Review Article

Toll-Like Receptors Signaling Contributes to Immunopathogenesis of HBV Infection

Yasuteru Kondo, Yoshiyuki Ueno, and Tooru Shimosegawa

Division of Gastroenterology, Tohoku University Hospital, 1-1 Seiryo-Machi, Aoba-ku, Miyagi, Sendai City 980-8574, Japan

Correspondence should be addressed to Yasuteru Kondo, yasuteru@ebony.plala.or.jp

Innate and adaptive immune systems have important role in the pathogenesis of acute and chronic infection with hepatitis B virus (HBV). These immune responses are mediated through complex interactions between the innate immune response and adaptive immune response. Toll-like receptors (TLRs) are a family of innate immune-recognition receptors that recognize the molecular patterns associated with microbial pathogens. So far, TLR1 to 13 were found in human or mice and investigated to detect the target molecules and the downstream mechanisms of these unique systems. Stimulation by their ligands initiates the activation of complex networks of intracellular signaling transduction and innate and adaptive immune-related cells (NK, NK-T, monocytes, dendritic cells, T cells, B cells, and Tregs, etc.). However, reports on such relationships between HBV and TLRs have been relatively rare in comparison to those on HCV and TLRs, but have recently been increasing. Thus, a review of TLRs involved in the pathogenesis of HBV infection may be needed toward better understanding of the immunopathogenesis of HBV infection.

1. Introduction

HBV is an enveloped, double-strand DNA virus that causes acute, chronic hepatitis and hepatocellular carcinoma (HCC) [1]. HBV infection continues to be a significant global health problem. There are approximately 400 million chronic HBV carriers in the world, and in approximately 5% of adults and 95% of neonates who become infected with HBV, persistent infection develops [2].

It has been shown that the innate immune system including natural killer (NK) cells, NK-T cells, monocytes, and intrahepatocyte-immune reaction in addition to the adaptive immune system including cytotoxic T lymphocyte (CTLs), Th1 CD4+ cells, CD4+CD25+FOXP3+ regulatory T cells (Tregs), and dendritic cells (DCs) plays an important role in the control of virus [3–8]. The hyporesponsiveness of NK cells, HBV-specific Th1 cells and distortion in regulatory functions of Tregs have been demonstrated in CHB patients [9, 10]. Lamivudine treatment in CHB has been reported to restore both CD4+ and CTL hyporesponsiveness following the decline of serum levels of HBV-derived Ag by many groups including us [11]. However, the other group reported

that this restoration of T-cell response might be transient [12]. We could not conclude whether the reduction of hepatitis B viral antigens could contribute to the better response of immune therapy. We previously reported that HBcAg-specific IL10 secreting Tregs could have important roles in the pathogenesis of CHB [13]. More recently, we found that one of the stress-related proteins produced by HBV-replicating hepatocytes could enhance the suppressive activity of Tregs via TLR2 [14]. In addition, HBV antigens and proteins related to HBV replication were reported to contribute to the TLR-related immune reactions [15].

TLRs are the most common groups among the pattern recognition receptors (PRRs), which differ from antigen-specific receptors in a number of ways. The TLRs were initially identified based on homology with the toll receptor from Drosophila. They are expressed on many kinds of cells including hepatocytes and effector cells of the innate immune and adaptive immune systems. Thus, TLRs might contribute not only to the innate immune systems but also to the adaptive immune systems. TLRs can be classified into two groups based on subcellular localization. The first group includes TLR1, 2, 4, 5, 6, 11, and 12, which are present

TABLE 1: TLR signaling affecting the immunopathogenesis of HBV infection.

TLRs	Localization	Ligand/Pathogen-associated molecular pattern	Contribution to the pathogenesis of HBV infection
TLR1	Plasma membrane	Triacyl lipopeptides, soluble factors	Ligand of TLR1 might suppress HBV replication
TLR2	Plasma membrane	Lipoprotein/lipopeptide	TLR2 expressions on monocytes and hepatocytes were reduced in HBeAg+ CHB sHSP60 produced from HBV-replicating hepatocyte enhanced Tregs activity via TLR2 HBeAg suppresses activation of the TLR2 signaling
TLR3	Endosomes	Double-strand RNA	HBV polymerase inhibits RIG-I- and TLR3-mediated beta interferon induction Ligand of TLR3 might suppress HBV replication Moderate adjuvant function
TLR4	Plasma membrane	LPS, fusion protein	Polymorphisms of TLR4 gene may be related to protection from HBV recurrence after liver transplantation Ligand of TLR4 might suppress HBV replication via CTL activation
TLR5	Plasma membrane	Flagellin	Moderate adjuvant function
TLR6	Plasma membrane	Diacyl lipopeptides	
TLR7	Endosomes	single-strand RNA	Moderate adjuvant function
TLR8	Endosomes	Imidazoquinolne, single-strand RNA	
TLR9	Endosomes	CpG DNA	Strongly enhances CTL activation; HBV vaccine with TLR9 ligand might be effective
TLR11	Plasma membrane	Profilins-like protein	
TLR12	Plasma membrane	not clear	
TLR13		Vesicular stomatitis virus	

