Hepatitis B and Pregnancy: Virologic and Immunologic Characteristics

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The hepatitis B virus (HBV) is an important human pathogen. Unvaccinated infants infected through mother-to-child transmission (MTCT) are at >95% risk of developing serum hepatitis B surface antigen-positive chronic hepatitis B (CHB). Despite complete passive-active HBV immunoprophylaxis, approximately 10% of infants born to mothers who are highly viremic develop CHB, and thus maternal treatment with nucleos(t)ide analogs (tenofovir disoproxil fumarate, lamivudine, or telbivudine) is recommended in the third trimester of pregnancy to reduce MTCT risk. Viral rebound usually occurs after stopping treatment and, in the context of maternal immunologic reconstitution postpartum, can also precipitate host immune-mediated hepatic (biochemical) flares. In this article, we review the epidemiology of HBV MTCT, discuss management and potential mechanisms of HBV vertical transmission, and highlight recent studies on virologic and immunologic aspects of hepatitis B in pregnancy and postpartum. (*Hepatology Communications* 2020;4:157-171).

Chronic hepatitis B (CHB) is a serious public health problem affecting approximately 250 million people worldwide, including 65 million women of childbearing age, leading to 8,00,000 deaths annually.[1] Acute hepatitis B virus (HBV) infection in healthy immunocompetent adults results in a self-limiting disease, with <2% to 3% progressing to serum hepatitis B surface antigen-positive (HBsAg)+ CHB.[2] However, acutely infected infants are at >95% risk of developing CHB, and hence vertical or mother-to-child transmission (MTCT) from mothers who are HBsAg+ is responsible for approximately 50% of the global disease burden. The World Health Organization recommends that all infants receive the birth dose HBV vaccine soon after birth, followed by two doses to complete the primary series. The implementation of universal childhood HBV vaccination with or without hepatitis B immune globulin (HBIG) in more than 200 countries since the 1990s has led to a significant decline in MTCT and the global incidence of CHB.[1] In this review, we summarize the current literature on HBV immunopathogenesis in pregnancy and proposed mechanisms of HBV MTCT.

EPIDEMIOLOGY OF HBV AND MTCT

The global prevalence of CHB (serum HBsAg+) is 3.6%, with the highest prevalence in Africa (8.8%) and the Western Pacific (5.2%). More than 75% of individuals with CHB worldwide are found in the Asia Pacific

Abbreviations: "a" determinant, antigenic determinant; ALT, alanine aminotransferase; anti-HBs, antibody to hepatitis B surface antigen; BCP, basal core promoter; cccDNA, covalently closed circular DNA; CD, clusters of differentiation; CHB, chronic hepatitis B; FDA, U.S. Food and Drug Administration; HBeAg, hepatitis B e antigen; HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; IL, interleukin; LMV, lamivudine; MTCT, mother-to-child transmission; NA, nucleos(t)ide analog; NK, natural killer; OBI, occult hepatitis B infection; PBMC, peripheral blood mononuclear cell; PC, precore; qHBsAg, quantitative hepatitis B surface antigen; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate; T_h, T helper.

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region, and vertical transmission is more common in Asia than Africa.[3] The risk of CHB following exposure to HBV varies from approximately 90% in infants to 30% to 50% in toddlers and young children up to 5 years of age. The rates of MTCT also vary depending on maternal hepatitis B e antigen (HBeAg) status, with a 70% to 90% transmission rate for mothers who are HBeAg+ versus 10% to 40% in HBeAg– infection.[4]

SUMMARY OF CURRENT CLINICAL GUIDELINES AND RECENT APPROACHES FOR MANAGEMENT OF CHB AND PREGNANCY

All HBV clinical practice guidelines recommend prenatal HBsAg screening in pregnancy and administering postnatal passive-active immunoprophylaxis with HBIG along with the three-dose HBV vaccine series to infants born to mothers who are HBsAg+ (Table 1). HBV immunoprophylaxis failures can occur in approximately 10% of infants born to mothers who are HBsAg+ and HBeAg+ with HBV-DNA 9 log10 copies/mL (1 IU = ~5 virus copies/mL). Current guidelines recommend initiation of oral antiviral (nucleos(t)ide analog [NA]) therapy in the third trimester in mothers who are highly viremic with HBV-DNA levels >6 log10 copies/mL to reduce the risk of MTCT.[5-8] According to the U.S. Food and Drug Administration (FDA), the three oral NAs considered safe in pregnancy are lamivudine (LMV; category class C) and class B drugs telbivudine and tenofovir disoproxil fumarate (TDF). However, it is important to note that animal reproductive toxicity studies are not always predictive of human response, thus NAs should be used during pregnancy only if potential benefits outweigh the risks. TDF is preferred due to a better resistance profile and safety data for treatment of HBV during pregnancy.[7,9] Although TDF is detected in breast milk, it has low oral bioavailability, and infants are exposed to minimal oral concentrations (<0.03% of recommended neonatal dose).[6] In nonpregnant patients with CHB, long-term TDF therapy is linked to metabolic bone disease and renal dysfunction, but to date no significant maternal safety concerns have been reported with NA therapy during pregnancy.[10] A new formulation of TDF (tenofovir alafenamide fumarate [TAF]) was recently approved for treatment of CHB and human immunodeficiency virus (HIV) infection. TAF is a TDF prodrug with lower serum bioavailability due to rapid cellular uptake into hepatocytes and lymphocytes, with associated lower risk of renal and metabolic bone effects in treatment of either CHB or HIV.[11,12] In women of childbearing age with CHB, viral kinetics over a 12-week treatment regimen with TAF or TDF showed comparable efficacy of both drugs in lowering serum viral loads from approximately 5 log10 IU/mL to <29 IU/mL.[13] TAF may be a future therapeutic option for MTCT prevention, although safety data are limited (pending more data in the Antiretroviral Pregnancy Registry). However, it is noted that TAF will not receive FDA classification because this specific ranking system for drug use in pregnancy ended in 2015.[14]

