行政院國家科學委員會補助專題研究計畫成果報告

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※ B型肝炎病毒重複感染與慢性B型肝炎急性發作之相關性研究 ※

※ Superinfection of Hepatitis B Virus and acute exacerbation of chronic ※

※ hepatitis B ※

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一、中文摘要

目前已知有七種 HBV 基因型，然而不同 HBV 基因型是否會發生重覆感染仍未明瞭。本計畫乃針對 244 例慢性活動性 B 型肝炎患者研究 HBV 基因型重覆感染和臨床上慢性肝炎急性發作之相關性。在 244 例患者中，103 例曾發生急性發作(年發生率約 13%)，其中 20 例為 IgM anti-HBc 陽性且較 IgM anti-HBc 陰性者具有較高的 HBV 基因型 C 感染率(65%比 40%，P<0.05)。此外，吾人又對 20 例 IgM anti-HBc 陽性急性發作者(A 組)，20 例 IgM anti-HBc 陰性急性發作者(B 組)和 20 例無急性發作者(C 組)作系列的 HBV 基因型和前表面基因(pre-S)核酸序列分析。結果發現 2 例 A 組患者有 HBV 重覆感染之病毒學證據，一例為異型病毒重覆感染，而另一例為同型病毒重覆感染。新感染的病毒在急性發作期後消失而原先之病毒又再出現。因之，B 型肝帶原者 HBV 重覆感染發生率約為 0.8%而在急性發作者之發生率約為 1.9%。吾人之研究結果顯示在 B 型肝炎盛行區，HBV 確會發生重覆感染且和小部分之急性發作有關。

關鍵字：B 型肝炎病毒、基因型、重覆感染、急性發作

二、Abstract

There are seven genotypes of hepatitis B virus (HBV). Whether superinfection of HBV carriers with different HBV genotypes occurs remains unknown. We therefore determined the HBV genotype and association between superinfection and acute exacerbation of disease in a cohort of 244 patients with chronic HBV infection who had elevated serum aminotransferase levels for at least 1 year. Within this group, 103 patients experienced acute exacerbation with an annual incidence of 13% and 20 of the 103 patients had IgM antibody to hepatitis B core antigen (IgM anti-HBc). These 20 patients had a higher prevalence of genotype C infection (65%) than the remaining 83 anti-core IgM negative patients (40%) who also had acute exacerbations ($p < 0.05$). Detailed analysis of HBV genotypes and sequences of the variable pre-S gene were determined in serial samples from 20 patients with IgM anti-HBc-positive acute exacerbations (group A); 20 patients with IgM anti-HBc-negative acute exacerbations (group B); and 20 patients without exacerbations (group C). Two (10%) of the group A patients had virological evidence of HBV superinfection during acute exacerbation, one superinfected with heterotypic virus and the other with homotypic virus. The newly introduced virus disappeared after the exacerbation and the original virus resumed thereafter. The calculated prevalence of HBV superinfection in the hepatitis B carriers and those with acute exacerbations was 0.8% (2/244) and 1.9% (2/103), respectively. In conclusion, superinfection of HBV on hepatitis B carriers indeed occurs and may cause acute exacerbations, albeit at a low frequency even in hyperendemic areas of HBV infection.

三、Introduction

Hepatitis B virus (HBV) infection is a global health problem. About 2 billion people in the world have been infected by HBV, 350 million of whom are chronic carriers of the virus.[1] The infection can cause acute and chronic hepatitis, liver cirrhosis and even hepatocellular carcinoma (HCC).[1,2] The liver cell injury associated with HBV infection is predominantly mediated through immune mechanisms,[1,3] and the natural history can thus be divided into three phases based on virus-host interactions.[1,2] In the first immune tolerance phase, patients are hepatitis B e antigen (HBeAg) positive and have high serum HBV DNA levels, but have no symptoms, normal serum aminotransferase levels and minimal histological activities. During the second immune clearance phase, a proportion of previously symptomless HBV carriers start to have bouts of symptoms and signs suggestive of acute hepatitis, i.e., the so-called "acute exacerbations", when previous immune tolerance no longer exists. In addition to the break of immune tolerance and subsequent seroconversion of HBeAg, acute hepatitis superimposed on chronic HBV infection could also be attributed to the superinfection by other hepatotropic viruses,[4] variability of HBV genome such as point mutations and deletion,[5] reactivation of chronic hepatitis B spontaneously or after cessation of immunosuppressive therapy,[6,7] and hepatotoxic agents exposure. In the third low-replicative or integration phase, serum hepatitis B surface antigen (HBsAg) persists, but HBeAg is no longer detectable and patients are usually asymptomatic and liver disease is inactive.

