JAC antiviral

Journal of Antimicrobial Chemotherapy (2005) **55**, 139–142
doi:10.1093/jac/dkh533
Advance Access publication 23 December 2004

Influence of hepatitis B virus genotypes on the response to antiviral therapies

Norio Akuta* and Hiromitsu Kumada

Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, 105-0001, Japan

Hepatitis B virus (HBV) has been classified into eight genotypes (A–H) based on genome sequence divergence. Genotypes of HBV have distinct geographical distributions, and two genotypes account for most HBV worldwide. Hepatitis B e antigen expression lasts longer and liver disease is more severe with graver outcomes in carriers of genotype C than B in Asia. Accumulating lines of evidence indicate a better response to interferon and lamivudine in patients with chronic hepatitis B who are infected with genotype B rather than C. The therapeutic response may differ, however, in patients infected with HBV of the same genotype. For example, the response to lamivudine is poorer in patients infected with subtype Ba, which contains a recombination with genotype C, than in those with subtype Bj without such a recombination. Influence of genotypes on therapeutic response needs to be examined in patients infected with the other genotypes, particularly in those with genotype A or D infection.

Keywords: chronic hepatitis, genotypes, hepatitis B e antigen, hepatitis B virus, interferon, lamivudine

Introduction

Worldwide, 350 million people are estimated to be persistently infected with hepatitis B virus (HBV),[1] and three-quarters of these people reside in Asia. The morbidity and mortality of persistent HBV infection are a major public health concern. More than one million deaths every year are due to end-stage HBV liver disease, such as decompensated liver cirrhosis and hepatocellular carcinoma (HCC). The carrier state of HBV is established mainly through mother-to-baby infection in Japan, where the prevalence of hepatitis B surface antigen carriage (HBsAg) in the general population used to be less than 2%, while horizontal transmission during infancy plays an additional role in the other countries in Asia and accounts for most persistent infections in Africa where HBsAg prevailed in >8% of the general population during the past. Although much less efficient, horizontal transmission can lead to persistent HBV infection in Western countries through sexual contact and illicit intravenous drug use. Individuals with persistent HBV infection need to be identified early and receive efficient antiviral therapy in order to prevent the development of serious liver disease.

Genotypes of HBV

The response to antiviral therapy in HBV infection is influenced by many host and viral factors. Recently, HBV genotypes have attracted increasing attention since they influence the activity and outcome of HBV-associated chronic liver disease, as well as the response to antiviral therapies. In 1988, four genotypes of HBV (A–D) were proposed based on sequence divergence of >8% in the entire HBV genome, which consists of approximately 3200 base pairs.[2] Later, an additional four genotypes (E–H) were identified by the same criteria.[3–5] HBV genotypes have distinct geographical distributions,[6] and the full picture awaits further epidemiological surveys in many as yet unexamined countries. Overall, genotype A prevails in Northwest Europe, sub-Saharan Africa, India and the United States, B and C are frequent in Southeast Asia, Japan and Oceania, and D is common in the Mediterranean countries. Genotype E is restricted to Africa, and F is found mainly in Central and South America. The distribution of genotypes G and H is yet to be determined. Since persistent HBV infection is frequent in Asia, genotypes B and C prevailing there have been studied most extensively with their clinical and therapeutic differences unfolding rapidly. Differences between genotypes A and D prevailing in Western countries, India and the United States, also, are increasingly coming to the fore.

Clinical manifestations of persistent HBV infection with distinct genotypes

Prospective, case-controlled and cross-sectional studies predominantly but not entirely indicate that the severity and outcome of chronic hepatitis B are more serious in patients infected with

*Corresponding author. Tel: +81-44-877-5111; Fax: +81-44-860-1623; E-mail: akuta-gi@umin.ac.jp

genotype C compared with B.[7–10] Liver cirrhosis and HCC are more frequent in carriers of genotype C than B.[7,11–13] Very recently, chronic liver disease was detected more frequently in Japanese individuals infected with genotype C than D [221/350 (63%) compared with 6/38 (16%), $P < 0.001$].[14]

The synthesis of hepatitis B e antigen (HBeAg) is regulated at both translational and transcriptional levels.[15] Mutations to create stop codons in the precore region, typified by the G-to-A mutation at nucleotide (nt) 1896 (G1896A), shut down the translation of HBeAg completely. The double mutation in the core promoter, A-to-T at nt 1762 and G-to-A at nt 1764 (A1762T/G1764A), interferes with the proper transcription of HBeAg precursor, thereby downregulating the synthesis of HBeAg. The precore stop codon mutation (G1896A) is detected more frequently in persons infected with genotype B than C; it is inhibited in those with genotype A, because it destabilizes the e encapsidation signal.[16] In remarkable contrast, the core promoter double mutation (A1762T/G1764A) is more common in those with genotype C than B. Overall, the ramifications of this are that the seroconversion to the loss of HBeAg takes longer in individuals infected with genotype C than B, and is accompanied by the development of severe liver disease.