In a multicenter randomized clinical trial, Jourdain et al.[15] demonstrated the successful prevention of MTCT by administration of the standard dose of HBIG and five instead of only three doses of the HBV vaccine (at 0, 1, 2, 4, and 6 months of age) in infants born to mothers who were HBeAg+. The study did not show a significant difference in immunoprophylaxis

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TABLE 1. SUMMARY OF MAJOR GUIDELINE RECOMMENDATIONS FOR HBV MANAGEMENT IN PREGNANCY

	AASLD 2018[6]	EASL 2017[7]	APASL 2016[8]
HBV-DNA threshold for treatment	>2 × 10^5 IU/mL (10^6 copies/mL) or HBsAg >4log IU/mL	>2 × 10^5 IU/mL (10^6 copies/mL)	10^6-10^7 IU/mL (5 × 10^6 copies/mL)
Treatment initiation gestational age	28-32 weeks	28-32 weeks	28-32 weeks
Preferred drug	TDF (LMV or TBV alternative)	TDF (LMV or TBV alternative)	TDF (LMV or TBV alternative)
Therapy discontinuation	At delivery or up to 12 weeks after delivery; postpartum ALT monitoring suggested every 3 months for 6 months	12 weeks after delivery	At delivery or 4-12 weeks after delivery
Breastfeeding	Not contraindicated. Risk of low-level antiviral exposure to infants should be discussed with mothers	Not contraindicated in untreated and TDF-treated women	Discouraged while mothers are on antiviral therapy
Mode of delivery	Cesarean section is not indicated	No comment	No comment

Abbreviations: AASLD, American Association for the Study of Liver Diseases; APASL, Asian Pacific Association for the Study of the Liver; EASL, European Association for the Study of the Liver; TBV, telbivudine.

failure rates between mothers who received placebo (n = 147) versus TDF (n = 147) in the third trimester. Although this study highlights the effectiveness of a more aggressive immunoprophylaxis strategy in preventing perinatal HBV transmission, given the robust data on safety of TDF in pregnancy, TDF remains the drug of choice to treat CHB and prevent MTCT.[15,16] Other studies have explored the potential of antenatal administration of multiple low and high doses of HBIG to reduce MTCT; however, well-designed clinical trials are necessary to compare HBIG versus antiviral therapy to draw meaningful conclusions.[17,18]

Expert guidelines recommend several viral markers (HBV-DNA, HBeAg) and clinical tests (i.e., alanine aminotransferase [ALT], liver histology, or noninvasive tests, such as liver stiffness measurement [LSM] by transient elastography [TE] or FibroScan) to determine the need for antiviral treatment and assess liver disease progression.[7,19] The key viral marker of HBV persistence is the resilient intrahepatic HBV covalently closed circular DNA (cccDNA) template. Due to the invasive nature of liver biopsy to assess HBV-cccDNA levels, there is significant interest in surrogate serum biomarkers in CHB.[19] Quantitative HBsAg (qHBsAg) levels may be a useful biomarker because it originates from both HBV-cccDNA (either secreted as subviral particles or from the intact virion) and integrated HBV.[20] We and others found that qHBsAg could be a surrogate marker for HBV-DNA in women who are HBeAg+ during pregnancy. Moreover, a cost-effectiveness analysis showed that qHBsAg testing was significantly more cost effective compared to HBV-DNA.[21,22] In other studies, no correlation was observed between HBV-DNA and qHBsAg.[23,24] These conflicting reports may be attributable to the data being limited in certain genotypes and the presence of integrated virus.[25] An interesting study that assessed correlation between qHBsAg levels and vertical transmission risk showed that a maternal HBsAg level >4.5 log10 IU/mL and a maternal viral load cutoff approximately 6 log10 IU/mL is associated with a higher risk of infant infection, with a sensitivity of 100% and specificity of 71%.[26] Given the overall lower risk of transmission in patients who are HBeAg–, qHBsAg testing for predicting high maternal viremia could be performed as a surrogate when HBV-DNA quantitation is not possible in mothers who are HBeAg+ in resource-limited regions. Additionally, circulating HBV-RNA has been proposed as a useful biomarker for monitoring response to NA therapy.[27] A strong correlation was observed between serum HBV-RNA and intrahepatic HBV-cccDNA levels. Our recent study found that in pregnant CHB carriers, serum HBV-RNA levels correlate with HBeAg, qHBsAg, and HBV-DNA and hence could be used as a complementary viral marker in assessing liver disease risk in pregnancy.[28] Currently, HBV-DNA is the most important viral marker for predicting HBV MTCT risk, maternal liver disease progression, and need for TDF therapy in pregnancy. Additional studies on novel HBV biomarkers and relevant host immunologic markers are needed to evaluate their prognostic and diagnostic potential in management of patients with CHB in pregnancy and postpartum.

