial. We searched for serum HBV DNA in 210 HBsAg-negative patients with hepatitis C virus (HCV)-related liver disease (110 patients with chronic hepatitis, 50 patients with cirrhosis, and 50 patients with hepatocellular carcinoma) by PCR. Most of the patients had detectable antibodies to HBsAg or HBV core antigen. All of the 110 chronic hepatitis C patients were treated with a combination therapy consisting of interferon plus ribavirin. In addition, 100 HBsAg-negative healthy adults served as controls. Thirty-one of the 210 patients (14.8%) had HBV DNA in their sera, as did 15 of the 100 healthy controls (15%). HBV DNA was not detected in the sera of those negative for serological markers of HBV infection. In patients with chronic HCV infection, the prevalence of occult HBV infection did not parallel the severity of liver disease (14.5% in patients with chronic hepatitis, 8% in patients with liver cirrhosis, and 22% in patients with hepatocellular carcinoma). In addition, the sustained response to combination therapy against hepatitis C was comparable between patients with and without occult HBV infection (38 versus 39%). In conclusion, these data suggest that occult HBV infection does not have clinical significance in chronic hepatitis C patients residing in areas where HBV infection is endemic.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for a substantial proportion of cases of chronic liver disease including chronic hepatitis, cirrhosis, and liver cancer. It is estimated that there are 350 million HBV carriers and 170 million HCV carriers worldwide (6). HBV and HCV are transmitted parenterally and share common routes of infection; thus, infection with both viruses may occur, particularly in areas where the two viruses are endemic and among people at high risk for parenteral infections (4, 15). The diagnosis of HBV infection is usually based on the detection of hepatitis B surface antigen (HBsAg), and the disappearance of this antigen indicates the clearance of HBV (6). However, previous studies have shown that HBV DNA can be detected in patients with chronic liver disease who are negative for HBsAg but positive for antibodies to hepatitis B core antigen (anti-HBc) (1, 12, 21). More recently, this so-called occult HBV infection has frequently been identified in patients with chronic HCV infection (5, 10, 19), and in such patients this occult infection may be associated with more severe liver damage and even the development of hepatocellular carcinoma (HCC) (3, 18, 20). In addition, several studies have suggested that occult HBV infection may correlate with a lack of response to interferon treatment in patients with chronic hepatitis C (3, 5, 22). Taken together, a low-level HBV infection not only may contribute to the severity of HCV-related liver disease but also may be of prognostic importance. However, whether the presence of such small amounts of HBV will lead to progressive liver disease has been questioned (2) and indeed

needs further confirmation from other parts of the world where HBV infection is rampant.

Taking advantage of the fact that HBV and HCV infections are common in Taiwan (4), we determined the prevalence of occult HBV infection in patients with HCV-related chronic liver disease and studied the possible influence of occult HBV infection on the clinical outcomes for the infected patients.

MATERIALS AND METHODS

Patients. Serum samples from 210 Taiwanese patients with histologically verified HCV-related chronic liver disease and 100 control subjects were retrospectively studied. These included (i) 100 healthy adults (52 men, 48 women; mean age, 40 ± 7 years) who had normal serum alanine aminotransferase (ALT) levels and who were negative for both HBsAg and antibodies against hepatitis C virus (anti-HCV); among them, 82 were positive for both antibodies against HBsAg (anti-HBs) and anti-HBc, 8 were positive for anti-HBc alone, and 10 were negative for the three serological markers of HBV infection; (ii) 50 patients (30 men, 20 women; mean age, 64 ± 9 years) with HCC; (iii) 50 patients (28 men, 22 women; mean age, 57 ± 10 years) with cirrhosis; and (iv) 110 patients (74 men, 36 women; mean age, 45 ± 13 years) with chronic hepatitis C who had received combination therapy with alfa-2b interferon (Intron A; Schering-Plough, Kenilworth, N.J.) at 3 million units thrice weekly plus oral ribavirin (ICN Pharmaceuticals, Inc., Costa Mesa, Calif.) at 1,000 to 1,200 mg daily for 24 weeks. The presence of HCV RNA and HBV DNA in serum was determined before the initiation of combination therapy, at the end of therapy, and 24 weeks after the therapy was discontinued. The response to combination therapy was classified into two patterns according to the serum ALT level and serum HCV RNA status. Patients who had normal serum ALT levels (≤ 40 U/liter) and in whose serum HCV RNA was undetectable at the end of therapy and during the follow-up period were considered to have a sustained response. A nonsustained response was defined as serum ALT levels that could not be normalized either at the end of therapy or during the follow-up period, without clearance of HCV RNA from serum. Those with chronic HCV infection were positive for both anti-HCV and HCV RNA and were negative for HBsAg. Of those individuals, 181 (86%) were positive for anti-HBc and 127 (60%) were positive for anti-HBs. To sum up, 20 (9.5%) of these patients with HCV-related chronic liver disease were negative for serological markers of HBV infection and 190 (90.5%) were positive for