at plasma membrane (Table 1). The second group includes TLR3, 7, 8, and 9, which localize to intracellular compartments such as endosomes [16]. Once the TLRs recognize a pathogen-associated molecular pattern, the effector cell is triggered to perform its function immediately, rather than after proliferation in the lymph nodes. Stimulation is started through the involvement of adaptor protein MyD88, protein kinase, and the transcription factor, nuclear factor kappa B (NF-κB), which can induce a variety of cytokines including TNF-α, IL-1, and IL-6. Thereafter, stimulation of TLRs could contribute to the coordination of inflammatory immune responses. So far, TLR2, 3, 4, 7, 8, and 9 might have roles in the pathogenesis of various kinds of viral infections including hepatitis B and C (Table 1).

2. TLRs and HBV Replicating Hepatocyte

The initial step of the innate immune response is induced by type 1 IFN secretion from infected hepatocytes via stimulation of TLRs in hepatitis C virus infection [17, 18]. However, the relationship between HBV and TLRs in hepatocyte has been relatively unclear in comparison to that between HCV and TLRs. It has been reported that the expression of TLR2 is regulated by the precore protein in CHB, and that there was

significantly reduced cytokine production (TNF-alfa) and phospho-p38 kinase expression in the presence of HBeAg [19]. More recently, the same group reported that HBeAg colocalized with the Toll/IL-1-receptor-(TIR-) containing proteins TRAM, MAL, and TLR at the subcellular level. Thus, HBeAg was able to disrupt TIR:TIR interaction critical for TLR-mediated signaling [20]. Another group reported that HBV polymerase might inhibit IFN-beta expression at the TBK/IKKe level. IFN-beta induction is one of the first-phase characteristics of the activation of the type I IFN system [21]. In addition to these reports, other groups have described that HBV could suppress TLRs signaling in hepatocytes as well as nonparenchymal liver cells [22]. These reports were very important, since direct disruption of TLRs signaling was demonstrated in CHB patients as seen in CHC patients (Figure 1). For example, HCV NS3/4A could cleave TRIF and IPS-1 and lead to a deficiency of IFN-beta induction in HCV-infected hepatocytes [18]. Not only TLRs but also retinoic acid-inducible gene I (RIG-I) might be involved in the pathogenesis of HBV infection. Kumar et al. reported that HBx protein (the 17 kDa HBV regulatory protein that plays an essential role in virus replication) could bind to adaptor protein IPS-1 and inhibit the dsDNA-induced IFN-beta [23].

Mechanisms of TLRs signaling suppression in hepatocyte

(1) The expressions of TLR2 were regulated by the precore protein
(2) HBeAg disrupts TIR:TIR interaction
(3) HBV polymerase inhibits IFN-beta at the TBK/IKKe level
(4) HBx protein binds IPS-1 and inhibits the dsDNA - induced IFN-beta