Sugauchi *et al.*[17] reported two subtypes of genotype B, one of which possesses a recombination with genotype C over the precore region plus core gene (subtype Ba) while the other does not (subtype Bj). The distribution of subtype Bj is restricted to Japan (hence the 'j' for Japan), in contrast to subtype Ba found in all Asian countries other than Japan ('a' for Asia, therefore). Since HBeAg and the double mutation in the core promoter (A1762T/G1764A) are significantly more frequent in carriers of subtype Ba than Bj,[18] subtypes of genotype B may influence the clinical outcome and the response to antiviral therapies for chronic hepatitis B.

To a lesser extent, clinical differences between genotype A and D infections have been reported from Europe, where these genotypes are frequent. HBV infection is contracted in adulthood in these countries, principally through sexual contacts and illicit drug use, and HBV infection is more likely to persist in persons infected with genotype A rather than D or the other genotypes.[19] These findings stand at variance with those of Sanchez-Tapias *et al.*[20] who found sustained biochemical remission and clearance of HBV DNA to be more frequent in infection with genotype A than genotype D (log-rank, 14.2, $P = 0.002$) or genotype F (log-rank, 4.2, $P = 0.03$); the rate of HBsAg clearance was also found to be higher in genotype A compared with D infection (log-rank, 4.06, $P = 0.03$). Likewise in a comparison between 60 and 63 patients in India infected with HBV genotype A or D, respectively, genotype D was significantly associated with severe liver disease (61% compared with 30%, $P < 0.05$) and tended to be more frequent in those with HCC below 40 years of age (63% compared with 44%, $P = 0.06$).[21] Clinical differences amongst HBV genotypes manifest themselves in the distribution of acute and chronic liver disease in those who visit hospitals. In our Toranomon Hospital in metropolitan Tokyo, 57 adult patients with acute hepatitis B and 1077 with chronic hepatitis B were admitted during the same period.[22] The distribution of genotypes were: genotype A (acute, 22.8% versus chronic, 1.9%; $P < 0.00001$); B (14.0% versus 9.4%); C (43.9% versus 87.7%, $P = 0.004$); D (1.8% versus 0.2%); F (1.8% versus 0.2%); and untypeable (15.8% versus 0.6%, $P = 0.001$).

Influence of HBV genotype on the response to antiviral therapy

Until lamivudine was developed for clinical use, interferon had remained the sole practical antiviral for chronic hepatitis B, ever since initial clinical trials by Hoofnagle and colleagues[23,24] in the mid-1980s. The response to interferon, judged by the loss of HBeAg from serum, is achieved in at most 20% of treated patients.[25] Moreover, Asian patients who have acquired the HBV carrier state at birth or in early infancy respond to interferon more poorly than Caucasian patients who contracted it in adulthood.[26] To make matters even worse, patients with chronic hepatitis B positive for anti-HBe antibodies are much less responsive to interferon than those with serum HBeAg.[27] Limited experience indicates that HBV genotypes make a difference in the response to interferon in patients with chronic hepatitis B.

Zhang *et al.* compared the response between 10 patients with genotype A infection and 21 patients with genotype D or E infection.[28] Since all patients they studied were positive for anti-HBe antibodies, the negative influence of genotype A on seroconversion to anti-HBe was excluded. They found the response to interferon was higher in patients infected with genotype A compared with D or E (70% versus 40%, $P = 0.001$).[28] Likewise, Kao *et al.*[29] reported the response to interferon to be higher in patients infected with genotype B rather than C [13/32 (41%) versus 4/26 (15%), $P = 0.045$]. More recently, Wai *et al.*[30] compared the response between patients randomized to interferon or placebo. They found the response was better in patients with genotype B than C infection who were allocated to interferon treatment [12/31 (39%) versus 7/42 (17%), $P = 0.034$]; the response rate did not differ in those who received placebo.

