Pathogenic immunity and protective immunity in chronic hepatitis B

Sheikh Mohammad Fazle Akbar[1], Mamun Al-Mahtab[2], Md. Sakirul islam Khan[3], Sheikh Mohammad Noor-E-Alam[4], Ahmed Lutful Moben[5], Md. Ashraful Alam[6]

[1]Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan

[2]Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

[3]Department of Anatomy and Embryology, Ehime University Graduate School of Medicine, Ehime, Japan

[4]Department of Hepatology, Shahid Tajuddin Ahmad Medical College, Gazipur, Bangladesh,

[5]Hepatologist, Kurmitola General Hospital, Tangail, Bangladesh

[6]Department of Hepatology, Dhaka Medical College, Dhaka, Bangladesh

Corresponding Author

Dr. Sheikh Mohammad Fazle Akbar, MD, PhD

Principal Investigator, Department of Medical Sciences,

Toshiba General Hospital

Higashi Oi 6-3-22, Shinagawa, Tokyo 140-8522, Japan

Phone: 81 3 3764 0511 (Ext 3913), Mobile Phone: 819099790098, Fax: 81 3 3764 8992

E. mail: sheikh.akbar@po.toshiba.co.jp

Abstract

Hepatitis B virus (HBV) infects human being and at present about 2 billion people of the world has been infected by HBV at some point of their life and about 240-370 million are chronically infected with HBV. The natural course of HBV infection exhibits considerable variability. In one hand, the majority of HBV-infected persons control HBV replication and liver damages after initial infection. On the other hand, considerable numbers of HBV-infected patients develop chronic hepatitis B (CHB) and complications like cirrhosis of liver (LC) and hepatocellular carcinoma (HCC). The mechanisms underlying these dichotomies have not been properly explored and accordingly evidence-based therapeutic approaches for chronic HBV infection could not be developed. HBV is a non-cytopathic virus with no direct role on liver damage in HBV-infected patients. In one hand, host immunity of HBV-infected patients has important roles in control of HBV and containment of liver damages. On the other hand, recent evidences have unmasked that host immunity is also responsible for ongoing viral replication and progressive liver damages. Thus, host immunity in HBV infection may be categorized in two broad headings; (1) pathogenic immunity and (2) protective immunity. In this review, a comprehensive discussion will be made about the nature of 'pathogenic immunity' and 'protective immunity' during chronic HBV infection and their implications for developing new and innovative therapy.

Key words: Antigen-specific immunocytes; chronic hepatitis B; HBV replication; immune therapy; non-antigen-specific immunocytes; pathogenic immunity; protective immunity, therapeutic immunity

Text

1. Natural course of HBV infection

1.1 Epidemiology of HBV infection

The natural course of HBV infection differs among individuals and is dependent on multiple viral and host factors. Epidemiological data indicate that of the 2 billion HBV-infected individuals worldwide, 80-90% (~1.7 billion at least) exhibit controlled viral replication and no liver damage [1]. On the other hand, 240-370 million HBV-infected individuals experience persistent HBV replication, and ~20% experience liver damage [1,2]. CHB patents are at risk of developing HBV-related complications, including liver cirrhosis (LC) and hepatocellular carcinoma (HCC), with 0.6-1.2 million individuals dying from these complications each year [3].

1.2 Liver damage by HBV

Approximately 20% of chronic HBV-infected patients experience persistent HBV replication and liver damage. Conversely, ~80% of chronic HBV-infected patients do not exhibit liver damage, despite harbouring HBV DNA, hepatitis B surface antigen (HBsAg), and hepatitis B e antigen (HBeAg) [4,5]. HBV is a non-cytopathic virus, and its direct role on liver damage has not been elucidated in animal models or CHB patients. Although multiple HBV transgenic mouse (HBV TM) lines containing different levels of HBV DNA, HBsAgs, and HBeAgs have been developed, none experienced liver damage [6-8]. Moreover, the levels of HBV DNA, HBsAg or HBeAg-positivity or HBeAg-negativity do not correlate with the extent of

liver damage in CHB patients [9-11].

