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LETTER

Is the function of the HBeAg really unknown?

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ABSTRACT

The immune response to the hepatitis B virus (HBV) vaccine in newborns of hepatitis B e antigen (HBeAg)-positive or HBeAg-negative mothers is the subject of Huang et al. The authors report no correlation between the HBeAg status of the mothers/cord blood and the newborns immune response to the vaccine, but, unfortunately, draw unfounded conclusions regarding the tolerogenic potential of *in utero* exposure to HBeAg. In this reply, I address the possible influence of *in utero* exposure to the HBeAg, and briefly review other characteristics of the HBeAg, that may promote HBV chronicity. I argue that the function of HBeAg should no longer be considered "unknown" and that immunotolerance/immunomodulation represent the dominant functions of the HBeAg in viral–host interactions.

The study by Huang et al.[1] examining antibody responses to hepatitis B vaccination in newborns from hepatitis B surface antigen (HBsAg)-positive mothers demonstrates with some rigor that "the anti-HBsAg response to hepatitis B vaccination between newborn infants positive for hepatitis B e antigen (HBeAg) in umbilical blood and those negative for HBeAg in umbilical blood" was equivalent in response rates and anti-HBs antibody levels. This result is consistent with a similar study, which reported no association between infant anti-HBs antibody responses and HBeAg-positive status and hepatitis B virus (HBV) DNA levels in the mother,[2] and the generally high efficiency rates for the hepatitis B vaccine in newborns (i.e., >90% protective levels of anti-HBs antibodies).[2,3]

The authors correctly conclude that "transplacental HBeAg in the fetus did not inhibit the immune response to the hepatitis B vaccine," as would be expected because the HBeAg and the HBsAg are separate and distinct antigens. However, the Huang et al.'s study differs dramatically from all other similar studies by stating: "that HBeAg appears not to be inducing immunotolerance to HBV;" "that our present study provides evidence against the concept of HBeAg as an immunotolerogen in the transmission of HBV from mothers to infants;" and "that the transplacentally acquired maternal HBeAg *in utero* may not be associated with the pathogenesis of chronic HBV infection after neonatal exposure to HBV." None of these three conclusions are supported by the Huang et al.'s study nor are they even addressed. For example, the authors did not measure immune tolerance to the HBeAg in HBeAg-positive and HBeAg-negative newborns nor determine the rate of chronic HBV infection in newborns born to HBeAg-positive and HBeAg-negative mothers in the absence of immunoprophylaxis.

I believe the authors began with the incorrect premise "that fetal HBeAg exposure can cause partial tolerance of newborn infant's immune system to HBV," which represents an incorrect interpretation of their Refs. [5–7]. The hypothesis they referenced states that exposure to HBeAg *in utero* and/or shortly after infection in the newborn elicits immune tolerance in T cells specific for HBeAg and in cross-reactive hepatitis B core antigen (HBcAg)-specific T cells, but *not* in T cells specific for other HBV antigens, and specifically *not* in HBsAg-specific T cells.[4-6] An exception may be in the case of relatively rare *in utero* HBV infection, which may elicit immune tolerance to all HBV antigens and has been proposed as a risk factor for vaccine failure.[7]

The authors cite their own study and a number of other studies documenting that the HBeAg does transverse the human placenta and is present in cord blood (their Refs. [18–21]). These and other studies note that the particulate HBsAgs do not efficiently cross the human placenta and are less frequently found in cord blood.[8,9] The efficient anti-HBs antibody responses to the hepatitis B vaccine reported in newborns by Huang et al. and previously by others demonstrate that any HBsAg present *in utero* is insufficient to elicit immune tolerance to the HBsAg or at least to the HBsAg as presented in the vaccine, even when the mothers have high circulating levels of HBV DNA, HBsAg, and HBeAg. In other words, these babies are not born immune tolerant to the HBsAgs, which is notable because they still cannot clear a perinatal infection thus emphasizing the role of HBeAg in promoting chronicity. Therefore, it is difficult to understand why Huang et al. believed that examining anti-HBs antibody responses to the HBsAg vaccine in newborns would reveal anything about the potential of *in utero* exposure to HBeAg to elicit immune tolerance in HBe/HBcAg-specific T cells or any subsequent effects on "the pathogenesis of chronic HBV infection after neonatal exposure to HBV."