* Corresponding author. Mailing address: Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, 7 Chung-Shan South Rd., Taipei 100, Taiwan. Phone: 886-2-23123456, ext. 7307. Fax: 886-2-23317624. E-mail: kjh@ha.mc.ntu.edu.tw.

TABLE 1. Sequences of primer pairs used for PCR to detect HBV genome

Gene and primer ^a	Sequence (5'→3')	Nucleotide position
Surface gene		
S-1s	AGAACATCGCATCAGGACTC	159–178
S-2a	CATAGGTATCTTGCAGAAAGC	642–623
S-3s	AGGACCCCTGCTCGTGTAC	181–200
S-4a	AGATGATGGGATGGGAATAC	619–600
Core gene		
C1s	CTGGGAGGAGTTGGGGGA	1730–1747
C2a	GTAGAAGAATAAAGCCC	2503–2487
C3s	GGTCTTTGTACTCGGAGGCTG	1763–1783
C4a	ATACTAACATTGACATTCCC	2455–2436
X gene		
X1s	CTAGCCGCTTGTTTTGCTCG	1282–1301
X2a	TTATGCCTACAGCCTCCTAG	1666–1647
X3s	GGTCTTACATAAGAGGACTC	1518–1537
X4a	GTTACAGGTGGTCTCCAT	1625–1608

^a s, sense; a, antisense.

anti-HBs and/or anti-HBc. The diagnosis of chronic liver disease was based on generally accepted clinical and pathological grounds including mild chronic hepatitis, moderate or severe chronic hepatitis, liver cirrhosis, and HCC.

None of the patients enrolled in the study had markers suggestive of autoimmune hepatitis including antinuclear antibodies, antimitochondrial antibodies, and anti-smooth muscle antibodies. None had a history of alcoholism (alcohol intake of >50 g/day), injection drug abuse, homosexuality, or hepatotoxic drug intake. Metabolic liver disease including hemochromatosis, Wilson's disease, or α_1 -antitrypsin deficiency was excluded by clinical and laboratory data. Serum samples taken from each subject were stored at -70°C until use.

Serological markers. Tests for HBsAg, anti-HBs, anti-HBc, and anti-HCV were done with commercially available kits (Abbott Laboratories, North Chicago, Ill.).

Detection of HCV RNA, genotyping of HCV, and quantitation of HCV RNA. Serum was assayed for HCV RNA by reverse transcription-PCR with primers from the most conserved 5' untranslated region of the viral genome, and identification of the HCV genotype was done with type-specific primers as described previously (9). To avoid false-positive results, the methods described by Kwok and Higuchi (11) to prevent cross contamination were applied. Serum HCV RNA levels were also quantified by using a second-generation branched DNA signal amplification assay (Quantiplex-HCV, version 2.0; Chiron) with a detection limit of 0.2 Meq/ml.

Detection of HBV DNA. Serum samples were assayed for HBV DNA by using three different PCR assays with primer pairs whose sequences were taken from the surface, core, and X genes of the viral genome (Table 1). Briefly, total DNA was extracted from 100 μl of serum with a QIAamp Blood kit (QIAGEN Ltd., Crawley, United Kingdom) and resuspended in 50 μl of elution buffer. For the first round of PCR, the DNA in 25 μl of a reaction mixture containing 2 μl of the DNA sample, $1\times$ PCR buffer (10 mM Tris-HCl [pH 9.0], 50 mM KCl, 1.5 mM MgCl_2 , 0.01% gelatin, 0.1% Triton X-100), each deoxynucleoside triphosphate at a concentration of 10 mM, 100 ng of each outer primer pair, and 1 U of *Taq* DNA polymerase was amplified in a thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) for 30 cycles. Each cycle entailed denaturation at 95°C for 60 s, primer annealing at 55°C for 30 s, and extension at 72°C for 60 s with a final extension step at 72°C for 7 min. After the first amplification, 1 μl of the PCR products was reamplified for another 30 cycles with 100 ng of each inner primer pair. The second round of PCR was done in the same manner as the first round. The amplified products were separated by electrophoresis in a 3% agarose gel and stained with ethidium bromide. The sensitivities of our PCR assays reached 10 copies of HBV DNA per specimen, as determined by testing serial 10-fold dilutions of HBV DNA transcripts with known amounts (10^8 copies/ml) as described previously (7). Serum samples reactive by at least two of the three PCR assays were considered HBV DNA positive.