Lamivudine [(−)-β-L-2′,3′-dideoxy-3′-thiacytidine] is a nucleoside analogue with a potent antiviral activity. Since its approval in 1998, lamivudine has gained wide popularity for the treatment of chronic hepatitis B due to high a efficacy with minimal untoward effects.[31–35] We believe in continued lamivudine treatment for patients with or without serum HBeAg,[36–39] and have accumulated experience with 286 patients including seven who have received lamivudine for 7 years or longer.[40] HBV genotype also makes a difference in the response to lamivudine in patients with chronic hepatitis B. Among the 16 patients who had received lamivudine for 3 years or longer, the virological response with the loss of HBV DNA detectable by non-amplified method was achieved in two of the three (67%) patients infected with genotype B and in seven of the 13 (54%) patients infected with genotype C.

Kao *et al.*[41] reported on the response to lamivudine in patients treated for 6–30 months infected with genotype B compared with C [3/13 (23%) versus 2/18 (11%), no significant differences]. They found resistance to lamivudine in two (15%) patients with genotype B and in four (22%) with genotype C. Chien *et al.*[42] reported that the sustained response to lamivudine was much higher in patients infected with genotype B compared with genotype C [38/62 (61%) versus 5/20 (20%), $P = 0.009$]. We monitored 213 patients on continued lamivudine treatment for drug-resistant HBV variants for mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the viral DNA polymerase/reverse transcriptase.[43] The emergence of YMDD mutants was no different amongst patients infected with genotype A, B or C. However, YMDD mutants developed significantly more frequently in patients infected with subtype Ba

JAC antiviral

than Bj during 2 years on lamivudine [3/4 (75%) versus 1/14 (7%), $P<0.05$]. Severe acute exacerbation of hepatitis occurred in four of the 185 (2%) patients with genotype C along with the emergence of YMDD mutants, but in none of the 28 patients with the other genotypes. In patients with chronic hepatitis B in Germany, risk of lamivudine resistance was significantly higher in carriers of HBsAg of serotype adw than ayw [7/13 (54%) versus 1/13 (8%), $P=0.03$];[44] serotype adw corresponded to genotype A and ayw to D.[45]

Conclusion

HBV genotypes influence the severity of liver disease and response to interferon and lamivudine. They are also expected to influence the response to adefovir dipivoxil, which has recently been approved for treatment of chronic hepatitis B,[46] as well as the emergence of resistant mutants;[47] although as yet no differences have been observed in the response to adefovir dipivoxil in relation to HBV genotypes.[48] Should poor responses to a given antiviral be predicted in patients infected with HBV of certain genotypes, they can be directed to the other therapeutic options to spare the cost and burden of treatment. In evaluating the association of HBV genotypes with the response to antiviral therapies, however, it needs to be taken into account that patients visiting hospitals are biased for severe liver disease. Moreover, once full-blown disease develops, it would become refractory to any antiviral treatments. Thus, genotype differences may be attenuated in patients with severe liver disease seen in hospitals,[49,50] probably due to exclusion of patients with less severe disease who can still benefit from treatments. This view would be supported by different distributions of genotypes B (12% and 54%, respectively) and C (84% and 47%) between patients visiting hospitals and individuals found with HBV infection at routine check-ups in the same district of Japan.[51,52] Therefore, in evaluating the influence of HBV genotypes on response to antiviral therapies, one has to keep in mind not only patients with liver disease who visit hospitals, but also those who have not and who may benefit from early treatment.

References

1. Lee, W. M. (1997). Hepatitis B virus infection. *New England Journal of Medicine* **337**, 1733–45.

2. Okamoto, H., Tsuda, F., Sakugawa, H. *et al.* (1988). Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *Journal of General Virology* **69**, 2575–83.

3. Norder, H., Courouce, A. M. & Magnius, L. O. (1994). Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* **198**, 489–503.

4. Stuyver, L., De Gendt, S., Van Geyt, C. *et al.* (2000). A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *Journal of General Virology* **81**, 67–74.

5. Arauz-Ruiz, P., Norder, H., Robertson, B. H. *et al.* (2002). Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *Journal of General Virology* **83**, 2059–73.