A way to determine if *in utero* exposure to HBeAg elicits immune tolerance would be to immunize newborns from

HBeAg-positive and HBeAg-negative mothers with the HBeAg but *not* with the HBsAg. This study has been performed in animal models but, understandably, not in humans. However, a study examining T cell responses to HBe/HBcAg in cord blood of HBeAg-positive and HBeAg-negative newborns reported no HBe/HBcAg-specific responses in T cells derived from HBeAg-positive cord blood, which is consistent with immune tolerance rather than immune priming by *in utero* exposure to the HBeAg.[9]

The authors also claim that "the role of HBeAg in inducing neonatal immunologic tolerance to HBV remains controversial" and the literature invariably asserts that "the function of the HBeAg is unknown." It is not clear which part of the hypothesis is controversial or if the function of the HBeAg is really unknown.

HBeAg characteristics determined in clinical studies

Many aspects of HBeAg have been studied in humans since its discovery in 1972.[10] Findings include:

(1) In the absence of prophylaxis, perinatal transmission of HBV is frequent when the mothers are HBeAg-positive (70–90% within 3 months) and significantly less frequent when the mothers are HBeAg-negative, which is associated with lower viral loads.

(2) HBV-infected newborns of HBeAg-positive mothers frequently become chronically infected (≥90%), whereas HBV-infected newborns of HBeAg-negative mothers rarely become chronically infected (<10%).[11]

(3) Numerous studies indicate that HBeAg can cross or be transported across the human placenta and disappears from serum within 6 months in most babies who are not infected.[8,9]

(4) Primary infections with HBeAg-negative HBV in adults as well as neonates rarely become chronic and are associated with an increased risk of severe acute hepatitis.[12-14]

(5) Fulminant hepatitis can be associated with an overwhelming B cell response specific for the HBcAg in HBeAg-negative HBV acute infection.[15,16]

(6) The propensity to develop precore and core promoter mutations that affect HBeAg expression is influenced by genotype/subgenotype, which is associated with clinical outcomes.[17] For example, in a Japanese study of adult acute HBV patients seeking medical care, 55% of patients infected with subgenotype HBV/Bj developed fulminant hepatitis, whereas none of the patients infected with subgenotype HBV/Ae developed fulminant hepatitis. The HBV/Bj subgenotype, HBeAg-negative status, and the precore stop codon G1896A mutation were independent risk factors for the development of fulminant hepatitis.[18]

(7) In chronic HBV infection, HBe/HBcAg T cell responses are significantly depressed as compared to acute infection, especially during the immune tolerant phase.[19]

(8) During HBeAg-positive immune activation or clearance phases, the HBe/HBcAgs are primary targets of the T cell response, especially during acute exacerbations.[20] A corallery being these HBe/HBcAg-specific T cell responses must have been silenced during the immune tolerant phase.

(9) In adult chronic hepatitis B (CHB) patients, HBcAg-specific T cell responses (proliferation, INFγ, and IL-10 production) are weaker in HBeAg-positive (3% responders) compared to HBeAg-negative patients (23% responders). Although *in vitro* blockade of PD-1 or CTLA4 increases T cell responses, the effect is weaker in HBeAg-positive than HBeAg-negative patients.[21]

(10) Plasmacytoid dendritic cells pulsed with HBe/HBcAg-peptides stimulate T cells derived from HBeAg-negative but not HBeAg-positive chronic HBV patients.[22]

(11) Emergence of a predominant precore negative genotype late in CHB infection can be associated with increased liver injury and the selection of additional mutations in the HBe gene.[23]

(12) A number of immunomodulatory functions have been described for the HBeAg including downregulation of TLR2 expression; suppression of IL-1β-induced TRAF6-dependent K63-linked ubiquitination of NEMO, causing downregulation of NF-κB activation and promotion of viral replication; downregulation of IL-18-mediated signaling of IFNγ expression; interaction with RIPK2 to regulate IL-6 expression; interaction with IL-1 receptor accessory protein to trigger IL-1 responses.[24-28]

HBeAg characteristics determined in animal studies

(1) The HBeAg is not required for HBV infection, replication, or assembly.[29-31]

(2) The HBeAg is conserved in all orthohepadnaviruses.[32]

(3) Infection of neonatal woodchucks with wildtype woodchuck hepatitis virus (WT WHV) elicits chronic infection, whereas infection with a WHeAg-negative virus elicits acute infection.[31]

(4) The HBeAg expressed as a transgene *in utero* or 3 d after birth elicits T cell tolerance to HBeAg and HBcAg.[5,6,33]

(5) The secreted HBeAg is more tolerogenic than the cytosolic HBcAg[6,34] (see Figure 1(a)).