2. Host immunity during HBV infection

2.1 Dichotomy of host immunity in HBV infection

Since the levels of HBV, or its antigens, do not correlate with liver damage in CHB patients, most investigations have focused on host factors. Circumstantial and experimental data suggest that the immune responses of HBV-infected hosts cause liver damage. Additionally, host immunity also appears to play a role in controlling viral replication. Thus, host immunity is dichotomous during CHB infection, altering between pathogenic and protective/therapeutic states. Understanding such states—and the control there of-is crucial for developing therapies against CHB, as well as for preventing HBV-related complications and deaths.

2.2 'Pathogenic Immunity' in Chronic HBV infection

A temporal relationship between host immunity and hepatocyte damage was shown by Ando *et al*. in an HBV TM model of infection [12], in which an accumulation of non-specific mononuclear cell infiltrates was observed in the damaged liver of HBV TM. The roles of pathogenic immunity in CHB patients were also investigated by Maini *et al.*, who showed that the levels of non-antigen-specific lymphocytes were elevated in the liver of CHB patients experiencing liver damage [13]. That study also revealed a significantly higher ratio of non-antigen-specific immunocytes to HBV antigen-specific immunocytes in CHB patients experiencing liver damage and HBV replication [13].

2.3 Natural killer (NK) cells and pathogenic immunity by non-antigen-specific immunocytes

The proportion of NK cells to total immunocytes is normally high in healthy liver; however, the biological significance of this phenomenon is unknown. Moreover, the number of NK cells in the liver of CHB patients is further elevated, which results in liver damage [14]. Dunn *et al*. investigated the mechanisms underlying NK-induced liver damage in CHB patients [15] and found that the majority of NK cells infiltrating damaged liver were activated. Additionally, the concentration of activated NK cells was significantly higher among CHB patients with high ALT levels compared with patients with low ALT levels. Based on those data, the authors suggested that non-antigen-specific immunocytes play an important role in liver damage in CHB patients [15]. This hypothesis has since been supported by additional investigations [16-18], and it seems likely that non-antigen-specific immunocytes induce pathogenic immunity in CHB patients.

2.4 Migration of non-antigen-specific immunocytes in the liver of CHB patients

Compared with healthy control patients, CHB patients exhibit elevated NK cell numbers in liver [15]; however, little is known regarding the underlying mechanisms of migration and localization. A role for cytokines in such mechanisms has been highlighted, since HBV-infected liver secretes interferon (IFN)-gamma-induced chemokines, including CXCL9 and CXCL10, which may influence the recruitment of antigen-non-specific immunocytes [19-21]. The recruitment of such non-antigen-specific immunocytes can cause chronic inflammation that may induce liver damage and chronic hepatitis [15,19-21].

2.5 Potential mechanisms of NK cell-mediated damage of hepatocytes in CHB, including tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated killing of hepatocytes by activated NK cells:

TNF-related apoptosis-inducing ligand (TRAIL) is a primary ligand involved in the NK-mediated destruction of hepatocytes. NK cells from control subjects express low levels of TRAIL [22]; however, expression is upregulated in NK cells from CHB patients [15]. The receptor for TRAIL is also upregulated on the surface of hepatocytes in CHB patients [23]; thus, NK cells expressing TRAIL may induce apoptosis of liver cells expressing the ligand [15]. In addition to a TRAIL-dependent mechanism of apoptosis, the Fas pathway has also been implicated in causing lysis of hepatocytes by NK cells in CHB patients [15,18].

2.6 The role of cytokines in liver damage

Multiple cytokines are involved in the pathogenesis of CHB, including interleukin (IL)-8, IFN-alpha, and IL-1beta, the expression of which is elevated in CHB patients, compared with healthy controls [23]. The expression levels of IL-6, TNF-alpha, transforming growth factor (TGF)-beta, hepatocyte growth factor, and epidermal growth factor are also elevated in CHB patients [24,25]. Moreover, IL-22 has been shown to increase the pro-inflammatory activity of TNF-alpha.