Mechanisms of TLRs signaling suppression in monocyte

(1) TLR2 expression was reduced in HBeAg+ CHB
(2) HBV-core antigen induces cytokine induction via TLR2
(3) TLR3/IFN-beta signaling in mDCs was decreased
(4) TLR4 expression was also affected by hepatitis B persistent infection
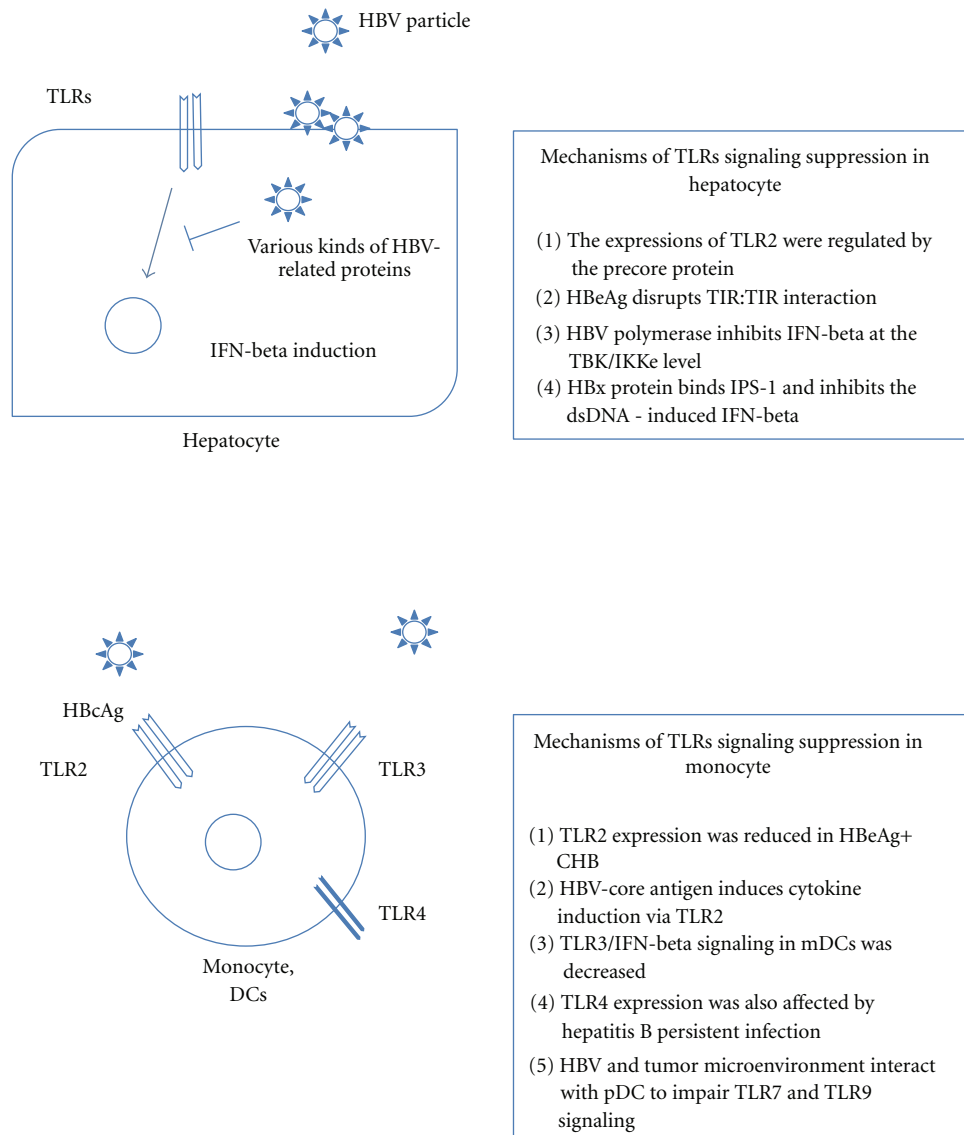(5) HBV and tumor microenvironment interact with pDC to impair TLR7 and TLR9 signaling

FIGURE 1: TLRs contribution to Hepatocellular- and monocyte-related immune systems.

3. TLRs Expression on Monocytes and DCs in HBV Infection

Monocytes including Kupffer cells play an important role in mediating the innate and adaptive immune responses. Various kinds of TLRs are highly expressed on monocytes in comparison to NK cells and CD3+ lymphocytes. It was reported that TLR2 expressions on peripheral monocytes were reduced in patients with HBeAg+ CHB [24]. In addition to the TLR2 expression, another group clearly demonstrated that the cytokine induction by the HBV capsid in macrophages is facilitated by the interaction of its arginine-rich domain with membrane heparan sulfate and involves signaling through TLR2. They found the mechanisms of cytokine induction by HBV core Ag (HBcAg), which serves as the structural subunit of the highly immunogenic capsid