(6) Mechanisms of tolerance can include clonal deletion, clonal anergy, and clonal ignorance depending on TCR avidity.[5,6,35]

(7) T cell tolerance to HBeAg can be reversed in the absence of the HBeAg.[6]

(8) Maternal-derived HBeAg alters macrophage function in non-transgenic offspring, which enables viral persistence and requires both maternal-derived HBeAg and the presence of HBeAg in the periphery.[35]

(9) Although secreted HBeAg is tolerogenic, cytosolic HBcAg is immunogenic and a target for HBe/HBcAg-
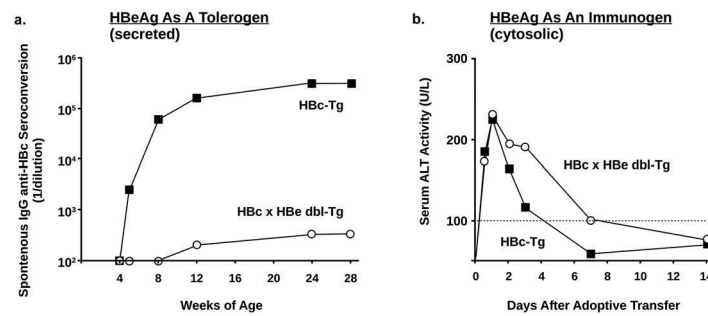
Figure 1. The dual roles of HBeAg. (a). Serum HBeAg inhibits spontaneous IgG anti-HBc Ab production *in vivo* in a HBc-Tg lineage that is not tolerant to the HBcAg. IgG anti-HBc Ab was measured in the serum of six HBc-Tg and six HBc × HBe double-Tg (dbl-Tg) mice on a genetic background of a HBe/HBcAg-specific, CD4+ TCR-Tg strain. Adapted from Ref. [6]. (b). Liver injury persists longer when the HBcAg and HBeAg are co-expressed in the liver. To mimic the period when HBeAg-specific tolerance is subsiding, activated polyclonal HBc/HBeAg-specific CTL cells were transferred into HBc-Tg or HBc × HBe dbl-Tg mice recipients and serum ALT levels were measured as a marker of liver injury. Adapted from Ref. [33].

specific CTL once HBeAg-specific tolerance subsides. Hepatocytes expressing both the HBeAg and the HBcAg are superior targets for HBe/HBcAg-specific CTL compared to hepatocytes expressing HBcAg alone *in vivo* (see Figure 1(b)). This may represent qualitative differences between the HBeAg and the HBcAg or quantitative differences between hepatocytes expressing both antigens as opposed to HBcAg alone. Currently, we favor the quantitative interpretation. Therefore, the HBeAg-negative mutation has a selective advantage and may represent a CTL escape mutant.[33] Most CTL escape mutants are point mutations that eliminate one CTL specificity at a time; however, because the HBeAg is dispensable for the virus the expression of the entire protein can be eliminated or reduced by precore and core promoter mutations.

Summary

The authors noted two points of controversy: the first being the question of whether the HBeAg can cross the murine placenta (their Refs. [5] and [10]). This point is now moot because another study has shown the effects of maternal HBeAg in non-transgenic pups and HBeAg-specific tolerance occurs in a transgenic lineage in which HBeAg is expressed 3 d after birth.[33,35] Most importantly, numerous studies have demonstrated that HBeAg can cross the human placenta.[8,9] The other point of controversy arises from a misunderstanding of T cell tolerance (their Refs. [11] and [17]). T cell tolerance like T cell activation is clonal; therefore, the deletion or tolerization of a single high avidity, highly functional HBV-specific T cell clone could theoretically prevent viral clearance, regardless of any number of low avidity, poorly functioning T cell clones that may have escaped tolerance. In fact, we have shown that low avidity HBe/HBcAg-specific CD4+ T cells can survive and co-exist with the HBe/HBcAgs in transgenic mice.[36] It is likely that this phenotype of HBe/HBcAg-specific T cell is the target of mechanisms of tolerance such as clonal exhaustion and expression of check point inhibitors that mediate non-deletional tolerance in the periphery and

eventually mediate immune clearance as tolerance mechanisms subside. Is the mechanism of immune tolerance any less functional if the tolerization of one or numerous T cell clones contributes to viral persistence after neonatal infection?