By using the phylogenetic tree analysis, viral genomes can be classified into distinct genotypes, and reinfection or mixed infection of different genotypes of the same virus has been documented in hepatitis C or D.[8-15] We have previously shown that hepatitis C carriers can be superinfected by heterotypic or homotypic HCV, and this may contribute to the hepatitis flares in some patients with chronic hepatitis C.[9,12,13] A similar genetic classification based on the comparison of complete genomes has recently defined seven genotypes of HBV (A to G);[16,17] however, the genotype-related differences in the pathogenicity and clinical relevance of HBV infection remain to be explored. Our recent studies indicated that genotypes B and C are the most prevalent HBV genotypes in Taiwan, and genotype C is associated with more severe liver disease whereas genotype B may be associated the development of HCC in young hepatitis B carriers.[18] In addition, genotype C has a poor response to interferon therapy compared to genotype B.[19] Taken together, these data suggest the possible pathogenic and therapeutic differences among HBV genotypes. Although the

clinical significance remains unclear, several molecular epidemiological studies have indicated the possible existence of mixed infection of different HBV genotypes in some hepatitis B carriers.[20,21] These lines of evidence prompted us to investigate whether HBV superinfection, heterotypic or homotypic, can occur on top of chronic hepatitis B carriers, as is in the case of chronic hepatitis C or hepatitis D infection, and its possible association with the acute exacerbation of chronic hepatitis B in Taiwan where HBV infection is rampant.

四、Patients and Methods

Patients

A cohort of 244 patients (172 men and 72 women; age 17 ~ 69 years) with chronic HBV infection who had elevated serum alanine aminotransferase (ALT) levels for at least 1 year was followed at the gastroenterological clinic of National Taiwan University Hospital. Chronic HBV infection was defined as a persistent seropositivity for hepatitis B surface antigen (HBsAg) during the entire follow-up period. These patients received liver tests including serum ALT activity every 3 months and more frequently, if indicated. Serial serum samples taken from each patient were stored at –20 ^{o}C until use. During their follow-up period, all patients were negative for antibodies to hepatitis C virus (anti-HCV) and hepatitis D virus (anti-HDV), and had no serologic markers suggestive of autoimmune disease. None had a history of alcohol abuse (>50 gm/day), parenteral drug use or hepatotoxin exposure. No specific antivirals or immune modulators were given in the study period. Among them, 103 (76 men, 27 women; mean age: 33±9 years) had acute exacerbations of the chronic hepatitis during the follow-up period. Acute exacerbation was defined as clinical symptoms along with an abrupt increase of serum ALT level (upper limit of normal, 40 U/L) to a level greater than 5 times the upper limit of normal (200 U/L) as previously described.[4] Of the 103 patients with episodes of acute exacerbation, 20 (13 men, 7 women; mean age: 34±10 years) were positive for IgM antibody to hepatitis B core antigen (IgM anti-HBc) (group A). Another 20 sex and age-matched patients (15 men, 5 women; mean age: 35±8 years) selected from the remaining 83 patients with acute exacerbations but negative for IgM anti-HBc (group B) and 20 (14 men, 6 women; mean age: 34±9 years) without exacerbation (group C) served as controls.

Hepatitis virus markers

Serum HBsAg and HBeAg were tested by Ausria-II and IMx HBe 2.0 (Abbott Laboratories, North Chicago, IL), respectively. Serum IgM anti-HBc was measured using an

AxSYM assay (AxSYM CORE™-M, Abbott Laboratories). Samples with index values greater than 1.2 are considered reactive for IgM anti-HBc. This cutoff index value allows delineation between acute and chronic HBV infection as previously described.[22] Anti-HCV and anti-HDV were tested by commercially available assays (HCV EIA II, Anti-Delta, Abbott Laboratories).

Genotyping of HBV

Sequential serum samples before, during and after acute exacerbations with serum ALT levels above 200 U/L (groups A and B patients) or peak serum ALT levels below 200 U/L (group C patients) were selected for genotyping of HBV by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the surface gene of HBV as previously described.[18] Six genotypes (A to F) of HBV could be identified by the restriction patterns of DNA fragments. To avoid false-positive results, instructions to prevent cross contaminations were strictly followed, and results were considered valid only when they were obtained in duplicate.