FACTORS INVOLVED IN INFANT HBV INFECTION

The dynamic course of CHB infection depends on age of infection, route of infection, host immune response, and viral factors, including genotypes and variants, as discussed below.

Age-Dependent Immune Response to HBV Infection

The "immaturity" and/or "immune tolerance" of the immune system during infancy was thought to be responsible for the weak HBV-specific immune response in neonates resulting in CHB. However, there is now a paradigm shift and reports suggesting that the immune system of newborns appears to be less proinflammatory, which is probably an adaption to avoid aggravated immune responses *in utero*.[29,30] Koumbi et al.[31] found HBV core-specific and HBsAg-specific T-cell responses in vaccinated uninfected infants born to mothers who were HBsAg+/HBeAg–, suggesting an *in utero* viral encounter that did not result in overt infection but led to priming of HBV-specific immunity. In preclinical studies, livers from young HBV transgenic mice produced much lower interleukin (IL)-21 than adult mice livers, resulting in a weaker clusters of differentiation (CD)8+ T-cell and B-cell response. The chemokine (C-X-C motif) ligand 13 is involved in germinal center activity and was expressed in an age-dependent manner in both adult mouse and human Kupffer cells.[32,33] In a mouse model of HBV infection, Chou et al.[34] demonstrated the role of gut microbiota and innate immunity in modulating HBV clearance with 12-week-old mice (carrying wild-type toll-like receptor 4) with stable microbiome clearing HBV within 6 weeks of hydrodynamic injection. In comparison, 6-week-old mice in which the gut microbiota was still developing remained HBsAg+ at 26 weeks after injection. Antibiotic treatment of mice from 6 to 12 weeks of age inhibited HBV clearance. Based on preclinical studies, it appears that switching from an anti-inflammatory to a proinflammatory milieu from infancy to adulthood, along with changes in the microbiome, impacts progression of CHB. Additional studies are needed in HBV-exposed newborns and children with CHB to delineate the innate and adaptive immune mechanism(s) early on in CHB.

Potential Modes and Risk Factors for HBV MTCT

HBV vertical transmission is the main cause of CHB, particularly in HBV-endemic countries that lack effective HBV immunization programs. HBV MTCT can occur prenatal or intrauterine, natal or at the time of birth, and postnatal or postpartum (Fig. 1).[35-51] Most HBV infections occur perinatally (at birth or soon after) in unvaccinated infants, but reports suggest that approximately 3% to 8% of infections may occur through the intrauterine route.[52] It is believed that intrauterine transmission of HBV is the most significant contributor to MTCT and immunoprophylaxis failure.[49] The potential mechanisms involved in MTCT and viral/host factors associated with MTCT risk are summarized below.

ROLE OF HBV PROTEINS IN MTCT

Maternal HBeAg seropositivity is a significant risk factor for MTCT. It is recognized that the HBeAg has an immunomodulatory role in virus–host interactions and that early *in utero* exposure may promote chronicity instead of viral clearance.[53] In infants infected by MTCT, *in utero* transfer of maternal HBeAg has been shown to be involved in persistence of HBV due to immunologic tolerance. Tian et al.[54] showed in a mouse model that conditioning of hepatic macrophages by maternal HBeAg generates anti-inflammatory macrophages following subsequent HBeAg exposure, leading to HBV persistence. However, in the absence of HBeAg preconditioning, macrophages acquire a proinflammatory phenotype, leading to HBV clearance by activated CD8+ T cells. In pregnant women with viral loads of >3 log10 copies/mL, functional hepatitis B X protein (HBx) produced in HBV-infected placenta cells could activate phosphoinositide 3-kinase in placenta, which signals inhibition of apoptosis in placental cells, allowing for HBV persistence in trophoblasts.[55] Overall, these studies shed light on the immunomodulatory role of HBeAg and also how other viral proteins (HBx) can impact the risk of MTCT.

MATERNAL VIRAL LOAD AND MTCT

High viral load (>8 log10 copies/mL) is a known significant risk factor for MTCT. In an Australian study of 313 pregnant women who were HBsAg+, it was found that 9% (4/47) of infants of



PRENATAL (INTRAUTERINE) *3%-8%*	NATAL (AT BIRTH) *~35%*	POSTNATAL (POSTPARTUM) *Variable rates reported depending on age of infection and country of origin*
1. **Placental infection** • Through maternal vascular endothelium to endovascular extravillous trophoblasts(35) • Infection of Hofbaeur cells (placental macrophages)(36) • Transcytosis – entry mechanism for HBV into trophoblasts; HBV-specific receptors may be present in only the early stages of infection(37) • Increased ASGPR (receptor involved in endocytosis of HBV) in placental and maternal circulating dendritic cells(38) • Paracellular routes from maternal blood to fetal capillaries – through placental leakage(39), transmission through HBV within maternal PBMC(40,41) 2. **Germline infection** HBV-infected oocytes and embryo(42,43)	1. **Microtransfusion – mixing of maternal and fetal blood**(44) 2. **Direct contact with infected fluid in maternal genital tract**(45) 3. **Swallowing of infected fluids**(44)	1. **Early horizontal transmission – from close contact with infected mothers**(46,47) 2. **Incomplete or delayed vaccination (immunoprophylaxis failure)**(48,49) 3. **Breastfeeding – HBsAg detected at high concentrations in breast milk; however, no significant difference in infection rates between breastfed and non-breastfed infants**(50,51)