shell. HBcAg harbors a unique arginine-rich C terminus that has been implicated in the immune responses induced by the capsid [15]. Although it has also been reported that no quantitative, phenotypic of functional impairment of mDCs or pDCs was found in CHB [25], TLR3/IFN-beta signaling was decreased significantly in mDCs from patients with chronic hepatitis B or acute-on-chronic hepatitis B liver failure, compared with normal controls [26] (Figure 1). Moreover, the existence of a relationship between TLR2/4 expressions on monotyes and Tregs in CHB patients was reported recently [27] (Figure 2). In addition to TLR2/4, impairment of TLR7 and TLR9 signaling might be associated with chronic viral infection including HIV-1, HBV, and HCV. One group reported that viruses and components of the tumor microenvironment interact with regulatory receptors on plasmacytoid dendritic cells to impair TLR7 and TLR9 signaling.
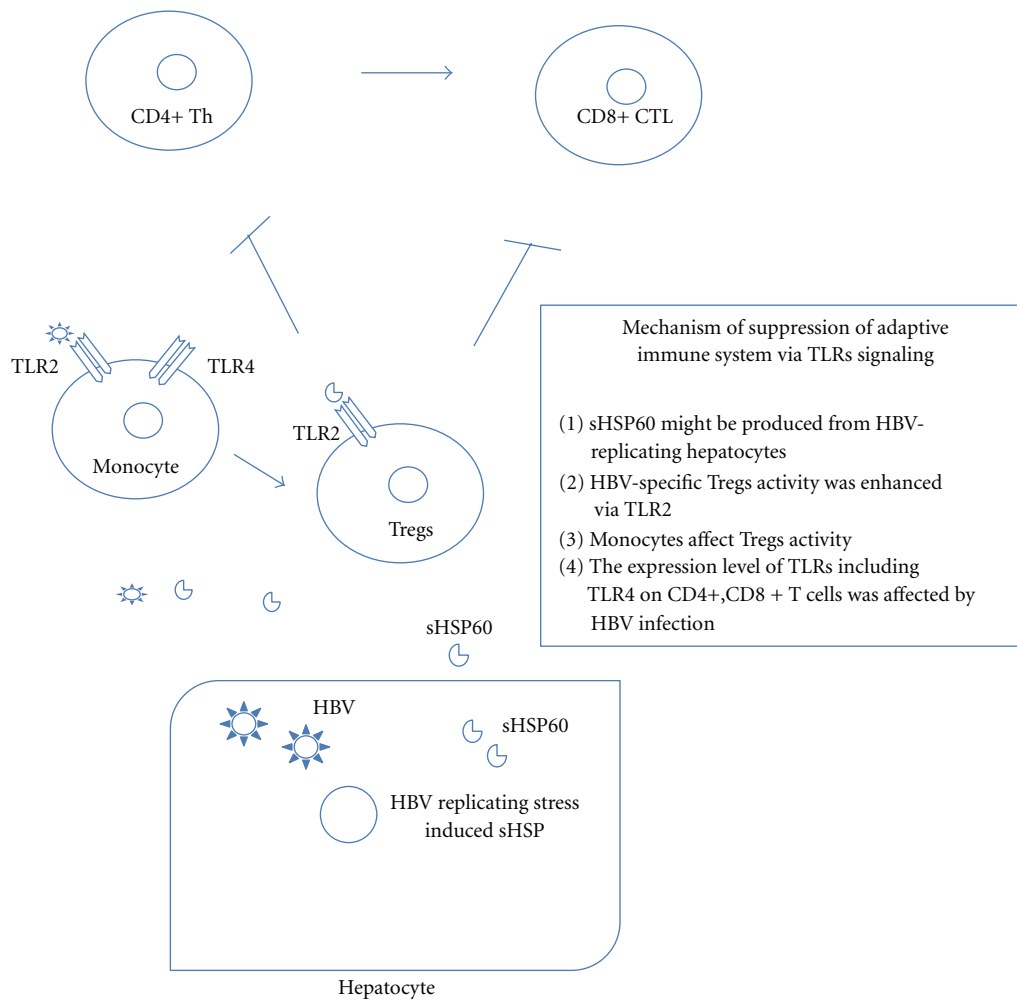
FIGURE 2: TLRs contribution to PBMC, CD3+/CD4+/CD8+ T cells, and Tregs-related immune systems.

4. TLRs Expression on PBMC, CD3+/CD4+/CD8+ T Cells, and Tregs in HBV Infection

For the resolution of viral infection, the efficient recognition of intracellular viruses is essential. It has been reported that CTLs and Th1 play a central role in the control of viral infection after the rapid response by NK and NK-T cells [4]. CTL recognizes viral antigens synthesized within infected cells in the form of oligopeptides that are presented on HLA class I molecules. Many groups including us have reported on the hyporesponsiveness of HBV-specific CTLs in the peripheral blood of patients with CHB [11, 28–30]. There may be several mechanisms in this CTLs hyporesponsiveness including anergy, exhaustion induced by the large amount of HBV antigens, PD-1: PD-L1 interaction, and excessive function of Tregs during CHB [3, 11, 13, 14, 31–36]. Among these immune cells that could contribute to the cellular immune responses, the involvement of Tregs was investigated intensely [13, 14, 27, 34–41]. However, although the expression levels of TLRs were relatively low in comparison to monocytes and DCs, the biological significance of TLRs