Amplification and sequencing of the pre-S gene

The entire pre-S gene (522 nucleotides, nucleotide positions 2848-154) was further amplified, and this region was chosen because the pre-S gene is one of the most variable regions of the HBV genome.[23] For the first stage PCR, a 25 μl of reaction mixture containing 2 μl of the cDNA sample, 1x PCR buffer (10 mM tris-HCl pH 9.0, 50mM KCl, 1.5 mM MgCl2, 0.01% gelatin and 0.1% Triton X-100), 10 mM of each dNTP, 100 ng of each outer primer (outer sense: PS1s 5'- GGGTCACCTTATTCTTGGGA -3', positions 2814–2833; outer antisense: PS2a 5'- CCCCGCCTGTAACACGAGCA -3', positions 208–189) and 1 unit of Taq DNA polymerase was amplified in a thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) 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 (inner sense: PS3s 5'-TTGGGAACAAGATCTACAGC -3', positions 2828–2847; inner antisense: PS4a 5'-GTCCTGATGCGATGTTCTCC -3', positions 176–157). Nucleotide sequences of the amplified products were directly determined by using fluorescence labelled primers with a 373A Sequencer (Applied Biosystems, Foster City, CA). Sequencing conditions were specified in the protocol for the Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The inner primer pair PS3s and PS4a was used as sequencing

primers for both directions.

Statistical Analysis

Fisher's exact test, chi-square test with Yates' correction, and Student's *t* test were used where appropriate. A P value of < 0.05 was considered statistically significant.

五、Results

Among 103 hepatitis B carriers with acute exacerbations of chronic hepatitis during the follow-up period, HBV genotypes B and C infection were identified in 57 (55%) and 46 (45%), respectively. In addition, the prevalence of genotype C infection in 20 patients with IgM anti-HBc-positive acute exacerbations was significantly higher than that of 83 patients with IgM anti-HBc-negative acute exacerbations [13 of 20 (65%) vs. 33 of 83 (40%), $P < 0.05$]. The demographic, clinical and laboratory data as well as changes of sequential HBV genotype before, during and after acute exacerbations of group A patients are shown in Table 1, and those of groups B and C patients are shown in Tables 2 and 3, respectively. Of the three groups of 60 patients, 33 (55%) were originally infected by genotype B virus, and 27 (45%) by genotype C virus. Emergence of a new HBV genotype during abrupt elevation of serum ALT levels was rarely found, in only 1 (5%) of the 20 patients with IgM anti-HBc-positive acute exacerbations (Table 1) and in none of 40 patients with IgM anti-HBc-negative acute exacerbations or without exacerbations (Tables 2 and 3). In the only patient (Case 20, Table 1) with heterologous HBV infection, genotype C virus emerged and predominated over the original genotype B virus during the acute exacerbation. However, the newly introduced genotype C virus disappeared 6 months after the acute exacerbation and the original genotype B virus reappeared thereafter (Table 1 and Figure 1).

The variable pre-S gene of HBV genome was amplified and directly sequenced to determine the master sequence from sequential serum samples before, during and after acute exacerbations of groups A and B patients who had no changes of HBV genotypes during their follow-up period. The comparison of nucleotide sequences between HBV isolates revealed that all but one isolates had > 99% nucleotide identity. The only patient (Case 13, Table 1) bore a lower degree (94.7%) of genetic homology between the HBV strains isolated before and during acute exacerbation for a period of 2 months (Figure 2A). The homologous strain found during the acute exacerbation was not present in the original HBV populations by using a strain-specific PCR, suggesting it was newly introduced to the host. The newly introduced

homotypic strain became undetectable 4 months after the acute exacerbation and the original strain resumed thereafter (Figure 2B).

Patients in groups A and B were comparable in terms of gender, mean age, positivity of HBeAg before acute exacerbation, and mean follow-up period (Table 4). Although patients with IgM anti-HBc-positive acute exacerbations had a higher mean serum ALT level and frequency of HBV superinfection than those with IgM anti-HBc-negative acute exacerbations, the difference was not statistically significant. Meticulous interview with both patients who had virological evidence of superinfection failed to identify any discernible source of the HBV superinfection.

六、Discussion

IgM anti-HBc is present in high titer in patients with acute HBV infection and this has been used as a specific serological marker for the diagnosis of acute hepatitis B.[24] Although low titers of IgM anti-HBc exist in chronic hepatitis B,[25] all available commercial assays have been designed to detect high titers of IgM anti-HBc that occur only during the acute phase of HBV infection.[26] Nevertheless, misclassification has been observed in up to 28% of chronic hepatitis B patients with acute exacerbations, and thus, a higher cut-off level to differentiate acute HBV infection from acute exacerbations of chronic HBV infection is proposed.[26] In a cohort of 244 Taiwanese patients with chronic HBV infection in the present study, 103 (42%) had episodes of acute exacerbation in a mean follow-up period of 4 years. Of those with acute exacerbations, 20 (19%) were positive for IgM anti-HBc by a commercial assay. Accordingly, the annual incidence of acute exacerbation in our study was 13%, and these data were consistent with previous reports.[26-28]