FIG. 1. Summary of proposed modes of HBV MTCT and underlying mechanisms. Abbreviation: ASGPR, asialoglycoprotein receptor.

mothers who were HBeAg+ with HBV-DNA >8 log10 copies/mL were perinatally infected despite appropriate immunoprophylaxis, whereas none of the infants born to mothers with viral loads <8 log10 copies/mL were infected.[56] A large study of more than a thousand mother–infant pairs from China stratified maternal HBV-DNA levels as <6 log10, 7 log10, 7 to 8 log10, and >8 log10 copies/mL and found that the corresponding rates of immunoprophylaxis failure were 0%, 3.2%, 6.7%, and 7.6%, respectively.[57] Xu et al.[35] found that placental infection occurs progressively through different cellular layers from maternal to the fetal side, with the depth of placental tissue infection in direct proportion to maternal viral load.

OCCULT HEPATITIS B INFECTION IN MTCT

The diagnosis of CHB is confirmed by the presence of HBsAg in serum for >6 months in infected infants. However, a covert form of HBV infection, occult hepatitis B infection (OBI), has been described. OBI is characterized by HBsAg negativity and natural immunity (with HBV anti-core and/or antibody to HBsAg [anti-HBs]) antibodies, low-level viremia within the liver, and serum and extrahepatic reservoirs (peripheral blood mononuclear cells [PBMCs] or lymphoid system).[58] OBI is implicated in liver fibrosis progression and the development of hepatocellular carcinoma (HCC).[59] Bai et al.[40] reported that 16/60 (27%) infants born to mothers with undetectable serum HBV-DNA but HBV-DNA positivity in PBMCs became infected with HBV. It should be noted, however, that the neonate blood collection occurred within 24 hours of birth and prior to administration of infant immunoprophylaxis. A prospective longitudinal study found that 42% of infants born to mothers with CHB developed OBI despite HBIG and HBV vaccination.[60] Taken together, these data imply that vertical transmission of OBI may occur despite appropriate immunoprophylaxis.

OBSTETRIC FACTORS IN HBV MTCT

HBV vertical transmission in mothers with viral load >7 log10 copies/mL undergoing amniocentesis

has been reported.[61] In another study, it was found that infants born to mothers who were HBeAg+ and who were delivered vaginally versus by cesarean section had a higher rate of CHB.[62] However, because appropriate and timely passive-active immunoprophylaxis and maternal antiviral therapy will greatly reduce the risk of MTCT, the mode of delivery should only be determined by obstetric indications.

HBV Genome Variability and Impact on HBV Management in Pregnancy

The HBV replicates by reverse transcription of an RNA intermediate. This reverse transcription is catalyzed by a virus-encoded polymerase that lacks proofreading ability, which leads to sequence heterogeneity.[63]

HBV GENOTYPES AND VARIANTS

The HBV is classified into eight major HBV genotypes (A-H) worldwide, with a distinct geographic distribution, which are identified by approximately 8% divergence across the HBV full genome.[64,65] In a Japanese study, genotype C was correlated with increased perinatal transmission rates compared to genotype B. However, the increased MTCT risk may be due to higher viral loads and HBeAg positivity in mothers with HBV genotype C infection.[66] Other studies did not find an association between MTCT risk and the HBV genotype.[67]

HBV PRECORE/BASAL CORE PROMOTER MUTANTS

HBV variants are selected under host and antiviral pressure, producing immune escape and drug resistance variants. HBV precore (PC) or basal core promoter (BCP) mutations introduce a stop codon that interferes with HBeAg protein translation without affecting viral replication.[68] The HBV PC mutant guanine (G) to adenine (A) at nucleotide position 1896 (G1896A) mutation is implicated in acute liver failure, and the dual BCP mutations adenine (A) to thymine (T) at nucleotide position 1762/G to A at nucleotide position 1764 (A1762T/G1764A) were strongly associated with cirrhosis and HCC regardless of the HBV genotype.[69-71] Papadakis et al.[72] found that in 32 pregnant women who were HBeAg– and carrying PC mutant G1896A and HBV-DNA levels of 4 to 5 log10 copies/mL, HBV transplacental transmission did not occur, as evidenced by HBV-DNA negativity in the analyzed placenta tissues. Additionally, passive-active immunoprophylaxis failure was not observed in the infants at 1 year of age. A retrospective study from China of 332 mothers with CHB compared HBV mutants in mothers with and without *in utero* transmission of HBV. Dual BCP mutants were found in mothers who did not transmit HBV to their neonates, suggesting a protective role in MTCT but also likely related to lower maternal viral loads.[73] Our recent study suggests that the presence of BCP/PC mutants in PBMCs of TDF-treated and untreated mothers did not cause an overt HBV infection in infants who received complete immunoprophylaxis and also did not increase the risk of maternal liver disease at 4 years of follow-up.[74] In summary, although combined maternal HBeAg positivity and high viral loads is an established risk factor for MTCT, HBV genotype, PC/BCP mutations, and risk of immunoprophylaxis failure are not well understood and may be an area of future studies.