expression on a variety of T cells was substantial [27, 34, 36–44]. It has been shown that TLR2 and 4 were important for the regulation of Tregs [42–44]. More recently, we have shown that the soluble form of heat shock protein 60 induced by HBV-replicating hepatocytes could enhance the HBcAg-specific Il10 secreting activity of Tregs via TLR2 (Figure 2). The suppression of HBV replication with entecavir therapy could reduce the excessive function of Tregs in CHB [14]. Based on other findings and those of our studies, not only the HBV antigens but also unknown proteins produced from HBV-replicating hepatocytes and other cells could affect the immune function via TLRs.

5. Immunological Therapy with TLR Agonist

Interferon and nucleos(t)ide analogs, such as lamivudine, adefovir, entecavir, and tenofovir, are the currently available treatments. Interferon-alfa is effective in approximately 30% of cases and even less so in younger cases [45]. The efficacy of nucleos(t)ide analogs is limited by viral reactivation because of the emergence of escaped mutants in cases of prolonged treatment [46, 47]. Immunotherapy is one of the

important options to control HBV replication. Various kinds of immune therapies including protein vaccines, DNA vaccine, T-cell epitope vaccines, and immunomodulating agents (cytokines, IFN, and TLR agonists) have been reported [48–51].

So far, a promising immune therapy that could eradicate or control HBV has not been developed. Cellular immune responses are suppressed by various kinds of mechanisms. Therefore, TLR agonists were strong candidates to activate and modulate the impaired immune responses with HBV epitopes, proteins, and DNA vaccines [52–57]. Whether TLR2, 3, 4, 5, 7, and 9 signaling could enhance the CTL response upon vaccination has been examined. The results indicated that TLR2 and 4 failed to increase the CTL response, whereas stimulation by TLR3, 5, and 7 exhibited a moderate adjuvant function. In contrast, stimulation of TLR9 dramatically increased the CTL responses [54]. On the other hand, TLR4 in addition to TLR 3, 5, 7, and 9 was reported to have the ability to suppress HBV replication in a mouse model [52, 53]. Thereafter, a vaccine based on HBV antigens and immunostimulatory DNA sequence, an adjuvant that acts as a TLR9 agonist, was developed [55]. However, to determine the potential of using TLR agonists to improve the HBV-specific immune response, future studies will be needed.

6. Concluding Remarks

Recently various studies about the immunopathogenesis of HBV infection, including adaptive and innate immune responses, have been reported, and the contribution of TLRs signaling to the pathogenesis of HBV infection is receiving increased interest. In this paper, we described the suppression of TLRs signaling in heptocytes and immune cells during HBV infection, however, reports concerning the suppression of TLRs signaling in HBV infection have been few in comparison to those on HCV infection. The involvement of TLRs signaling suppression during HBV persistent infection should be considered more precisely. For example, the relationship between HBV genotypes and the level of TLRs suppression is not clear yet. As for immune therapy, the TLRs agonists, especially TLR9 agonist, with various kinds of HBV vaccines could be strong candidates to activate the HBV-specific immune responses that are suppressed during HBV persistent infection. The combination of TLR agonists and appropriately timed immune therapy should be considered in future studies.

Acknowledgment

References

[1] P. Tiollais, C. Pourcel, and A. Dejean, "The hepatitis B virus," *Nature*, vol. 317, no. 6037, pp. 489–495, 1985.

[2] C. L. Lai, V. Ratziu, M. F. Yuen, and T. Poynard, "Viral hepatitis B," *Lancet*, vol. 362, no. 9401, pp. 2089–2094, 2003.

[3] F. V. Chisari and C. Ferrari, "Hepatitis B virus immunopathogenesis," *Annual Review of Immunology*, vol. 13, pp. 29–60, 1995.

[4] D. Kägi, B. Ledermann, K. Bürki, R. M. Zinkernagel, and H. Hengartner, "Molecular mechanisms of lymphocyte-mediated cytotoxicity and their role in immunological protection and pathogenesis in vivo," *Annual Review of Immunology*, vol. 14, pp. 207–232, 1996.

[5] S. Reignat, G. J. M. Webster, D. Brown et al., "Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection," *Journal of Experimental Medicine*, vol. 195, no. 9, pp. 1089–1101, 2002.

[6] J. L. Baron, L. Gardiner, S. Nishimura, K. Shinkai, R. Locksley, and D. Ganem, "Activation of a nonclassical NKT cell subset in a transgenic mouse model of hepatitis B virus infection," *Immunity*, vol. 16, no. 4, pp. 583–594, 2002.