Although serological and genotypic classifications of HBV have been well documented,[16,17] the clinical significance of HBV genotypes in terms of clinical outcomes and therapeutic response to antiviral therapy in patients with chronic HBV infection remains largely unknown until recently. Our studies suggested that HBV genotype C is associated with the development of cirrhosis and HCC, and genotype B may be associated the development of HCC in young patients.[18] In addition, HBV genotype C is associated with a higher frequency of core promoter mutation and a lower response rate to interferon alfa therapy as compared to genotype B.[19] Previous studies have shown that the frequency and severity of acute exacerbation of chronic hepatitis are closely associated with the

development of liver cirrhosis in hepatitis B carriers;[29] however, little is known about the genotype-related differences in the frequency of acute exacerbation. Our findings indicated that the distribution of HBV genotypes B and C infection in patients with acute exacerbations was comparable to that in those without exacerbation. Nevertheless, the prevalence of genotype C virus infection in patients with IgM anti-HBc-positive acute exacerbations was higher than that of those with IgM anti-HBc-negative acute exacerbations (65% vs. 40%, $P < 0.05$), implying patients with HBV genotype C infection might have more severe acute exacerbations than those with genotype B infection. However, further large studies are needed to confirm this preliminary finding.

In chronic hepatitis B, superinfection with other hepatitis viruses and reactivation of the original hepatitis B virus are two major causes of clinical exacerbations.[4,6] In hepatotropic viruses, although superinfection with different strains of HCV or HDV on top of chronic HCV or HDV carriage has been demonstrated before,[8-15] the situation in HBV is far from clear. Mixed infection of different HBV genotypes has been observed in a small proportion of hepatitis B carriers;[20,21] however, whether superinfection of homologous or heterologous HBV strains occurs on top of hepatitis B carriers, as is in chronic HCV or HDV infection, remains unknown. Although the PCR-RFLP typing method is useful for the classification of HBV genotypes,[18,30] it fails to differentiate viral strains of the same genotype, and for this purpose, analysis of the variable region, like the pre-S gene of the HBV genome, remains the method of choice.[13] Thus by using the HBV genotyping with PCR-RFLP and direct sequencing of the variable pre-S gene of HBV genome, our results showed that 2 (10%) of the 20 chronic hepatitis B patients with IgM anti-HBc-positive acute exacerbations had virological evidence of HBV superinfection, one superinfected with heterotypic virus and the other with homotypic virus. The calculated prevalence of HBV superinfection on top of all hepatitis B carriers and those with acute exacerbations was 0.8% and 1.9%, respectively, based on the total cohort of 244 patients. These findings indicated that HBV superinfections contribute little to the clinical exacerbations of chronic hepatitis B, even in an HBV hyperendemic area like Taiwan. The stability of HBV genotypes in chronic HBV infection observed in the present study can also be reasoned by the concept of "replication space" defined as the potential of the liver to accommodate replicating virus or new genotypes of virus.[31] It has been hypothesized that the replication space is severely restricted in patients with chronic HBV infection. In addition, most of the patients with IgM anti-HBc-positive acute exacerbations were negative for virological evidence of HBV superinfection, suggesting

the presence of high-titered IgM anti-HBc in such patients is associated with continued replications of their original HBV strains.[25]

Given the high prevalence of HBV infection in the general population of Taiwan,[2] it is not surprising to speculate that multiple exposures to HBV is likely to occur and our data documented that superinfection of a new HBV indeed exists in chronic hepatitis B carriers. This fact also suggests that the immune system of an HBV persistently infected individual fails to prevent the host from reinfection with homologous or heterologous strains of HBV. Thus efforts to prevent superinfection in patients with chronic hepatitis B should not be overlooked, especially in areas endemic for HBV infection.

Interference between different hepatitis viruses as well as different HCV subtypes has been documented.[8-10,12,13,32] Taken together, it seems that the timing or sequence of infection is a factor influencing the outcome of viral interactions. In our study, the HBV superinfection induced viral suppression whereby only a single genotype of HBV was detected in both patients with HBV superinfection. Of the patient with heterologous HBV superinfection (Case 20, Table 1), genotype C virus emerged and predominated over the original genotype B virus during the acute exacerbation of chronic hepatitis. However, the newly introduced genotype C virus became undetectable after the acute exacerbation and the original genotype B virus resumed thereafter (Figure 1). Of the patient with homologous HBV superinfection (Case 13, Table 1), the newly introduced homotypic strain (Y3 isolate) became undetectable after acute exacerbation and the original genotype C strains (Y1, Y2, Y4 to Y7 isolates) reappeared thereafter (Figure 2B). Our findings therefore lent support to the speculation that the newcomer virus may suppress the pre-existing virus.[32] It has been well-known that immunocompetent adults with acute HBV infection rarely evolve into persistent infection because of their vigorous, polyclonal, and multispecific humoral as well as cellular immune response against the intruding virus.[33] And thus, the elimination of the newly introduced HBV strains as observed in our two patients is not surprising. Our previous observation that HBsAg carriers in Taiwan may bear antibodies heterotypic to their HBsAg also support this hypothesis.[34]