Statistical analysis. Data were analyzed by the chi-square test with Yates' correction or Student's *t* test where appropriate. A *P* value of less than 0.05 was considered statistically significant.

TABLE 2. Prevalence of HBV DNA in sera of 100 healthy controls and 210 HCV carriers by serological patterns and liver status

Group and serological pattern or liver status	No. of individuals studied	No. (%) of individuals positive for HBV DNA ^a
Healthy controls		
Negative for all HBV markers	100	15 (15.0)
Positive for all HBV markers	10	0 (0)
Positive for anti-HBc alone	8	2 (25.0)
Positive for anti-HBc and anti-HBs	82	13 (15.9)
HCV-infected patients		
Negative for all HBV markers	210	31 (14.8)
Positive for all HBV markers	20	0 (0)
Positive for anti-HBc alone	63	16 (25.4)
Positive for anti-HBc and anti-HBs	127	15 (11.8)
HCV-infected patients		
Chronic hepatitis	210	31 (14.8)
Liver cirrhosis	110	16 (14.5)
Hepatocellular carcinoma	50	4 (8.0)
	50	11 (22.0)

^a HBV DNA was detected by PCR with primer pairs whose sequences were taken from the surface, core, or X gene. Serum samples reactive by at least two of the three PCR assays were considered HBV DNA positive.

RESULTS

Of 210 patients with chronic HCV infection, the sera of 31 (14.8%) were positive for HBV DNA by the different PCR assays, documenting an occult HBV infection. The prevalence of occult HBV infection in HCV carriers was comparable to that in healthy adults (15%) (Table 2). In addition, none of the 10 healthy adults or 20 hepatitis C patients negative for all three serological markers of HBV infection was positive for HBV DNA by our PCR assays.

Among the 100 healthy adults and 210 HCV carriers with different serological patterns for HBV infection, the prevalence of occult HBV infection ranged from 0 to 25% among subjects negative for all markers, those positive for anti-HBc alone, and those positive for both anti-HBs and anti-HBc (Table 2). The overall prevalence was significantly higher for those positive for anti-HBc alone than for those negative for all markers ($P = 0.001$).

In 210 patients with HCV-related chronic liver diseases, the prevalence of occult HBV infection did not parallel the severity of chronic liver disease (Table 2). It was highest in patients with HCC (22%) and lowest in patients with cirrhosis (8%), but the difference was not significant. The mean age of HCC patients with HCV infection and occult HBV infections was similar to that of patients with HCV infection alone (60.1 ± 8.5 versus 60.1 ± 7.9 years).

Among 110 patients with chronic hepatitis C, HCV genotypes 1b, 2a, and 2b and mixed infection were found in 67, 29, 9, and 5 patients, respectively (Table 3). When these patients were stratified by the presence or absence of occult HBV infection, there was no significant difference in clinicopathologic features including gender distribution, mean age, percentage of patients with a transfusion history, mean peak serum ALT level, histological severity, and distribution of HCV genotypes (Table 3). Although patients with HCV and occult HBV infections had higher mean serum HCV RNA levels than those with HCV infection alone, the difference was not statistically significant. In addition, the biochemical and virological

TABLE 3. Clinicopathologic data and response to interferon plus ribavirin in 110 chronic hepatitis C patients with and without HBV DNA in their sera

Characteristic	Serum HBV DNA ^a	
	Positive	Negative
No. of patients	16	94
Sex (no. of M/no. of F) ^b	10/6	64/30
Age (yr)	47 ± 16	45 ± 11
No. (%) of patients who had received a transfusion history	5/(31)	24/(26)
Peak ALT level (U/liter)	151 ± 33	124 ± 78
Histology (no. [%] of patients)		
Mild chronic hepatitis	13 (81)	66 (70)
Moderate or severe chronic hepatitis	3 (19)	28 (30)
No. (%) of patients infected with HCV genotype:		
1b	11 (69)	56 (60)
2a	3 (19)	26 (28)
2b	1 (6)	8 (8)
Mixed	1 (6)	4 (4)
Serum HCV titer (Meq/ml)	2.1 ± 4.2	1.6 ± 3.8
No. (%) of patients who responded to interferon plus ribavirin		
Sustained response	6 (38)	37 (39)
Nonsustained response	10 (62)	57 (61)

^a The difference between HBV DNA-positive and -negative groups for each characteristic was not statistically significant.