HOST AND VIRAL FACTORS IN HBV IMMUNOPROPHYLAXIS FAILURE

Hepatitis B Vaccine-Specific Responses in Infants Born to Mothers Who Were HBsAg+

There are few reports on the long-term efficacy of postnatal passive-active HBV vaccination. In a study from Taiwan, approximately 16% of the subjects who had received the HBV vaccine as infants were found to have anti-HBs at 15 years of age.[75] However, the study did not assess memory B- or T-cell response to the vaccine. Transplacental passage of HBsAg and transient HBsAg positivity in infants was associated with blunted immune response in infants born to mothers who were HBsAg+/HBeAg+.[76] In infants born to mothers with HBeAg positivity, increasing the vaccine dose from 10 μg to 20 μg improved vaccine immunogenicity.[77] Studies have also shown that the HBV vaccine alone is effective in preventing HBV infection or reactivation in infants born to mothers who were HBeAg– compared to passive-active prophylaxis with HBIG+ vaccine.[78] These studies highlight the importance of administering HBV vaccine to all newborns of mothers with HBsAg positivity regardless of their HBeAg status, especially in resource-limited countries. Most studies

assessing long-term response to postnatal vaccination have focused on anti-HBs response as a measure of vaccine immunity. There are few studies analyzing T-cell responses in vaccinated uninfected infants born to mothers with CHB. In infants who received complete immunoprophylaxis, lower amounts of IL-2 (T helper 1 [T_h1] cytokine) secretion in an *in vitro* stimulation assay were associated with vaccine failure.[79] Interestingly, Koumbi et al.[31] found HBV-specific T-cell responses in uninfected vaccinated infants born to mothers who were HBsAg+/HBeAg−, suggesting *in utero* encounter to HBV antigens. Further, they found that this exposure did not impair the neonatal B-cell and T-cell vaccine response.

A large multicenter study by Chen et al.[80] of 1,063 mothers who were HBsAg−/anti-HBs+ and their infants showed a strong negative correlation between maternal anti-HBs and infant anti-HBs titers in vaccinated infants. Further, up to 23% of infants born to mothers with protective anti-HBs titers >10 IU/L did not respond to the standard vaccination series. These findings may have implications regarding the age of infancy/childhood vaccination. Some studies support these data[81,82]; however it was noted by others that, despite negative or low anti-HBs levels, HBV-specific T-cell responses were found in children 10 years after their primary infant vaccination.[83] In 2017, the FDA approved a new two-dose HBV vaccine, HEPLISAV B (Dynavax Technologies), which consists of CpG-adjuvanted recombinant HBsAg, for use in adults. Although this vaccine was tested in pregnant rats and found to be safe, no studies have been conducted in pregnant women or infants and may be an area for future clinical trials.[84]

In summary, poor vaccine response, vaccine failure, and breakthrough HBV infection have been reported up to 15 years after adequate infantile immunoprophylaxis. Although the evidence is limited, all successfully vaccinated infants born to mothers with HBsAg positivity (especially HBeAg+) should be followed long term (once every 5 years) until adolescence or early adulthood. It may also be prudent to provide an HBV booster, especially if at risk of ongoing exposure to HBsAg+ individuals (close household contacts).

HBV Immune Escape Mutants

The anti-HBV surface antibodies induced by the current recombinant vaccine predominantly target the hydrophilic region of the major HBsAg protein, known as the antigenic determinant ("a" determinant) (amino acid residues 124-147). A glycine to arginine substitution (G145R) is the archetypal immune escape mutant that markedly reduces the affinity of anti-HBs binding and is able to survive despite high anti-HBs titers.[65,85] Mutations that affect antigenicity outside of the major "a" determinant region could also affect the binding of circulating antibodies. These "downstream mutations" could affect the conformation of the major HBV S antigenic region and binding of neutralizing antibodies induced by the current vaccine.[86,87] Mutations, such as threonine to lysine at 118 (T118K), lysine to glutamine at 141 (K141E), aspartic acid to glycine at 144 (D144G), cysteine to arginine at 147 (C147R), and cysteine to arginine at 149 (C149R), were reported in association with vaccine escape.[88] Overall, these mutants appear to replicate very slowly and have not significantly impacted the global immunization programs.[89] Development of anti-HBs antibodies requires T cell help. Therefore, mutations in CD4+ T-cell epitopes can result in immune escape mutations affecting humoral immune response.[90] Some of these include threonine to alanine at 23 (T23A), phenylalanine to serine at 20 (F20S), and phenylalanine to cysteine at 85 (F85C). Mutations in CD8+ T-cell epitopes, such as proline to serine at 29 (P29S), serine to leucine at 34 (S34L), and glutamine to arginine at 181 (Q181R), have also been discovered.[91,92]