Because of the limitations of amplification technology, both the PCR-RFLP genotyping and direct sequencing methods used in the present study can only detect the dominant genotype or strain. Accordingly, the possibility of transient appearance of heterotypic or homotypic strains in the course of chronic HBV infection cannot be excluded.

In summary, our data suggest that patients with HBV genotype C infection might have

more severe acute exacerbations. Superinfection of a new HBV is not common in chronic HBV carriers and plays a minor etiological role in acute exacerbations of chronic hepatitis B, even in those positive for IgM anti-HBc. In addition, viral interference among different HBV genotypes does occur and the newcomer may suppress the original virus transiently.

七、References

1. Kao JH, Chen DS. Overview of Hepatitis B and C Viruses. In: Infectious Causes of Cancer: Targets for Intervention. Ed. Goedert JJ. Totowa: Humana Press Inc.; 2000, pp 313-330.
2. Chen DS. From hepatitis to hepatoma: lessons from type B viral hepatitis. Science 1993;262:369-370.
3. Chisari FV. Cytotoxic T cells and viral hepatitis. J Clin Invest 1997;99:1472-1477.
4. Liaw YF. Acute exacerbation and superinfection in patients with chronic viral hepatitis. J Formos Med Assoc 1995;94:521-528.
5. Torre F, Naoumov NV. Clinical implications of mutations in the hepatitis B virus genome. Eur J Clin Invest 1998;28:604-614.
6. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. Gastroenterology 1984;86:230-235.
7. Hoofnagle JH, Dusheiko GM, Schafer DF, Jones EA, Micetich KC, Young RC, Costa J. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Ann Intern Med 1982;96:447-449.
8. Farci P, Alter HJ, Govindarajan S, Wong DC, Engle R, Lesniewski RR, Mushahwar IK, et al. Lack of protective immunity against reinfection with hepatitis C virus. Science 1992;258:135-140.
9. Kao JH, Chen PJ, Lai MY, Chen DS. Superinfection of heterologous hepatitis C virus in a patient with chronic type C hepatitis. Gastroenterology 1993;105:583-587.
10. Okamoto H, Mishiro S, Tokita H, Tsuda F, Miyakawa Y, Mayumi M. Superinfection of chimpanzees carrying hepatitis C virus of genotype II/1b with that of genotype III/2a or I/1a. HEPATOLOGY 1994;20:1131-1136.
11. Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH, Chen DS. Genotypes of hepatitis C virus in Taiwan and the progression of liver disease. J Clin Gastroenterol 1995;21:233-237.

12. Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH, Chen DS. Mixed infections of hepatitis C virus as a factor in acute exacerbation of chronic type C hepatitis. J Infect Dis 1994;170: 1128-1133.

13. Kao JH, Chen PJ, Lai MY, Yang PM, Wang TH, Chen DS. Superinfection of homotypic virus in hepatitis C virus carriers: studies on patients with posttransfusion hepatitis. J Med Virol 1996;50:303-308.

14. Wu JC, Choo KB, Chen CM, Chen TZ, Huo TI, Lee SD. Genotyping of hepatitis D virus by restriction-fragment length polymorphism and relation to outcome of hepatitis D. Lancet 1995;346:939-941.

15. Wu JC, Huang IA, Huang YH, Chen JY, Sheen IJ. Mixed genotypes infection with hepatitis D virus. J Med Virol 1999;57:64-67.

16. Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 1994;198:489-503.

17. Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 2000;81:67-74.

18. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000; 118: 554-559.

19. Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. J Hepatol 2000;33:998-1002.

20. Usuda S, Okamoto H, Iwanari H, Baba K, Tsuda F, Miyakawa Y, Mayumi M. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. J Virol Methods 1999;80:97-112.

21. Grandjacques C, Pradat P, Stuyver L, Chevallier M, Chevallier P, Pichoud C, Maisonnas M, et al. Rapid detection of genotypes and mutations in the pre-core promoter and the pre-core region of hepatitis B virus genome: correlation with viral persistence and disease severity. J Hepatol 2000;33:430-439.

22. Eble K, Clemens J, Krenc C, Rynning M, Stojak J, Stuckmann J, Hutten P, et al. Differential diagnosis of acute viral hepatitis using rapid, fully automated immunoassays. J Med Virol 1991;33:139-150.