^b M, males; F, females.

responses to combination therapy with interferon and ribavirin did not differ between patients with and without occult HBV infection (Table 3). Overall, the sustained response rates were 38 and 39% in patients with HBV DNA in their sera and those without HBV DNA in their sera, respectively.

Of the 16 patients with occult HBV coinfection before initiation of combination therapy for chronic hepatitis C, all were negative for HBV DNA in their sera at the end of therapy, as determined by PCR assays, and the sera of 14 (88%) remained HBV DNA negative after the therapy had been stopped for 6 months. No correlation between the sustained normalized serum ALT level and the loss of HBV DNA from serum was found. On the contrary, a correlation was seen between a sustained normalized serum ALT level and a sustained loss of HCV RNA from serum.

DISCUSSION

HBV infection is diagnosed when circulating HBsAg is detected (6). However, a unique persistent infection known as occult HBV infection, which is characterized by positivity for HBV DNA in serum by using nested PCR assays, has been identified in HBsAg-negative patients with or without serological markers of previous HBV infection (anti-HBs or anti-HBc) (1, 2, 12, 21). Several recent studies have indicated that

this occult HBV infection can be found in patients with chronic HCV infection at various frequencies (50 to 87%) (2, 3, 5, 10, 19, 20, 22). The high prevalence of the occult HBV infection in such patients has been suggested to have clinical implications in the pathogenesis of HCV-induced chronic liver disease. Nevertheless, by using highly sensitive PCR assays (Table 1), we found that the prevalences of occult HBV infection were comparable in patients with chronic HCV infection (14.8%) and healthy adults (15%), implying that the risk of occult HBV infection among HCV carriers is not increased compared to that for the general population in Taiwan. These findings are not unanticipated because most adults in Taiwan contracted HBV infection during childhood, and superinfection with other viruses including HCV may occur thereafter (8). In addition, the low-level replication of HBV in patients with occult HBV infection does not appear to result from interference by HCV because the occult HBV infection is not necessarily accompanied by HCV infection but is observed at the same rate in healthy controls (17). However, the lower prevalence of occult HBV infection in our HCV-infected patients (14.8%) compared to that in previous studies (50 to 87%) may be explained by a more strict definition of positivity for HBV DNA in the present study.

The relationship between occult HBV infection and serological markers of HBV infection has been studied before, and the prevalence of occult HBV infection was usually higher in subjects positive for either anti-HBs or anti-HBc or for both anti-HBs and anti-HBc than in those negative for all serological markers (46 to 80% versus 20 to 50%) (2, 3, 5). Although the data were not statistically significant, our data also showed that healthy adults positive for either anti-HBs or anti-HBc or for both anti-HBs and anti-HBc had higher rates of positivity for HBV DNA in their sera than those negative for all serological markers (16 to 25% versus 0%; Table 2). The possibility of persistent HBV infection in anti-HBc-positive individuals has been supported by recent studies showing that traces of HBV are often detectable in the blood for many years after clinical recovery from acute hepatitis, despite the presence of antibodies against HBV and HBV-specific cytotoxic T lymphocytes in serum (14, 16).

The clinical significance of occult HBV infection alone or in combination with HCV infection remains unsettled (2). Previous epidemiologic and molecular studies have indicated that persistent HBV infection may be associated with the development of HCC in HBsAg-negative patients and in woodchucks that had once been infected with woodchuck hepatitis virus even after the apparent clearance of the virus (13, 20). Recently, occult HBV infection has been shown to correlate significantly with the development of cirrhosis among HCV-infected patients (3). The earlier data (3) suggested that a masked HBV infection may interfere with the clinical outcome of chronic hepatitis C and favor or accelerate the evolution to cirrhosis. Since cirrhosis is generally the most important risk factor for the development of HCC (6), occult HBV infection may thus favor neoplastic transformation in HCV-infected patients through its contribution to cirrhosis. In contrast, our results showed that the prevalence of occult HBV infection did not parallel the severity of chronic liver disease, and the mean age of HCC patients with HCV and occult HBV coinfection was comparable to that of patients with HCV infection alone.