2.7 Pathogenic immunity in CHB and progression to complications

Persistent inflammation in CHB patients activates hepatic stellate cells, myofibroblasts, and fibroblasts, which initiate collagen, laminin, fibronectin, and proteoglycan production and deposition, leading to liver

fibrosis. The activation of these cells is regulated by proinflammatory cytokines such as TGF-β, IL-6, TNF-α, and CCL-21, among other stimuli [27]. Thus, LC is caused by the collective dysregulation of liver regeneration processes [28].

3. Protective Immunity in Chronic HBV Infection

3.1 Nature of protective immunity

An increase in HBV antigen-specific immunocytes has been observed in the liver of patients with CHB who experience limited HBV replication and reduced liver damage [12,13]. Studies have provided evidence of a therapeutic role for HBV antigen-specific immunocytes [33-36] in CHB patients that may stem from the elevated levels of hepatitis B core antigen (HBcAg)-specific and HBsAg-specific cytotoxic T lymphocytes (CTL). Also, antigen-specific immunity may induce suppression of HBV replication by non-cytotoxic mechanisms. However, the cellular mechanisms related to liver protection by antigen-specific immunocytes need to be elucidated in CHB patients.

4. Proof of concept:

4.1 Protective Immunity by HBV antigen-specific immune modulators

Although high levels of antigen-specific immunocytes have been detected in the liver of CHB patients who experience controlled HBV replication and reduced liver damage, direct causation for this correlation is lacking. Several investigators have assessed the safety and therapeutic potential of HBV antigen-specific immune modulation in CHB patients, and Pol *et al.* (1994) revealed that administration of HBsAg was safe

and effective at reducing the concentration of HBV DNA and liver damage [37]. Subsequent clinical trials also showed that immunity induced by HBV antigen-specific immune modulators was safe and effective in CHB patients [38-50].

In addition to HBsAgs, a combination of HBsAg and anti-HBs was administered to Chinese CHB patients by Wen and colleagues [39-41]. A combination of antiviral drugs and therapeutic vaccines have also been used to induce antigen-specific immunity in CHB patients and were found to limit HBV replication and control liver damage [42-45]. An HBcAg epitope-based vaccine has also been deemed safe and effective in CHB patients [47,48], and recently, a phase I/II clinical trial using an antigen-specific immune modulator containing HBsAg and HBcAg has begun. The HBsAg/HBcAg-based vaccine is safe in CHB patients in whom it reduced HBV DNA in 50% of individuals. Normalization of ALT was also observed in all vaccine recipients [49]. A phase-III clinical trial of the nasally and subcutaneously administered HBsAg/HBcAg-based vaccine has revealed comparable therapeutic efficacy to that of pegylated IFN [Registered; ClinicalTrials.gov; NCT01374308] [50].

5. **Compilation**

Elucidation of the dichotomous nature of immunity in CHB is increasing, and a temporal relationship between the pathogenic effects of non-antigen-specific immunity and the protective effects of HBV antigen-specific immunity has also been observed. Inducible HBV antigen-specific immunity has not been shown to have adverse effects in CHB patients and is non-pathogenic; however, long-term assessments

of such patients-with a particular focus on autoimmune phenomena-have not been performed.

Although inducible non-antigen-specific immunity seems to be pathogenic in nature, NUCs and IFN have also shown protective and therapeutic effects in limited numbers of CHB patients [51-54]. These observations raise several important questions regarding the immunity of CHB patients, including those surrounding the nature of immunocytes conferring pathogenic and protective immunity, the conversion of non-antigen-specific to antigen-specific immunity, the differences between antigen-specific and pathogenic immunity, and the role of the hepatic microenvironment that supports different types of immunity.

The definition of CTL as it pertains to CHB patients should also be reviewed. CTLs are used to identify a population of cells that kill their target cells; however, HBcAg-specific and HBsAg-specific CTLs do not kill hepatocytes, but rather play a protective role in containing liver damage and HBV replication. Although the mechanisms underlying these activities are unknown, antigen-specific CTLs may produce cytokines that influence viral replication in a non-cytopathic manner. It is possible that the observed effects are due to a combination of traditional CTLs with cytotoxic properties and CD8 T+ cells with protective properties.