23. Uy A, Wunderlich G, Olsen DB, Heermann KH, Gerlich WH, Thomssen R. Genomic variability in the preS1 region and determination of routes of transmission of hepatitis B virus. J Gen Virol 1992;73:3005-3009.

24. Hoofnagle JH, Ponzetto A, Mathiesen LR, Waggoner JG, Bales ZB, Seeff LB. Serological diagnosis of acute viral hepatitis. Dig Dis Sci 1985;30:1022-1027.

25. Sjogren M, Hoofnagle JH. Immunoglobulin M antibody to hepatitis B core antigen in patients with chronic type B hepatitis. Gastroenterology 1985;89:252-258.

26. Tassopoulos NC, Papatheodoridis GV, Kalantzakis Y, Tzala E, Delladetsima JK, Koutelou MG, Angelopoulou P, et al. Differential diagnosis of acute HBsAg positive hepatitis using IgM anti-HBc by a rapid, fully automated microparticle enzyme immunoassay. J Hepatol 1997;26:14-19.

27. Liaw YF, Tai DI, Chu CM, Pao CC, Chen TJ. Acute exacerbation in chronic type B hepatitis: comparison between HBeAg and antibody-positive patients. HEPATOLOGY 1987;7:20-23.

28. Lok AS, Lai CL. Acute exacerbations in Chinese patients with chronic hepatitis B virus (HBV) infection. Incidence, predisposing factors and etiology. J Hepatol 1990;10:29-34.

29. Liaw YF, Tai DI, Chu CM, Chen TJ. The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. HEPATOLOGY 1988;8:493-496.

30. Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus–large-scale analysis using a new genotyping method. J Infect Dis 1997;175:1285-1293.

31. Zhang YY, Summers J. Low dynamic state of viral competition in a chronic avian hepadnavirus infection. J Virol 2000;74:5257-5265.

32. Liaw YF, Yeh CT, Tsai SL. Impact of acute hepatitis B virus superinfection on chronic hepatitis C virus infection. Am J Gastroenterol 2000;95:2978-2980.

33. Rehermann B. Intrahepatic T cells in hepatitis B: viral control versus liver cell injury. J Exp Med 2000;191:1263-1268.

34. Chen DS, Lai MY, Sung JL. Anti-HBs reactivity in hepatitis B surface antigen positive serum samples – with special emphasis on heterotypic antibody. J Formosan Med Assoc 1982;81:1357-1364.

Table 1. Clinical, laboratory and sequential HBV genotype data of 20 chronic hepatitis B patients with acute exacerbations and IgM anti-HBc positivity (Group A).

Patient no.	Sex/Age (years)	HBeAg before acute exacerbation	Peak ALT (U/L)	HBV Genotype pre-/on/post-acute exacerbation	Period of follow-up (months)
1	F/31	+	209	C/C/C	132
2	M/23	+	1050	C/C/C	32
3	M/24	+	1060	C/C/C	17
4	M/45	+	222	C/C/C	24
5	F/45	+	372	C/C/C	14
6	F/52	+	357	C/C/C	84
7	M/41	+	868	C/C/C	39
8	F/43	+	314	C/C/C	49
9	M/45	+	292	C/C/C	13
10	M/25	+	524	C/C/C	14
11	M/34	+	327	C/C/C	13
12	M/39	+	590	C/C/C	50
13*	M/30	+	912	C/C/C	79
14	M/22	+	1049	B/B/B	103
15	M/32	+	548	B/B/B	13
16	F/25	+	349	B/B/B	15
17	F/20	+	231	B/B/B	26
18	M/33	+	1197	B/B/B	41
19	F/24	+	910	B/B/B	16
20#	M/45	–	846	B/C/B	191

HBeAg, hepatitis B e antigen; ALT, serum alanine aminotransferase.

*Further illustrated in Figure 2.

Further illustrated in Figure 1.

Table 2. Clinical, laboratory and sequential HBV genotype data of 20 chronic hepatitis B patients with acute exacerbations and IgM anti-HBc negativity (Group B).

Patient no.	Sex/Age (years)	HBeAg before acute exacerbation	Peak ALT (U/L)	HBV Genotype pre-/on/post-acute exacerbation	Period of follow-up (months)
1	M/35	+	532	C/C/C	32
2	M/54	+	685	C/C/C	60
3	M/29	+	298	C/C/C	69
4	M/49	+	661	C/C/C	114
5	F/41	+	203	C/C/C	44
6	M/37	+	266	C/C/C	101
7	F/39	+	348	C/C/C	55
8	M/31	+	817	B/B/B	30
9	M/30	+	386	B/B/B	43
10	M/24	+	933	B/B/B	47
11	M/37	+	1070	B/B/B	72
12	M/29	+	935	B/B/B	37
13	M/24	+	207	B/B/B	41
14	F/39	+	884	B/B/B	94
15	F/44	+	300	B/B/B	75
16	M/39	+	496	B/B/B	81
17	M/33	+	572	B/B/B	55
18	M/35	+	205	B/B/B	46
19	M/37	+	583	B/B/B	54
20	F/28	+	556	B/B/B	90

HBeAg, hepatitis B e antigen; ALT, serum alanine aminotransferase.