In addition, among patients with chronic hepatitis C, the demographic, clinical, histological, and virological features were comparable between those with and those without occult HBV coinfection (Table 3). Taken together, our observations suggest that occult HBV infection may have little influence on the clinicopathologic course of chronic HCV infection, at least in Taiwanese patients. The discrepancy in the clinical outcomes for chronic hepatitis C patients with occult HBV infection between those reported previously and those reported in the present study may be explained by the fact that most of our patients were infected with HBV at birth or in early childhood, which induces an immune tolerance to HBV. This may therefore modulate the influence of occult HBV infection on the histological damage in chronic hepatitis C patients in Taiwan. In addition, occult HBV infection has been claimed to promote HCV replication (5, 19). However, we did not observe such an association in our study (Table 3).

Occult HBV infection has been suggested to jeopardize the response to interferon therapy in patients with chronic hepatitis C (3, 5, 22); however, its impact on the response to combination therapy remains unknown. Our results showed that the sustained rates of response to combined alpha interferon and ribavirin therapy were similar between chronic hepatitis C patients with occult HBV infection and those without occult HBV infection (Table 3), and thus, low-level HBV infection does not interfere with the response to combination therapy against HCV. In addition, there are no data on the use of antiviral treatment in patients with occult HBV infection, and whether HBV has a sustained response to interferon plus ribavirin remains unexplored. Our data showed that all of the 16 patients with HCV and occult HBV coinfection became negative for HBV DNA in serum at the end of the combination therapy, and 14 (88%) had a sustained virological remission after therapy had been stopped for 6 months. These results suggest that low-level HBV is sensitive to interferon and ribavirin, and whether this combination therapy also works for the treatment of usual chronic active hepatitis B needs to be studied further.

In summary, we found that occult HBV infection is common in healthy adults seropositive for HBV markers as well as in patients with chronic hepatitis C in Taiwan, where HBV infection is hyperendemic; nevertheless, low-level HBV coinfection has no effect on the clinicopathologic status of patients with chronic hepatitis C or the therapeutic response to combination therapy.

ACKNOWLEDGMENTS

This study was supported by grants from the Department of Health and the National Science Council, Executive Yuan, Taiwan.