The important cellular and molecular events regarding pathogenic and protective immunity in CHB patients cannot be investigated in humans due to ethical and scientific limitations. Thus, HBV TM models are used for such studies; however, mice do not experience liver damage. Therefore, the development of other animal models, including Tupai and Woodchuck, for such studies is required. Finally,

pathogenic and protective immunities may not be mutually exclusive events, as one type may convert to the other based on the hepatic microenvironment or other HBV-related factors.

Acknowledgement

The study has been supported by Japan Agency for Medical Research and Development (AMED), Japan

References

1. Iloeje UH, Yang HI, Chen CJ. Natural history of chronic hepatitis B: what exactly has REVEAL revealed? Liver Int 2012;32(9):1333-4

2. Hadziyannis SJ, Vassilopoulos D, Hadziyannis E. The natural course of chronic hepatitis B virus infection and its management. Adv Pharmacol 2013; 67: 247-91

3. Wang FS, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China. Hepatology 2014;60(6):2099-2108

4. Manesis EK, Papatheodoridis GV, Tiniakos DG, et al. Hepatitis B surface antigen: relation to hepatitis B replication parameters in HBeAg-negative chronic hepatitis B. J Hepatol 2011;55(1):61-8

5. Al-Mahtab M, Akbar SM, Rahman S, Kamal M, Khan MSI. Biochemical, virological, immunological and histopathological features of 702 incidentally detected chronic hepatitis B virus carriers in Bangladesh. Digestion 2012;86(1):1-5

6. Farag MM, Tedjokusumo R, Flechtenmacher C, et al. Immune tolerance against HBV can be overcome in HBV transgenic mice by immunization with dendritic cells pulsed by HBVsvp. Vaccine 2012; 30(42): 6034-9

7. Buchmann P, Dembek C, Kuklick L ,et al. A novel therapeutic hepatitis B vaccine induces cellular and humoral immune responses and breaks tolerance in hepatitis B virus (HBV) transgenic mice. Vaccine. 2013;31(8):1197-203.

8. Akbar SM, Chen S, Al-Mahtab M, Abe M, Hiasa Y, Onji M.. Strong and multi-antigen specific immunity by hepatitis B core antigen (HBcAg)-based vaccines in a murine model of chronic hepatitis B: HBcAg is a candidate for a therapeutic vaccine against hepatitis B virus. Antiviral Res. 2012;96(1):59-64.

9. Chu CM, Chen YC, Tai DI, Liaw YF. Level of hepatitis B virus DNA in inactive carriers with persistently normal levels of alanine aminotransferase. Clin Gastroenterol Hepatol. 2010;8(6):535-40.

10. Kumar M, Sarin SK, Hissar S, et al. Virologic and histologic features of chronic hepatitis B virus-infected asymptomatic patients with persistently normal ALT. Gastroenterology 2008;134(5): 1376-84

11. Tseng TC, Liu CJ, Yang HC, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low hepatitis B virus loads. Hepatology 2013;57(2):441-50

12. Ando K, Moriyama T, Guidotti LG, et al. Mechanisms of class I restricted immunopathology. A transgenic mouse model of fulminant hepatitis. J Exp Med 1993; 178:1541–54

13. Maini MK, Boni C, Lee CK, et al. The role of virus- specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med 2000; 191: 1269–80

14. Zheng Q, Zhu YY, Chen J, et al. Activated natural killer cells accelerate liver damage in patients with chronic hepatitis B virus infection. Clin Exp Immunol 2015;180(3):499-508

15. Dunn C. Brunetto M, Reynolds G, et al. Cytokines-induced during chronic hepatitis B virus infection promote a pathway for NK cell-mediated liver damage. J Exp Med 2007; 20: 667–680

16. Zhang Z, Zhang S, Zou Z, et al. Hypercytolytic activity of hepatic natural killer cells correlates with liver injury in chronic hepatitis B patients. Hepatology 2011; 53: 73–85.