6. Miyakawa, Y. & Mizokami, M. (2003). Classifying hepatitis B virus genotypes. *Intervirology* **46**, 329–38.

7. Kao, J. H., Chen, P. J., Lai, M. Y. *et al.* (2000). Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* **118**, 554–9.

8. Orito, E., Mizokami, M., Sakugawa, H. *et al.* (2001). A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. *Hepatology* **33**, 218–23.

9. Chu, C. J., Hussain, M. & Lok, A. S. (2002). Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* **122**, 1756–62.

10. Nakayoshi, T., Maeshiro, T., Nakasone, H. *et al.* (2003). Difference in prognosis between patients infected with hepatitis B virus with genotype B and those with genotype C in the Okinawa Islands: a prospective study. *Journal of Medical Virology* **70**, 350–4.

11. Tsubota, A., Arase, Y., Ren, F. *et al.* (2001). Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. *Journal of Medical Virology* **65**, 257–65.

12. Fujie, H., Moriya, K., Shintani, Y. *et al.* (2001). Hepatitis B virus genotypes and hepatocellular carcinoma in Japan. *Gastroenterology* **120**, 1564–5.

13. Lee, C. M., Chen, C. H., Lu, S. N. *et al.* (2003). Prevalence and clinical implications of hepatitis B virus genotypes in southern Taiwan. *Scandinavian Journal of Gastroenterology* **38**, 95–101.

14. Duong, T. N., Horiike, N., Michitaka, K. *et al.* (2004). Comparison of genotypes C and D of the hepatitis B virus in Japan: a clinical and molecular biological study. *Journal of Medical Virology* **72**, 551–7.

15. Miyakawa, Y., Okamoto, H. & Mayumi, M. (1997). The molecular basis of hepatitis B e antigen (HBeAg)-negative infections. *Journal of Viral Hepatitis* **4**, 1–8.

16. Li, J. S., Tong, S. P., Wen, Y. M. *et al.* (1993). Hepatitis B virus genotype A rarely circulates as an HBe-minus mutant: possible contribution of a single nucleotide in the precore region. *Journal of Virology* **67**, 5402–10.

17. Sugauchi, F., Orito, E., Ichida, T. *et al.* (2002). Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *Journal of Virology* **76**, 5985–92.

18. Sugauchi, F., Orito, E., Ichida, T. *et al.* (2003). Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. *Gastroenterology* **124**, 925–32.

19. Mayerat, C., Mantegani, A. & Frei, P. C. (1999). Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? *Journal of Viral Hepatitis* **6**, 299–304.

20. Sanchez-Tapias, J. M., Costa, J., Mas, A. *et al.* (2002). Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology* **123**, 1848–56.

21. Thakur, V., Guptan, R. C., Kazim, S. N. *et al.* (2002). Profile, spectrum and significance of HBV genotypes in chronic liver disease patients in the Indian subcontinent. *Journal of Gastroenterology and Hepatology* **17**, 165–70.

22. Kobayashi, M., Arase, Y., Ikeda, K. *et al.* (2002). Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. *Journal of Medical Virology* **68**, 522–8.

23. Dooley, J. S., Davis, G. L., Peters, M. *et al.* (1986). Pilot study of recombinant human alpha-interferon for chronic type B hepatitis. *Gastroenterology* **90**, 150–7.

24. Hoofnagle, J. H., Peters, M., Mullen, K. D. *et al.* (1988). Randomized, controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. *Gastroenterology* **95**, 1318–25.

25. Wong, D. K., Cheung, A. M., O'Rourke, K. *et al.* (1993). Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Annals of Internal Medicine* **119**, 312–23.

26. Liaw, Y. F., Leung, N., Guan, R. *et al.* (2003). Asian-Pacific consensus statement on the management of chronic hepatitis B: an update. *Journal of Gastroenterology and Hepatology* **18**, 239–45.

27. Brunetto, M. R., Giarin, M. M., Oliveri, F. *et al.* (1991). Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. *Proceedings of the National Academy of Sciences, USA* **88**, 4186–90.

28. Zhang, X., Zoulim, F., Habersetzer, F. *et al.* (1996). Analysis of hepatitis B virus genotypes and pre-core region variability during interferon treatment of HBe antigen negative chronic hepatitis B. *Journal of Medical Virology* **48**, 8–16.

29. Kao, J. H., Wu, N. H., Chen, P. J. *et al.* (2000). Hepatitis B genotypes and the response to interferon therapy. *Journal of Hepatology* **33**, 998–1002.