Although there is evidence that HBeAg functions as an immunomodulator even in adult acute infections and can moderate the degree of liver injury, chronicity does not usually occur outside the context of an HBeAg-positive neonatal infection, an immature immune system and an immature liver microenvironment.[37] Reciprocally, in the absence of the secreted HBeAg, an immature immune system and immature liver microenvironment are not sufficient to explain chronicity because viral clearance including acute fulminant hepatitis can occur in neonates infected with the HBeAg-negative mutant virus. Further, any explanation for the establishment of chronicity must also explain the evolution from the immune tolerant phase through immune activation to eventual immune-mediated viral clearance as well as the selection of HBeAg-negative mutants. The dual functions of secreted HBeAg as a tolerogen during the immune tolerant phase and cytosolic HBeAg as an immunogenic target of HBe/HBc-specific CTL once the tolerance has waned as a function of age provide an explanation for the progression through the various stages of CHB infection and possibly the selection of HBeAg-negative mutants.[38] A tolerogen is an immunogen presented in a non-immunogenic context. After all, self-antigens are tolerogens until they become immunogens as targets of an autoimmune process, which is an appropriate perspective for the neo-self HBeAg, if acquired *in utero*.

Although this list of HBeAg characteristics is not exhaustive and unknown characteristics likely exist, we believe the current knowledge base is sufficient to establish immunotolerance/immunomodulation as a primary and very important function of the secreted HBeAg, without suggesting that the "real" function is unknown.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

1. Huang H, Ning M, Liu J, Chen J, Feng J, Dai Y, Hu Y, Zhou YH. Comparison of antibody response to hepatitis B vaccination in infants with positive or negative maternal hepatitis B e antigen (HBeAg) in cord blood: implication for the role of HBeAg as an immunotolerogen, Human Vaccines & Immunotherapeutics. 2019, in press. doi:10.1080/21645515.2019.1575712.

2. Zou H, Chen Y, Duan Z, Zhang H. Protective effect of hepatitis B vaccine combined with two-dose hepatitis B immunoglobulin on infants born to HBsAg-positive mothers. PLoS One. 2011;6: e26748. doi:10.1371/journal.pone.0026748.

3. Stevens CE, Toy P, Kamili S, Taylor PE, Tong MJ, Xia G-L, Vyas GN. Eradicating hepatitis B virus: the critical role of preventing perinatal transmission. Biol J Int Assoc Biol Stand. 2017;50:3–19.

4. Milich DR, Jones JE, Hughes JL, Price J, Raney AK, McLachlan A. Is a function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? Proc Natl Acad Sci U S A. 1990;87:6599–603. doi:10.1073/pnas.87.17.6599.

5. Milich DR, Chen MK, Hughes JL, Jones JE. The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. J Immunol. 1998;160:2013–21.

6. Chen MT, Billaud J-N, Sällberg M, Guidotti LG, Chisari FV, Jones J, Hughes J, Milich DR. A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. Proc Natl Acad Sci U S A. 2004;101:14913–18. doi:10.1073/pnas.0406282101.

7. Shao Z-J, Zhang L, Xu J-Q, Xu D-Z, Men K, Zhang J-X, Cui H-C, Yan Y-P. Mother-to-infant transmission of hepatitis B virus: a Chinese experience. J Med Virol. 2011;83:791–95. doi:10.1002/jmv.v83.5.

8. Wang Z, Zhang J, Yang H, Li X, Wen S, Guo Y, Sun J, Hou J. Quantitative analysis of HBV DNA level and HBeAg titer in hepatitis B surface antigen positive mothers and their babies: HBeAg passage through the placenta and the rate of decay in babies. J Med Virol. 2003;71:360–66. doi:10.1002/(ISSN)1096-9071.

9. Hsu HY, Chang MH, Hsieh KH, Lee CY, Lin HH, Hwang LH, Chen PJ, Chen DS. Cellular immune response to HBcAg in mother-to-infant transmission of hepatitis B virus. Hepatology. 1992;15:770–76. doi:10.1002/hep.1840150505.

10. Magnius LO, Espmark JA. New specificities in Australia antigen positive sera distinct from the Le Bouvier determinants. J Immunol. 1972;109:1017–21.