Ayres et al.[9] found significantly increased viral quasi-species diversity in pregnancy despite short duration of LMV but not TDF therapy. Our study in 21 CHB carriers (5 on TDF) identified minor variants at residues associated with vaccine escape, drug resistance, and liver disease in pregnancy and/or postpartum,[93] but no overt immunoprophylaxis failures were reported. It is unclear if vaccine failure mutants arise *de novo* in infants born to mothers who are HBsAg+ as a result of host immune response or if the mutants are transmitted by mothers and replicate in infants. One preliminary study of 4 mother–infant pairs with infant immunoprophylaxis failure showed low (<10%) amino acid substitutions in the "a" determinant of surface antigen in infants versus mothers ($P > 0.05$), suggesting passage of minor vaccine escape mutants from mother to infant.[94] A recent report documented that, despite appropriate immunoprophylaxis in 44 children of mothers who were

HBsAg+, 3/44 infants at 1 year of age showed OBI, including 1 child who subsequently developed overt (HBsAg+) CHB at 5 to 7 years of follow-up.[95] It should be noted that the mothers of children with OBI had high (>8 log10 copies/mL) antenatal viral loads. A previous randomized controlled trial in 259 vaccinated infants (n = 128 on HBIG; n = 131 on placebo) born to mothers with HBeAg+ CHB found that 42% of infants in the HBIG group developed OBI at 2 years of age,[60] suggesting that follow-up (every 5-10 years) HBsAg testing should be considered in infants born to mothers who were highly viremic, as discussed above.

Immune Response and Trained Immunity in Neonates With CHB

An important mechanism of HBV persistence is from exhaustion of HBV-specific CD8+ T-cell responses, whereas robust polyclonal CD4+ and CD8+ T-cell responses are associated with HBV clearance.[96] Diminished expression of CD3+ T-cell receptor zeta chain was associated with functionally defective CD8+ T cells producing less interferon-γ (IFN-γ) and reduced expression of the cytotoxicity marker CD107a in newborns who were HBsAg+ versus HBsAg– and healthy newborns.[97] Shrivastava et al.[98] demonstrated higher prevaccination levels of immature transitional B cells in 12 newborns who were HBsAg+ compared with infants who were HBsAg– born to mothers who were HBsAg+. The frequency of immature transitional B cells declined at 12 months after vaccination in newborns who were HBsAg+, whereas no changes were observed in HBsAg– and uninfected healthy newborns. These studies suggest weak T-cell and B-cell responses in infants with CHB. More convincing evidence now points toward a fully functional T-cell response in infants. Serum cytokine profiling from neonates born to mothers with CHB revealed a cytokine signature compatible with a T_h1-like response (high levels of IL-12 p40 and low levels of T_h2 cytokines IL-4, IL-5, IL-13, and IL-10) and a decrease in proinflammatory cytokine profile (IL-1β and IL-6). Further, cord blood mononuclear cells from neonates who were HBV+ showed a stronger response compared to healthy controls to an unrelated bacterial challenge. Overall, these data suggest that HBV is able to induce "trained immunity" rather than a tolerogenic state in these infants.[99] This also supports the findings of Tian et al.[54] that maternal HBeAg "trains" Kupffer cells *in utero*, leading to HBV persistence, highlighting the immunomodulatory role of HBeAg. The concept of trained immunity, or priming of innate response by a prior pathogen or its components, is well established in many other infection and vaccination scenarios (Fig. 2).[100-102]

VIROLOGIC AND BIOCHEMICAL FLARES IN PREGNANCY AND RISK OF MATERNAL LIVER FIBROSIS

Globally, especially in developed countries, more women are choosing to have children at an older age (>35 years).[103] CHB carriers in the third and fourth decades are more likely to transition to HBeAg–/anti-HBe+ (with undetectable or low HBV-DNA) or harbor PC/BCP mutants (with fluctuating levels of moderate to high HBV-DNA). In the latter scenario, monitoring for liver disease progression is important given the recognized risk of HBeAg– CHB in fibrosis progression and HCC development.

The clearance of HBV from infected cells is due to a complex interplay between different immune effector cell types. CD4+ T cells secrete cytokines that are responsible for development of efficient CD8+ T cells, which kill HBV-infected hepatocytes through cytolytic and noncytolytic mechanisms. HBV-specific CD4+ T cells also prime B cells to produce antibodies that neutralize free virus. However, this antiviral response is unsuccessful in patients with CHB.[104] Immune-mediated liver injury in HBV infection is initiated by antigen nonspecific cells, such as natural killer (NK) cells, whereas HBV-specific CD4+ and CD8+ T cells are functionally exhausted.[105]

It is unknown if immune changes in pregnancy and postpartum impact the natural history of CHB. Higher rates of HBeAg loss (and HBsAg clearance) and biochemical-hepatic flares with increased ALT levels have been reported, especially during early postpartum when immune reconstitution occurs.[106-110] Most flares are self-limited and do not require therapy, but some can be severe, resulting in liver failure.[111,112] The reported rates of ALT flares postpartum are variable owing to different definitions of ALT flare, patient characteristics, and antiviral therapy (Table 2). Studies from our group (N = 138; 60% Asian, 30% African) and other retrospective