Table 3. Clinical, laboratory and sequential HBV genotype data of 20 chronic hepatitis B patients without acute exacerbations (Group C).

Patient no.	Sex/Age (years)	HBeAg before peak ALT level	Peak ALT (U/L)	HBV Genotype pre-/on/post-peak ALT level	Period of follow-up (months)
1	F/35	+	45	C/C/C	95
2	M/34	+	46	C/C/C	90
3	M/40	+	95	C/C/C	66
4	M/37	+	85	C/C/C	69
5	M/35	+	160	C/C/C	72
6	M/24	+	54	C/C/C	31
7	F/34	+	46	C/C/C	33
8	M/35	+	92	B/B/B	72
9	M/34	+	67	B/B/B	36
10	M/33	+	143	B/B/B	22
11	M/51	+	90	B/B/B	34
12	M/29	+	129	B/B/B	25
13	M/22	+	60	B/B/B	24
14	F/39	+	63	B/B/B	36
15	F/23	+	148	B/B/B	49
16	F/20	+	71	B/B/B	48
17	F/54	–	47	B/B/B	54
18	M/38	–	49	B/B/B	64
19	M/48	+	48	B/B/B	70
20	M/24	+	79	B/B/B	142

HBeAg, hepatitis B e antigen; ALT, serum alanine aminotransferase.

Table 4. Comparison of clinical and laboratory data in two groups of chronic hepatitis B patients with IgM anti-HBc-positive or –negative acute exacerbations.

Features	Group A	Group B	P Value
Number	20	20	
Gender (M/F)	13/7	15/5	NS
Age (years, mean±SD)	34±10	35 ±8	NS
Positivity of HBeAg	19	20	NS
Follow-up period (months)	48±47	62±23	NS
Peak serum ALT (U/L)	611±329	547±273	NS
HBV superinfection (%)	2 (10%)	0	NS

HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase.

Group A: patients with IgM anti-HBc-positive acute exacerbations (serum ALT > 200 U/L, normal < 40 U/L) during follow-up period.

Group B: patients with IgM anti-HBc-negative acute exacerbations during follow-up period.

八、Figure Legends

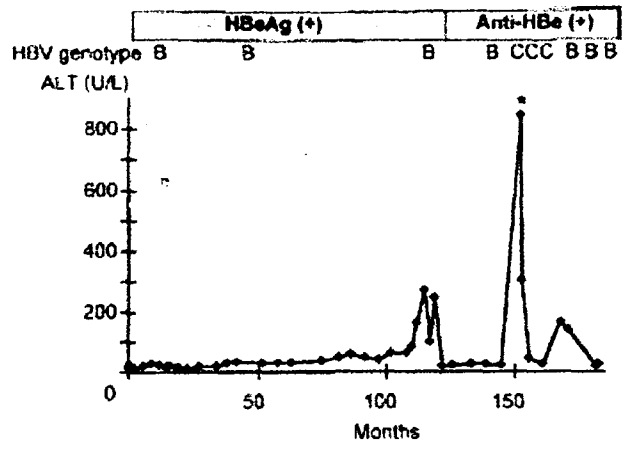

Figure 1. The clinical course of a 45-year-old man (Case 20, Table 1) originally persistently infected with HBV genotype B. He had seroconverted from HBeAg to anti-HBe with subsequent normalization of serum aminotransferase activity after an episode of IgM anti-HBc-negative acute exacerbation. During another bout of IgM anti-HBc-positive acute exacerbation, a genotype C virus emerged and predominated over the original genotype B virus. However, the newly introduced genotype C virus became undetectable 6 months after the acute exacerbation and the original genotype B virus reappeared thereafter. HBeAg: hepatitis B e antigen; anti-HBe: antibody against hepatitis B e antigen; ALT: alanine aminotransferase; *: positive for IgM antibody against hepatitis B core antigen.