11. Hadziyannis SJ, Papatheodoridis GV. Hepatitis B e antigen-negative chronic hepatitis B: natural history and treatment. Semin Liver Dis. 2006;26:130–41. doi:10.1055/s-2006-939751.

12. Terazawa S, Kojima M, Yamanaka T, Yotsumoto S, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Hepatitis B virus mutants with precore-region defects in two babies with fulminant hepatitis and their mothers positive for antibody to hepatitis B e antigen. Pediatr Res. 1991;29:5–9. doi:10.1203/00006450-199101000-00002.

13. Chen H-L, Chang C-J, Kong M-S, Huang F-C, Lee H-C, Lin -C-C, Liu -C-C, Lee I-H, Wu T-C, Wu S-F, et al. Pediatric fulminant hepatic failure in endemic areas of hepatitis B infection: 15 years after universal hepatitis B vaccination. Hepatology. 2004;39:58–63. doi:10.1002/hep.20006.

14. Fagan EA, Smith PM, Davison F, Williams R. Fulminant hepatitis B in successive female sexual partners of two anti-HBe-positive males. Lancet. 1986;2:538–40.

15. Farci P, Diaz G, Chen Z, Govindarajan S, Tice A, Agulto L, Pittaluga S, Boon D, Yu C, Engle RE, et al. B cell gene signature with massive intrahepatic production of antibodies to hepatitis B core antigen in hepatitis B virus-associated acute liver failure. Proc Natl Acad Sci U S A. 2010;107:8766–71. doi:10.1073/pnas.0910097107.

16. Chen Z, Diaz G, Pollicino T, Zhao H, Engle RE, Schuck P, Shen C-H, Zamboni F, Long Z, Kabat J, et al. Role of humoral immunity against hepatitis B virus core antigen in the pathogenesis of acute liver failure. Proc Natl Acad Sci U S A. 2018;115: E11369–78. doi:10.1073/pnas.1809028115.

17. Kramvis A, Kostaki E-G, Hatzakis A, Paraskevis D. Immunomodulatory function of HBeAg related to short-sighted evolution, transmissibility, and clinical manifestation of hepatitis B virus. Front Microbiol. 2018;9:2521. doi:10.3389/fmicb.2018.02521.

18. Ozasa A, Tanaka Y, Orito E, Sugiyama M, Kang J-H, Hige S, Kuramitsu T, Suzuki K, Tanaka E, Okada S, et al. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology. 2006;44:326–34. doi:10.1002/hep.21249.

19. Ferrari C, Penna A, Bertoletti A, Valli A, Antoni AD, Giuberti T, Cavalli A, Petit MA, Fiaccadori F. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. J Immunol. 1990;145:3442–49.

20. Tsai SL, Chen PJ, Lai MY, Yang PM, Sung JL, Huang JH, Hwang LH, Chang TH, Chen DS. Acute exacerbations of chronic type B hepatitis are accompanied by increased T cell responses to hepatitis B core and e antigens. Implications for hepatitis B e antigen seroconversion. J Clin Invest. 1992;89:87–96. doi:10.1172/JCI115590.

21. Park -J-J, Wong DK, Wahed AS, Lee WM, Feld JJ, Terrault N, Khalili M, Sterling RK, Kowdley KV, Bzowej N, et al. Hepatitis B virus–specific and global T-cell dysfunction in chronic hepatitis B. Gastroenterology. 2016;150:684–695 e5.

22. Martinet J, Leroy V, Dufeu-Duchesne T, Larrat S, Richard M-J, Zoulim F, Plumas J, Aspord C. Plasmacytoid dendritic cells induce efficient stimulation of antiviral immunity in the context of chronic hepatitis B virus infection. Hepatology. 2012;56:1706–18. doi:10.1002/hep.25938.

23. Maruyama T, Mitsui H, Maekawa H, Yamada H, Hirayama M, Iino S, Yasuda K, Koike K, Kimura S, Milich DR. Emergence of the precore mutant late in chronic hepatitis B infection correlates with the severity of liver injury and mutations in the core region. Am J Gastroenterol. 2000;95:2894–904. doi:10.1111/j.1572-0241.2000.03381.x.

24. Visvanathan K, Skinner NA, Thompson AJV, Riordan SM, Sozzi V, Edwards R, Rodgers S, Kurtovic J, Chang J, Lewin S, et al. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. Hepatology. 2007;45:102–10. doi:10.1002/hep.21482.