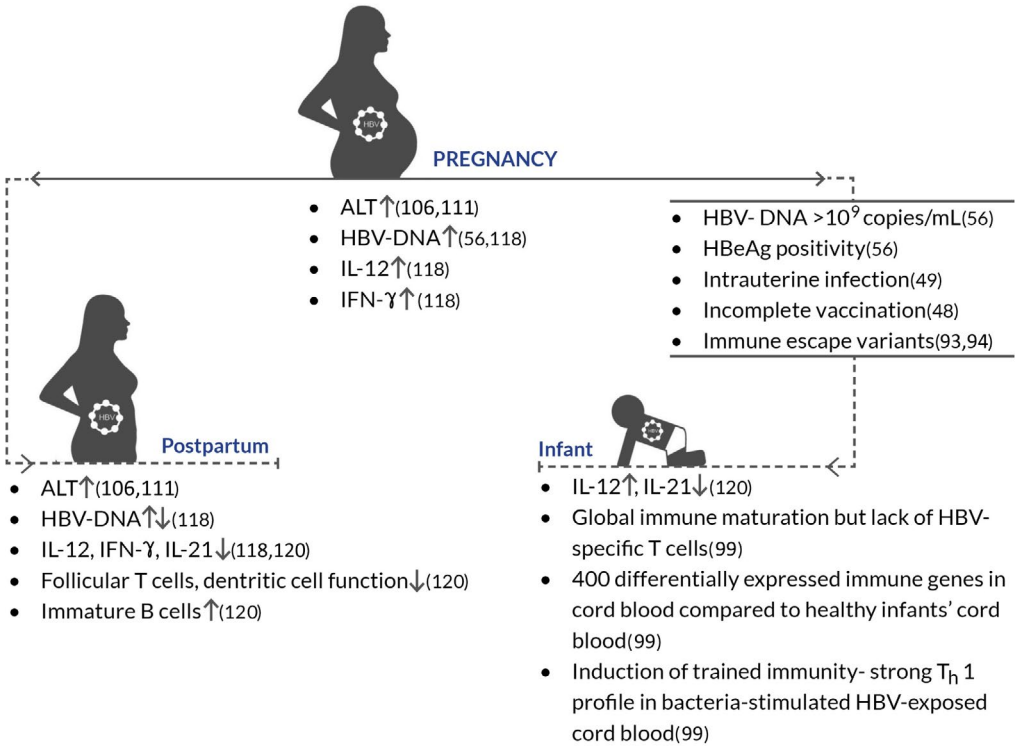

FIG. 2. Schematic representation of immunologic changes in the peripartum period in mothers with CHB and their infants.

studies conducted in the United States and Australia (N = ~100; ~80% Asian) have reported hepatic flares during late pregnancy and early postpartum, even in the absence of antiviral treatment.[106,107,113,114] ALT flares are reported to be more common in HBeAg+ carriers versus women with HBeAg– CHB. Interestingly, a Taiwanese study determined that titers of HBeAg <1:650 in women who were HBeAg+ were associated with postpartum HBeAg clearance.[115] Taken together, biochemical and virologic flares can occur in late pregnancy and postpartum in women with CHB, potentially increasing fibrosis risk, especially at an older maternal age and HBeAg– CHB. Thus, maternal postnatal monitoring for exacerbations of liver disease is necessary.

IMMUNOLOGIC CHANGES IN THE PERIPARTUM PERIOD IN MOTHERS WITH CHB

In pregnancy, immune alterations, such as regulatory T-cell (Treg) expansion and a distinct regulation of T_h1, T_h2, and T_h17 cytokines, occur to prevent fetus rejection. This tolerance is reversed around parturition.[116,117] Our data show an increase in T_h1 cytokines, IFN-γ and IL-12, and mild ALT flares in pregnant untreated HBeAg– carriers (n = 26) compared to healthy pregnant controls. A significant decline in these levels was noted postpartum in women with CHB (n = 12). Despite changes in the cytokine milieu in pregnancy versus postpartum, we found no impact on liver fibrosis risk, as determined by LSM using TE (FibroScan) in the peripartum period.[117] Additionally, there was no significant change in serum viral load and qHBsAg levels. These results suggest inefficient antiviral responses by the maternal host immune system that prioritizes elimination of virus over fetal rejection. Interestingly, levels of proinflammatory chemokines (monocyte chemoattractant protein 1 and macrophage-derived chemokine) increased postpartum, which might partially explain the mild ALT flares noted in our cohort.[118] Both larger studies as well as *ex vivo* characterization of immune response using nonpregnant and pregnant PBMC samples are necessary to confirm the proposed mechanism.