[7] P. Bonorino, M. Ramzan, X. Camous et al., "Fine characterization of intrahepatic NK cells expressing natural killer receptors in chronic hepatitis B and C," *Journal of Hepatology*, vol. 51, no. 3, pp. 458–467, 2009.

[8] K. Kakimi, L. G. Guidotti, Y. Koezuka, and F. V. Chisari, "Natural killer T cell activation inhibits hepatitis B virus replication in vivo," *Journal of Experimental Medicine*, vol. 192, no. 7, pp. 921–930, 2000.

[9] Y. Ju, N. Hou, J. Meng et al., "T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B," *Journal of Hepatology*, vol. 52, no. 3, pp. 322–329, 2010.

[10] P. T. F. Kennedy, A. J. Gehring, A. Nowbath et al., "The expression and function of NKG2D molecule on intrahepatic CD8$^+$ T cells in chronic viral hepatitis," *Journal of Viral Hepatitis*, vol. 15, no. 12, pp. 901–909, 2008.

[11] Y. Kondo, S. Asabe, K. Kobayashi et al., "Recovery of functional cytotoxic T lymphocytes during lamivudine therapy by acquiring multi-specificity," *Journal of Medical Virology*, vol. 74, no. 3, pp. 425–433, 2004.

[12] C. Boni, A. Penna, A. Bertoletti et al., "Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B," *Journal of Hepatology*, vol. 39, no. 4, pp. 595–605, 2003.

[13] Y. Kondo, K. Kobayashi, Y. Ueno et al., "Mechanism of T cell hyporesponsiveness to HBcAg is associated with regulatory T cells in chronic hepatitis B," *World Journal of Gastroenterology*, vol. 12, no. 27, pp. 4310–4317, 2006.

[14] Y. Kondo, Y. Ueno, K. Kobayashi et al., "Hepatitis B virus replication could enhance regulatory T cell activity by producing soluble heat shock protein 60 from hepatocytes," *Journal of Infectious Diseases*, vol. 202, no. 2, pp. 202–213, 2010.

[15] A. Cooper, G. Tal, O. Lider, and Y. Shaul, "Cytokine induction by the hepatitis B virus capsid in macrophages is facilitated by membrane heparan sulfate and involves TLR2," *Journal of Immunology*, vol. 175, no. 5, pp. 3165–3176, 2005.

[16] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[17] K. MacHida, "TLRs, alcohol, HCV, and tumorigenesis," *Gastroenterology Research and Practice*, vol. 2010, Article ID 518674, 8 pages, 2010.

[18] T. Saito, D. M. Owen, F. Jiang, J. Marcotrigiano, and M. Gale, "Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA," *Nature*, vol. 454, no. 7203, pp. 523–527, 2008.

[19] K. Visvanathan, N. A. Skinner, A. J. V. Thompson et al., "Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein," *Hepatology*, vol. 45, no. 1, pp. 102–110, 2007.

[20] T. Lang, C. Lo, N. Skinner, S. Locarnini, K. Visvanathan, and A. Mansell, "The Hepatitis B e antigen (HBeAg) targets and suppresses activation of the Toll-like receptor signaling pathway," *Journal of Hepatology*, vol. 55, no. 4, pp. 762–769, 2011.

[21] S. Yu, J. Chen, M. Wu, H. Chen, N. Kato, and Z. Yuan, "Hepatitis B virus polymerase inhibits RIG-I- and toll-like receptor 3-mediated beta interferon induction in human hepatocytes through interference with interferon regulatory factor 3 activation and dampening of the interaction between TBK1/IKKε and DDX3," *Journal of General Virology*, vol. 91, no. 8, pp. 2080–2090, 2010.

[22] J. Wu, Z. Meng, M. Jiang et al., "Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells," *Hepatology*, vol. 49, no. 4, pp. 1132–1140, 2009.

[23] M. Kumar, S. Y. Jung, A. J. Hodgson, C. R. Madden, J. Qin, and B. L. Slagle, "Hepatitis B virus regulatory HBx protein binds to adaptor protein IPS-1 and inhibits the activation of beta interferon," *Journal of Virology*, vol. 85, no. 2, pp. 987–995, 2011.

[24] S. M. Riordan, N. Skinner, J. Kurtovic, S. Locarnini, and K. Visvanathan, "Reduced expression of Toll-like receptor 2 on peripheral monocytes in patients with chronic hepatitis B," *Clinical and Vaccine Immunology*, vol. 13, no. 8, pp. 972–974, 2006.