30. Wai, C. T., Chu, C. J., Hussain, M. *et al.* (2002). HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* **36**, 1425–30.

31. Nevens, F., Main, J., Honkoop, P. *et al.* (1997). Lamivudine therapy for chronic hepatitis B: a six-month randomized dose-ranging study. *Gastroenterology* **113**, 1258–63.

32. Lai, C. L., Ching, C. K., Tung, A. K. *et al.* (1997). Lamivudine is effective in suppressing hepatitis B virus DNA in Chinese hepatitis B surface antigen carriers: a placebo-controlled trial. *Hepatology* **25**, 241–4.

33. Suzuki, Y., Kumada, H., Ikeda, K. *et al.* (1999). Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *Journal of Hepatology* **30**, 743–8.

34. Dienstag, J. L., Schiff, E. R., Wright, T. L. *et al.* (1999). Lamivudine as initial treatment for chronic hepatitis B in the United States. *New England Journal of Medicine* **341**, 1256–63.

35. Liaw, Y. F., Leung, N. W., Chang, T. T. *et al.* (2000). Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. *Gastroenterology* **119**, 172–80.

36. Suzuki, F., Suzuki, Y., Tsubota, A. *et al.* (2002). Mutations of polymerase, precore and core promoter gene in hepatitis B virus during 5-year lamivudine therapy. *Journal of Hepatology* **37**, 824–30.

37. Suzuki, Y., Arase, Y., Ikeda, K. *et al.* (2003). Histological improvements after a three-year lamivudine therapy in patients with chronic hepatitis B in whom YMDD mutants did not or did develop. *Intervirology* **46**, 164–70.

38. Suzuki, F., Tsubota, A., Arase, Y. *et al.* (2003). Efficacy of lamivudine therapy and factors associated with emergence of resistance in chronic hepatitis B virus infection in Japan. *Intervirology* **46**, 182–9.

39. Kumada, H. (2004). Lamivudine for patients with chronic hepatitis B: how long? *Journal of Gastroenterology*, in press.

40. Kumada, H. (2003). Continued lamivudine therapy in patients with chronic hepatitis B. *Intervirology* **46**, 377–87.

41. Kao, J. H., Liu, C. J. & Chen, D. S. (2002). Hepatitis B viral genotypes and lamivudine resistance. *Journal of Hepatology* **36**, 303–4.

42. Chien, R. N., Yeh, C. T., Tsai, S. L. *et al.* (2003). Determinants for sustained HBeAg response to lamivudine therapy. *Hepatology* **38**, 1267–73.

43. Akuta, N., Suzuki, F., Kobayashi, M. *et al.* (2003). The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. *Journal of Hepatology* **38**, 315–21.

44. Zollner, B., Petersen, J., Schroter, M. *et al.* (2001). 20-fold increase in risk of lamivudine resistance in hepatitis B virus subtype adw. *Lancet* **357**, 934–5.

45. Zollner, B., Petersen, J., Puchhammer-Stockl, E. *et al.* (2004). Viral features of lamivudine resistant hepatitis B genotypes A and D. *Hepatology* **39**, 42–50.

46. Qaqish, R. B., Mattes, K. A. & Ritchie, D. J. (2003). Adefovir dipivoxil: a new antiviral agent for the treatment of hepatitis B virus infection. *Clinical Therapeutics* **25**, 3084–99.

47. Angus, P., Vaughan, R., Xiong, S. *et al.* (2003). Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology* **125**, 292–7.

48. Westland, C., Delaney, W. T., Yang, H. *et al.* (2003). Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil. *Gastroenterology* **125**, 107–16.

49. Sumi, H., Yokosuka, O., Seki, N. *et al.* (2003). Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* **37**, 19–26.

50. Yuen, M. F., Wong, D. K., Sablon, E. *et al.* (2003). Hepatitis B virus genotypes B and C do not affect the antiviral response to lamivudine. *Antiviral Therapy* **8**, 531–4.

51. Orito, E., Ichida, T., Sakugawa, H. *et al.* (2001). Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* **34**, 590–4.

52. Ishikawa, K., Koyama, T. & Masuda, T. (2002). Prevalence of HBV genotypes in asymptomatic carrier residents and their clinical characteristics during long-term follow-up: the relevance to changes in the HBeAg/anti-HBe system. *Hepatology Research* **24**, 1–7.