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. 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, and the generally high efficiency rates for the hepatitis B vaccine in newborns (i.e., >90% protective levels of anti-HBs antibodies).

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 HBsAg-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 immunonprophylaxis.

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. 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.

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. 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 HBsAg-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.15 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/HBcAg s are primary targets of the T cell response, especially during acute exacerbations.20 A corollary 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 genotyping 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-1p-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 HBcAg6,34 (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 HBeAg is immunogenic and a target for HBe/HBcAg-
The other point of controversy arises in vivo. A tolerogen is an immunogen. 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.

**Specificity**

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. 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. Most importantly, numerous studies have demonstrated that HBeAg can cross the human placenta. 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/HBCag in transgenic mice. 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. 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 cytotoxic 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. 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.
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