[25] S. Tavakoli, I. Mederacke, S. Herzog-Hauff et al., "Peripheral blood dendritic cells are phenotypically and functionally intact in chronic hepatitis B virus (HBV) infection," *Clinical and Experimental Immunology*, vol. 151, no. 1, pp. 61–70, 2008.

[26] N. Li, Q. Li, Z. Qian, Y. Zhang, M. Chen, and G. Shi, "Impaired TLR3/IFN-β signaling in monocyte-derived dendritic cells from patients with acute-on-chronic hepatitis B liver failure: relevance to the severity of liver damage," *Biochemical and Biophysical Research Communications*, vol. 390, no. 3, pp. 630–635, 2009.

[27] Y. Zhang, J. Q. Lian, C. X. Huang et al., "Overexpression of Toll-like receptor 2/4 on monocytes modulates the activities of CD4$^+$CD25$^+$ regulatory T cells in chronic hepatitis B virus infection," *Virology*, vol. 397, no. 1, pp. 34–42, 2010.

[28] P. A. Wentworth, E. Celis, C. Crimi et al., "In vitro induction of primary, antigen-specific CTL from human peripheral blood mononuclear cells stimulated with synthetic peptides," *Molecular Immunology*, vol. 32, no. 9, pp. 603–612, 1995.

[29] B. Rehermann, C. Pasquinelli, S. M. Mosier, and F. V. Chisari, "Hepatitis B virus (HBV) sequence variation in cytotoxic T lymphocyte epitopes is not common in patients with chronic HBV infection," *Journal of Clinical Investigation*, vol. 96, no. 3, pp. 1527–1534, 1995.

[30] Y. Kondo, K. Kobayashi, S. Asabe et al., "Vigorous response of cytotoxic T lymphocytes associated with systemic activation of CD8$^+$ T lymphocytes in fulminant hepatitis B," *Liver International*, vol. 24, no. 6, pp. 561–567, 2004.

[31] L. Frelin, T. Wahlström, A. E. Tucker et al., "A mechanism to explain the selection of the hepatitis e antigen-negative mutant during chronic hepatitis B virus infection," *Journal of Virology*, vol. 83, no. 3, pp. 1379–1392, 2009.

[32] H. Maier, M. Isogawa, G. J. Freeman, and F. V. Chisari, "PD-1:PD-L1 interactions contribute to the functional suppression

of virus-specific CD8$^+$ T lymphocytes in the liver," *Journal of Immunology*, vol. 178, no. 5, pp. 2714–2720, 2007.

[33] F. V. Chisari and C. Ferrari, "Hepatitis B virus immunopathology," *Springer Seminars in Immunopathology*, vol. 17, no. 2-3, pp. 261–281, 1995.

[34] J. P. Wang, Y. Zhang, X. Wei et al., "Circulating toll-like receptor (TLR) 2, TLR4, and regulatory T cells in patients with chronic hepatitis C," *APMIS*, vol. 118, no. 4, pp. 261–270, 2010.

[35] J. Q. Lian, X. Q. Wang, Y. Zhang, C. X. Huang, and X. F. Bai, "Correlation of circulating TLR2/4 expression with CD3$^+$/4$^+$/8$^+$ T cells and treg cells in HBV-related liver cirrhosis," *Viral Immunology*, vol. 22, no. 5, pp. 301–308, 2009.

[36] J. N. Stoop, R. G. Van Der Molen, C. C. Baan et al., "Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection," *Hepatology*, vol. 41, no. 4, pp. 771–778, 2005.

[37] X. P. Nan, Y. Zhang, H. T. Yu et al., "Circulating CD4$^+$CD25 high regulatory T cells and expression of PD-1 and BTLA on CD4$^+$ T cells in patients with chronic hepatitis B virus infection," *Viral Immunology*, vol. 23, no. 1, pp. 63–70, 2010.

[38] X. Li, Y. Chen, Z. Ma, B. Ye, W. Wu, and L. Li, "Effect of regulatory T cells and adherent cells on the expansion of HBcAg-specific CD8$^+$ T cells in patients with chronic hepatitis B virus infection," *Cellular Immunology*, vol. 264, no. 1, pp. 42–46, 2010.

[39] J. Li, W. Wu, G. Peng et al., "HBcAg induces interleukin-10 production, inhibiting HBcAg-specific Th17 responses in chronic hepatitis B patients," *Immunology and Cell Biology*, vol. 88, no. 8, pp. 834–841, 2010.