REFERENCES

- Brechot, C., F. Degos, C. Lugassy, V. Thiers, S. Zafrani, D. Franco, H. Bismuth, C. Trepo, J. P. Benhamou, and J. Wands. 1985. Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. *N. Engl. J. Med.* **312**:270–276.
- Bréchet, C., V. Thiers, D. Kremsdorf, B. Nalpas, S. Pol, and P. Paterlini-Brechot. 2001. Persistent hepatitis B virus: clinically significant or purely occult? *Hepatology* **34**:194–203.
- Cacciola, I., T. Pollicino, G. Squadrito, G. Cerenzia, M. E. Orlando, and G. Raimondo. 1999. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N. Engl. J. Med.* **341**:22–26.
- Chen, D. S., G. Kuo, J. L. Sung, M. Y. Lai, J. C. Sheu, P. J. Chen, P. M. Yang, H. M. Hsu, M. H. Chang, C. J. Chen, L. C. Hahn, Q. L. Choo, T. H. Wang, and M. Houghton. 1990. Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic liver disease: the Taiwan experience. *J. Infect. Dis.* **162**:817–822.
- Fukuda, R., N. Ishimura, M. Niigaki, S. Hamamoto, S. Satoh, S. Tanaka, Y. Kushiya, Y. Uchida, S. Iihara, S. Akagi, M. Watanabe, and Y. Kinoshita. 1999. Serologically silent hepatitis B virus coinfection in patients with hepatitis C virus-associated chronic liver disease: clinical and virological significance. *J. Med. Virol.* **58**:201–207.
- Kao, J. H., and D. S. Chen. 2000. Overview of hepatitis B and C viruses, p. 313–330. In J. J. Goedert (ed.), *Infectious causes of cancer: targets for intervention*. Humana Press Inc., Totowa, N.J.
- Kao, J. H., P. J. Chen, M. Y. Lai, and D. S. Chen. 2000. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* **118**:554–559.
- Kao, J. H., P. J. Chen, M. Y. Lai, W. Chen, D. P. Liu, J. T. Wang, M. C. Shen, and D. S. Chen. 1997. GB virus-C/hepatitis G virus infection in an area endemic for viral hepatitis, chronic liver disease, and liver cancer. *Gastroenterology* **112**:1265–1270.
- Kao, J. H., W. Chen, P. J. Chen, M. Y. Lai, and D. S. Chen. 2000. TT virus infection in patients with chronic hepatitis B or C: influence on clinical, histological and virological features. *J. Med. Virol.* **60**:387–392.
- Koike, K., M. Kobayashi, M. Gondo, I. Hayashi, T. Osuga, and S. Takada. 1998. Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients. *J. Med. Virol.* **54**:249–255.
- Kwok, S., and R. Higuchi. 1989. Avoiding false positive with PCR. *Nature* **339**:237–238.
- Liang, T. J., Y. Baruch, E. Ben-Porath, R. Enat, L. Bassan, N. V. Brown, N. Rimon, H. E. Blum, and J. R. Wands. 1991. Hepatitis B virus infection in patients with idiopathic liver disease. *Hepatology* **13**:1044–1051.
- Michalak, T. I., I. U. Pardoe, C. S. Coffin, N. D. Churchill, D. S. Freake, P. Smith, and C. L. Trelogan. 1999. Occult lifelong persistence of infectious hepadnavirus and residual liver inflammation in woodchucks convalescent from acute viral hepatitis. *Hepatology* **29**:928–938.
- Penna, A., M. Artini, A. Cavalli, M. Levrero, A. Bertoletti, M. Pilli, F. V. Chisari, B. Rehmann, G. Del Prete, F. Fiaccadori, and C. Ferrari. 1996. Long-lasting memory T cell responses following self-limited acute hepatitis B. *J. Clin. Invest.* **98**:1185–1194.
- Pontisso, P., M. G. Ruvoletto, G. Fattovich, L. Chemello, A. Gallorini, A. Ruol, and A. Alberti. 1993. Clinical and virological profiles in patients with multiple hepatitis virus infections. *Gastroenterology* **105**:1529–1533.
- Rehmann, B., C. Ferrari, C. Pasquinelli, and F. V. Chisari. 1996. The hepatitis B virus persists for decades after patient's recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat. Med.* **2**:1104–1108.
- Sheen, I. S., Y. F. Liaw, D. Y. Lin, and C. M. Chu. 1994. Role of hepatitis C and delta viruses in the termination of chronic hepatitis B surface antigen carrier state: a multivariate analysis in a longitudinal follow-up study. *J. Infect. Dis.* **170**:358–361.
- Sheu, J. C., G. T. Huang, L. N. Shih, W. C. Lee, H. C. Chou, J. T. Wang, P. H. Lee, M. Y. Lai, C. Y. Wang, P. M. Yang, and D. S. Chen. 1992. Hepatitis C and B viruses in hepatitis B surface antigen-negative hepatocellular carcinoma. *Gastroenterology* **103**:1322–1327.
- Uchida, T., Y. Kaneita, K. Gotoh, H. Kanagawa, H. Kouyama, T. Kawanishi, and S. Mima. 1997. Hepatitis C virus is frequently coinfecting with serum marker-negative hepatitis B virus: probable replication promotion of the former by the latter as demonstrated by in vitro cotransfection. *J. Med. Virol.* **52**:399–405.
- Yotsuyanagi, H., Y. Shintani, K. Moriya, H. Fujie, T. Tsutsumi, T. Kato, K. Nishioka, T. Takayama, M. Makuuchi, S. Iino, S. Kimura, and K. Koike. 2000. Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. *J. Infect. Dis.* **181**:1920–1928.
- Zhang, Y. Y., B. G. Hansson, L. S. Kuo, A. Widell, and E. Nordenfelt. 1993. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the presence of antibodies to HBsAg. *Hepatology* **17**:538–544.
- Zignego, A. L., R. Fontana, S. Puliti, S. Barbagli, M. Monti, G. Careccia, F. Giannelli, C. Giannini, G. Buzzelli, M. R. Brunetto, F. Bonino, and P. Gentilini. 1997. Relevance of inapparent coinfection by hepatitis B virus in alpha interferon-treated patients with hepatitis C virus chronic hepatitis. *J. Med. Virol.* **51**:313–318.