17. Zheng SJ, Wang P, Tsabary G, Chen YH. Critical roles of TRAIL inhepatic cell death and hepatic inflammation. J Clin Invest 2004; 113: 58–64

18. Zou Y, Chen T, Han M, et al. Increased killing of liver NK cells by Fas/Fas ligand and NKG2D/NKG2D ligand contributes to hepatocyte necrosis in virus-induced liver failure. J Immunol 2010; 184:466–75

19. Wu HL, Kao JH, Chen TC, et al. Serum cytokine/chemokine profiles in acute exacerbation of chronic hepatitis B: clinical and mechanistic implications. J Gastroenterol Hepatol. 2014;29(8):1629-36

20. Bayard F, Godon O, Nalpas B, et al. T-cell responses to hepatitis B splice-generated protein of hepatitis B virus and inflammatory cytokines/chemokines in chronic hepatitis B patients. ANRS study: HB EP 02 HBSP-FIBRO. J Viral Hepat. 2012 ;19(12):872-80.

21. Zhou Y, Wang S, Ma JW, et al. Hepatitis B virus protein X-induced expression of the CXC chemokine IP-10 is mediated through activation of NF-kappaB and increases migration of leukocytes. J Biol Chem 2010;285(16):12159-68.

22. Ishiyama, K., H. Ohdan, M. Ohira, H. Mitsuta, K. Arihiro, and T. Asahara. Difference in cytotoxicity against hepatocellular carcinoma between liver and periphery natural killer cells in humans. Hepatology 2006; 43:362–72.

23. Peppa D, Gill US, Reynolds G, et al. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. J Exp Med 2013; 210(1):99-114

24. Ramadori G, Saile B. Inflammation, damage repair, immune cells, and liver fibrosis: specific or nonspecific, this is the question. Gastroenterology 2004; 127: 997-1000.

25. Larrubia JR, Benito-Martínez S, Miquel-Plaza J, et al. Cytokines - their pathogenic and therapeutic role in chronic viral hepatitis. Rev Esp Enferm Dig 2009;101(5):343-51

26. Cobleigh MA, Robek MD. Protective and pathological properties of IL-22 in liver disease: Implication for viral hepatitis. Am J Pathol 2013;182:21-28.

27. Taub R, Greenbaum LE, Peng Y. Transcriptional regulatory signals define cytokine-dependent and -independent pathways in liver regeneration. Semin Liver Dis 1999; 19: 117-27.

28. Ramadori G, Saile B. Inflammation, damage repair, immune cells, and liver fibrosis: specific or nonspecific, this is the question. Gastroenterology 2004; 127: 997-1000.

29. Tilg H, Vogel W, Tratkiewicz J et al. Pilot study of natural human interleukin-2 in patients with chronic hepatitis B. Immunomodulatory and antiviral effects. J Hepatol 1993; 19(2):259-67.

30. Artillo S, Pastore G, Alberti A, et al. Double-blind, randomized controlled trial of interleukin-2 treatment of chronic hepatitis B. J Med Virol 1998; 54:167-172

31. Ruiz-Moreno M, García R, Rua MJ, et al. Levamisole and interferon in children with chronic hepatitis B. Hepatology 1993; 18(2):264-9

32. Woltman AM, Ter Borg MJ, Binda RS et al. Alpha-galactosylceramide in chronic hepatitis B infection: results from a randomized placebo-controlled Phase I/II trial. Antivir Ther 2009; 14(6), 809-18

33. Bertoletti A, Maini MK, Ferrari C. The host-pathogen interaction during HBV infection: immunological controversies. Antivir Ther 2010;15 Suppl 3:15-24.

34. Tan AT, Koh S, Goh W, Zhe HY, Gehring AJ, Lim SG, Bertoletti A. A longitudinal analysis of innate and adaptive immune profile during hepatic flares in chronic hepatitis B. .J Hepatol 2010;52(3):330-9

35. Maini MK, Peppa D. NK cells: a double-edged sword in chronic hepatitis B virus infection. Front Immunol 2013;4:57

36. Bertoletti A, Maini MK. Protection or damage: a dual role for the virus-specific cytotoxic T lymphocyte response in hepatitis B and C infection? Curr Opin Microbiol 2000;3(4):387-92.