[40] G. Peng, S. Li, W. Wu, Z. Sun, Y. Chen, and Z. Chen, "Circulating CD4$^+$ CD25$^+$ regulatory T cells correlate with chronic hepatitis B infection," *Immunology*, vol. 123, no. 1, pp. 57–65, 2008.

[41] G. Yang, A. Liu, Q. Xie et al., "Association of CD4$^+$CD25$^+$ Foxp3$^+$ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B," *International Immunology*, vol. 19, no. 2, pp. 133–140, 2007.

[42] A. Zanin-Zhorov, L. Cahalon, G. Tal, R. Margalit, O. Lider, and I. R. Cohen, "Heat shock protein 60 enhances CD4$^+$CD25$^+$ regulatory T cell function via innate TLR2 signaling," *Journal of Clinical Investigation*, vol. 116, no. 7, pp. 2022–2032, 2006.

[43] B. Schaub, M. Campo, H. He et al., "Neonatal immune responses to TLR2 stimulation: influence of maternal atopy on Foxp3 and IL-10 expression," *Respiratory Research*, vol. 7, article 40, 2006.

[44] D. Kabelitz, D. Wesch, and H. H. Oberg, "Regulation of regulatory T cells: role of dendritic cells and toll-like receptors," *Critical Reviews in Immunology*, vol. 26, no. 4, pp. 291–305, 2006.

[45] R. Guan, "Interferon monotherapy in chronic hepatitis B," *Journal of Gastroenterology and Hepatology*, vol. 15, pp. E34–E40, 2000.

[46] P. Angus, R. Vaughan, S. Xiong et al., "Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase," *Gastroenterology*, vol. 125, no. 2, pp. 292–297, 2003.

[47] Y.-F. Liaw, N. W.Y. Leung, T.-T. Chang et al., "Effects of extended lamivudine therapy in asian patients with chronic hepatitis B," *Gastroenterology*, vol. 119, no. 1, pp. 172–180, 2000.

[48] R. S. Ross, S. Viazov, and M. Roggendorf, "Clinical and immunological efficacy of intradermal vaccine plus lamivudine with or without interleukin-2 in patients with chronic

hepatitis B," *Journal of Medical Virology*, vol. 66, no. 4, pp. 452–460, 2002.

[49] Y. Horsmans, T. Berg, J. P. Desager et al., "Isatoribine, an agonist of TLR7, reduces plasma virus concentration in chronic hepatitis C infection," *Hepatology*, vol. 42, no. 3, pp. 724–731, 2005.

[50] S. H. Yang, C. G. Lee, S. H. Park et al., "Correlation of antiviral T-cell responses with suppression of viral rebound in chronic hepatitis B carriers: a proof-of-concept study," *Gene Therapy*, vol. 13, no. 14, pp. 1110–1117, 2006.

[51] Y. Kondo, Y. Ueno, and T. Shimosegawa, "Immunopathogenesis of hepatitis B persistent infection: implications for immunotherapeutic strategies," *Clinical Journal of Gastroenterology*, vol. 2, no. 2, pp. 71–79, 2009.

[52] W. W. Chang, I. J. Su, M. D. Lai, W. T. Chang, W. Huang, and H. Y. Lei, "Toll-like receptor 4 plays an anti-HBV role in a murine model of acute hepatitis B virus expression," *World Journal of Gastroenterology*, vol. 11, no. 42, pp. 6631–6637, 2005.

[53] M. Isogawa, M. D. Robek, Y. Furuichi, and F. V. Chisari, "Toll-like receptor signaling inhibits hepatitis B virus replication in vivo," *Journal of Virology*, vol. 79, no. 11, pp. 7269–7272, 2005.

[54] K. Schwarz, T. Storni, V. Manolova et al., "Role of toll-like receptors in costimulating cytotoxic T cell responses," *European Journal of Immunology*, vol. 33, no. 6, pp. 1465–1470, 2003.

[55] J. J. Y. Sung and H. L. Y. Chan, "HBV-ISS (Dynavax)," *Current Opinion in Molecular Therapeutics*, vol. 8, no. 2, pp. 150–155, 2006.

[56] J. Wu, M. Lu, Z. Meng et al., "Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice," *Hepatology*, vol. 46, no. 6, pp. 1769–1778, 2007.

[57] D. Yerly, L. Di Giammarino, F. Bihl, and A. Cerny, "Targets of emerging therapies for viral hepatitis B and C," *Expert Opinion on Therapeutic Targets*, vol. 10, no. 6, pp. 833–850, 2006.