25. Wang Y, Cui L, Yang G, Zhan J, Guo L, Chen Y, Fan C, Liu D, Guo D. Hepatitis B e antigen inhibits NF-κB activity by interrupting K63-linked ubiquitination of NEMO. J Virol. 2018. doi:10.1128/JVI.00667-18.

26. Jegaskanda S, Ahn SH, Skinner N, Thompson AJ, Ngyuen T, Holmes J, De Rose R, Navis M, Winnall WR, Kramski M, et al. Downregulation of interleukin-18-mediated cell signaling and interferon gamma expression by the hepatitis B virus e antigen. J Virol. 2014;88:10412–20. doi:10.1128/JVI.00111-14.

27. Wu S, Kanda T, Imazeki F, Nakamoto S, Tanaka T, Arai M, Roger T, Shirasawa H, Nomura F, Yokosuka O. Hepatitis B virus e antigen physically associates with receptor-interacting serine/threonine protein kinase 2 and regulates IL-6 gene expression. J Infect Dis. 2012;206:415–20. doi:10.1093/infdis/jis363.

28. Yang C-Y, Kuo T-H, Ting L-P. Human hepatitis B viral e antigen interacts with cellular interleukin-1 receptor accessory protein and triggers interleukin-1 response. J Biol Chem. 2006;281:34525–36. doi:10.1074/jbc.M510981200.

29. Chang C, Enders G, Sprengel R, Peters N, Varmus HE, Ganem D. Expression of the precore region of an avian hepatitis B virus is not required for viral replication. J Virol. 1987;61:3322–25.

30. Schlicht HJ, Salfeld J, Schaller H. The duck hepatitis B virus pre-C region encodes a signal sequence which is essential for synthesis and secretion of processed core proteins but not for virus formation. J Virol. 1987;61:3701–09.

31. Chen HS, Kew MC, Hornbuckle WE, Tennant BC, Cote PJ, Gerin JL, Purcell RH, Miller RH. The precore gene of the

woodchuck hepatitis virus genome is not essential for viral replication in the natural host. J Virol. 1992;66:5682–84.

32. Revill P, Yuen L, Walsh R, Perrault M, Locarnini S, Kramvis A. Bioinformatic analysis of the hepadnavirus e-antigen and its precursor identifies remarkable sequence conservation in all orthohepadnaviruses. J Med Virol. 2010;82:104–15. doi:10.1002/jmv.v82:1.

33. Frelin L, Wahlström T, Tucker AE, Jones J, Hughes J, Lee BO, Billaud J-N, Peters C, Whitacre D, Peterson D, et al. A mechanism to explain the selection of the hepatitis e antigen-negative mutant during chronic hepatitis B virus infection. J Virol. 2009;83:1379–92. doi:10.1128/JVI.02516-08.

34. Chen M, Sällberg M, Hughes J, Jones J, Guidotti LG, Chisari FV, Billaud J-N, Milich DR. Immune tolerance split between hepatitis B virus precore and core proteins. J Virol. 2005;79:3016–27. doi:10.1128/JVI.79.20.12905-12913.2005.

35. Tian Y, Kuo C-F, Akbari O, Ou J-HJ. Maternal-derived hepatitis B virus e antigen alters macrophage function in offspring to drive viral persistence after vertical transmission. Immunity. 2016;44:1204–14. doi:10.1016/j.immuni.2016.04.008.

36. Chen M, Sällberg M, Thung SN, Hughes J, Jones J, Milich DR. Nondeletional T-cell receptor transgenic mice: model for the CD4 (+) T-cell repertoire in chronic hepatitis B virus infection. J Virol. 2000;74:7587–99. doi:10.1128/JVI.74.16.7587-7599.2000.

37. Publicover J, Gaggar A, Nishimura S, Van Horn CM, Goodsell A, Muench MO, Reinhardt RL, van Rooijen N, Wakil AE, Peters M, et al. Age-dependent hepatic lymphoid organization directs successful immunity to hepatitis B. J Clin Invest. 2013;123:3728–39. doi:10.1172/JCI68182.

38. Milich D, Liang TJ. Exploring the biological basis of hepatitis B e antigen in hepatitis B virus infection. Hepatology. 2003;38:1075–86. doi:10.1053/jhep.2003.50453.