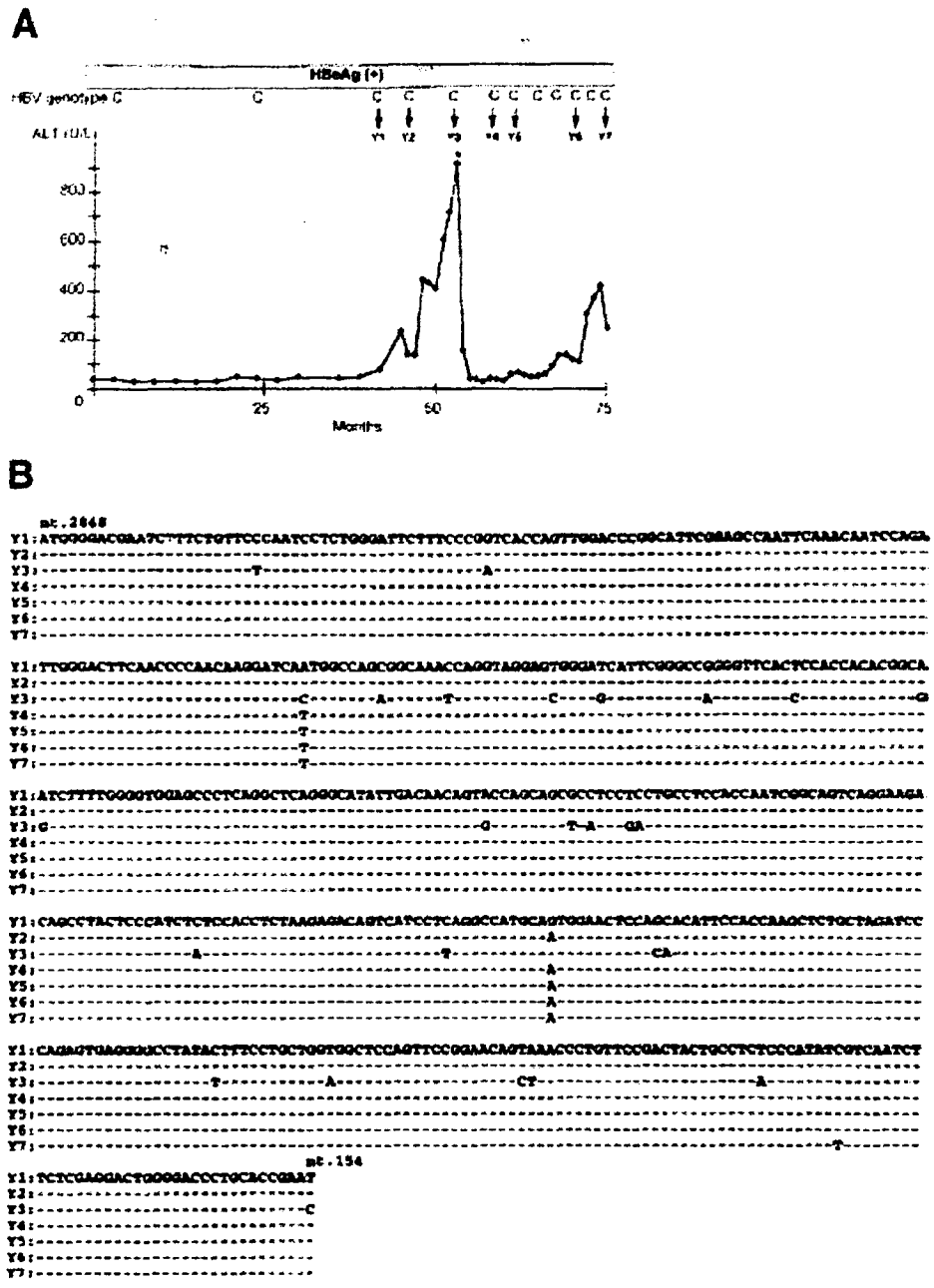

Figure 2. (A) The clinical course of a 30-year-old man (Case 13, Table 1) originally infected with HBV genotype C. He was persistently positive for HBeAg and had several episodes of acute exacerbations during the follow-up period. The seven time points (Y1 to Y7) when the amplified pre-S genes were used for direct sequencing of nucleotides are marked by arrows. The Y3 isolate was obtained from the only IgM anti-HBc-positive acute exacerbation. HBeAg: hepatitis B e antigen; ALT: alanine aminotransferase; *: positive for IgM antibody against hepatitis B core antigen. (B) Comparison of the complete sequences of the variable pre-S gene of 5 sequential isolates (Y1 to Y7) of HBV DNA from the same patient. The dashes

indicate identical sequences compared with the Y1 isolate. The Y1, Y2, Y4 to Y7 isolates show > 99.5% nucleotide identity, but the HBV strains isolated before (Y1 and Y2) and during acute exacerbation (Y3) within a period of 2 months show a lower degree (94.7%) of genetic homology. nt.: nucleotide.