37. Pol S, Driss F, Michel ML, et al. Specific vaccine therapy in chronic hepatitis B infection. Lancet 1994; 344(8918): 342

38. Senturk H, Tabak F, Akdogan M, et al. Therapeutic vaccination in chronic hepatitis B. J Gastroenterol. Hepatol 2002; 17(1):72–76

39. Wang XY, Zhang XX, Yao X, et al. Serum HBeAg sero-conversion correlated with decrease of HBsAg and HBV DNA in chronic hepatitis B patients treated with a therapeutic vaccine. Vaccine 2010; 28(51), 8169-74

40. Wen YM, Wu XH, Hu DC, et al. Hepatitis B vaccine and anti-HBs complex as approach for vaccine

therapy. Lancet 1995; 345(8964): 1575–1576.

41. Xu DZ, Zhao K, Guo LM, et al. A randomized controlled phase IIb trial of antigen-antibody immunogenic complex therapeutic vaccine in chronic hepatitis B patients. PLoS ONE 2008; 3:e2565,
42. Vandepapelière P, Lau GK, Leroux-Roels G, et al. Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. Vaccine 2007; 25(51); 8585-8597
43. Horiike N, Fazle Akbar SM, Michitaka K, et al. In vivo immunization by vaccine therapy following virus suppression by lamivudine: a novel approach for treating patients with chronic hepatitis B. J Clin Virol 2005; 32(2),156-61
44. Wang XY, Zhang XX, Yao X, et al. Serum HBeAg sero-conversion correlated with decrease of HBsAg and HBV DNA in chronic hepatitis B patients treated with a therapeutic vaccine. Vaccine 2010; 28(51), 8169-8174
45. Hoa PT, Huy NT, Thu le T, et al. Randomized controlled study investigating viral suppression and serological response following pre-S1/pre-S2/S vaccine therapy combined with lamivudine treatment in HBeAg-positive patients with chronic hepatitis B. Antimicrob Agents Chemother 2009; 53(12): 5134-40
46. Yang FQ, Yu YY, Wang GQ, et al. A pilot randomized controlled trial of dual-plasmid HBV DNA

vaccine mediated by in vivo electroporation in chronic hepatitis B patients under lamivudine chemotherapy. J Viral Hepat 2012; 19(8),581-93

47. Heathcote J, McHutchison J, Lee S, et al. A pilot study of the CY-1899 T-cell vaccine in subjects chronically infected with hepatitis B virus. The CY1899 T Cell Vaccine Study Group. Hepatology 1999; 30(2): 531–6.

48. Livingston BD, Alexander J, Crimi C, et al. Altered helper T lymphocyte function associated with chronic hepatitis B virus infection and its role in response to therapeutic vaccination in humans. J Immunol 1999; 161(5):3088-95

49. Al-Mahtab M, Akbar SM, Aguilar JC, Uddin MH, Khan MS, Rahman S. Therapeutic potential of a combined hepatitis B virus surface and core antigen vaccine in patients with chronic hepatitis B. Hepatol Int 2013; 7(4), 981-9

50. Akbar SM, Al-Mahtab M, Rahman S et al. A phase III clinical trial with a therapeutic vaccine containing both HBsAg and HBcAg administered via both mucosal and parenteral routes in patients with chronic hepatitis B. Presented at: 64th Annual Meeting of American Association for the Study of the Liver, Washington DC, USA, 1st November-5th November 2013. ** Only phase III clinical trial with HBsAg/HBcAg-based therapeutic vaccine in chronic hepatits B

51. Yang YF, Zhao W, Zhong YD, Xia HM, Shen L, Zhang N. Interferon therapy in chronic hepatitis B reduces progression to cirrhosis and hepatocellular carcinoma: a meta-analysis. J Viral Hepat 2009 ;16(4):265-71.

52. Moon JC, Kim SH, Kim IH, et al. Disease progression in chronic hepatitis B patients under long-term antiviral therapy. Gut Liver 2015; 9(3): 395-404

53. Chiang CJ, Yang YW, Chen JD, et al. Significant reduction in end-stage liver diseases burden through the national viral hepatitis therapy program in Taiwan. Hepatology 2015; 61(4): 1154-1162

54. Kim WR, Loomba R, Berg T et al. Impact of long-term tenofovir disoproxil fumarate on incidence of hepatocellular carcinoma in patients with chronic hepatitis B. Cancer 2015; 121(20): 3631-.8