TABLE 2. RECENT STUDIES EVALUATING BIOCHEMICAL FLARES IN CHB DURING THE PERIPARTUM PERIOD

Study	Country	No. of Patients	Time Points for Reporting ALT Flares	Definition of Flares	Proportion of Flares (Untreated)	Proportion of Flares (Treated)	Time to Resolution of Flares After Delivery (Definition)	Rate of HBeAg Loss
Bzowej et al., 2019[111]	USA	158	Twice in pregnancy <4, 4-8, and 8-12 months after delivery	>5 × ULN (<20 U/L)	5/149 (3.4%) in pregnancy 4/92 (4.3%) postpartum	1/49 (2%) in pregnancy 2/36 (5.6%) postpartum 5/29 (17.2%) (7-14 weeks after NA cessation)	Not reported	1/149 untreated 1/29 after treatment withdrawal
Jourdain et al., 2018[15]	Thailand	154 (on TDF) 157 (placebo)	Postpartum ~6 months	>10 × ULN	N/A (study cohort includes TDF- or placebo-treated patients)	9/154 (6%) after TDF cessation 5/157 (3%) after placebo cessation	Not reported	Not reported
Kushner et al., 2018[114]	USA	310	During pregnancy or within 6 months after delivery	>2 × ULN	42/311 (14%) in pregnancy 22/134 (16%) postpartum*	4/19 (21%) on NA therapy before pregnancy 3/19 (15%) after delivery	Not reported	Not reported
Chang et al., 2017[110]	USA	56	Twice in pregnancy ~3-6 months postpartum	>5 × ULN (19 U/L) or >3× baseline, whichever was higher	7/43 (16%) in pregnancy 0/15 postpartum	4/13 (31%) in pregnancy 3/9 (33%) postpartum (NA cessation at delivery) 4/18 (22%) who continued therapy postpartum 2/7 (29%) postpartum (NA cessation in the first trimester)	Within 12 months postpartum (<2 × ULN or similar to baseline ALT)	1/9 (after therapy withdrawal postpartum)
Chang et al., 2016[106]	USA	113	Twice in pregnancy and up to ~6 months postpartum	>5 × ULN (19 U/L) or >3× baseline, whichever was higher	7/112 (6%) in pregnancy 5/51 (10%) postpartum	N/A (untreated women recruited to the study)	Within 12 months postpartum (<2 × ULN or similar to baseline ALT)	Not reported
Samadi Kochaksaraei et al., 2016[113]	Canada	161	second trimester and ~3 months postpartum	>2 × ULN (19 U/L)	7/138 (5%) postpartum	4/23 (17.3%) postpartum	Not reported	Not reported
Giles et al., 2015[107]	Australia	126	Twice (early and late) in pregnancy, 1.5-3 months and 12 months postpartum	>2 × ULN or 2 × baseline ALT if higher than normal	2/126 (1.6%) in pregnancy 27/108 (25%) postpartum	4/7 (57%) postpartum	9-12 months	2/30
Nguyen et al., 2014[108]	USA	101	Pregnancy, at birth, <3 months postpartum, and >3 months postpartum	5 × ULN (19 U/L)	4/14 (29%) postpartum	22/44 (50%) postpartum, NA withdrawal at delivery 17/43 (40%) postpartum, NA withdrawal 3 months after delivery	11-12 months (normal or baseline)	1/14 (untreated) 5/44 (early NA withdrawal) 1/43 (late NA withdrawal)

*Data for untreated flares not reported but includes 348/388 (90%) untreated cases.
Abbreviations: N/A, not applicable; ULN, upper limit of normal (defined as <19 or 20 U/L).

A recent study by Li et al.[119] evaluated cell proportions from PBMCs of women with HBeAg– (n = 23) versus HBeAg+ (n = 10) CHB alongside 190 healthy controls and studied their association with pregnancy outcomes. Compared to patients who were HBeAg–, a higher proportion of CD19+ B cells but lower frequency of CD3+CD4+ T cells was identified in patients who were HBeAg+. Peripheral NK cell inhibition was noted in HBeAg+ cases, as evidenced by reduced cytotoxicity against target cells, lower expression of activation receptor NK group 2, member D (NKG2D), and decreased production of cytotoxic molecules (granzyme B and perforin). These findings clearly point toward a differential immune response in women of childbearing age who were HBeAg– versus HBeAg+. No differences were seen in pregnancy outcomes (clinical pregnancy or early miscarriage) in women infected with HBV versus controls. Impaired dendritic cell function and decreased levels of follicular T cells (CD4+C-X-C chemokine receptor type 5+), plasma B cells (CD19+CD38+) within PBMCs, and low serum IL-21 were found in mothers in association with MTCT (n = 22), whereas mothers who did not transmit HBV to their infants (n = 28) had a more functional immune cell profile (Fig. 2). Interestingly, newborns showed transcriptomic imprints of their mothers, suggesting that mothers' immune signatures could be a potential marker for MTCT.[120]

Acute liver failure has been reported during pregnancy in CHB.[112,121] It is known that cortisol levels peak at term and delivery. A sudden decrease in cortisol levels postpartum is thought to have similar effects as withdrawal of steroid therapy causing HBV reactivation. There is little understanding regarding mechanisms of acute liver failure in pregnancy in the context of hepatitis B. There is some evidence that HBV core-specific T cells may be involved[114,122] and hence indicate a defective HBV-specific T-cell response in the peripartum period. Although there is limited literature on immune response during the peripartum period in CHB, the data show differences in immune cell functions during pregnancy versus postpartum and also between mothers who were HBeAg+ versus HBeAg–. Further studies are necessary in a larger cohort and, if possible, serially collected samples from mothers and infants to clearly understand the dynamics of the host immune response in association with HBV flares and MTCT in these groups.

Discussion

Despite the availability of an effective HBV vaccine, approximately 10% of children born to mothers who are HBsAg+ with high serum HBV-DNA levels (without antiviral therapy) during pregnancy are reported to develop CHB. Prior reports of vaccine failure may be related to incomplete or nonadherence to the recommended immunoprophylaxis schedule. The risk of OBI in successfully vaccinated infants is unclear.

Pregnancy impacts the host immune system, and it is unknown whether patients with CHB in the postpartum period are at greater (or lower) risk of immune-mediated flares and liver disease (vs. before and during pregnancy). The risk of liver fibrosis progression in pregnant women may also be affected by advanced maternal age and the obesity epidemic in developed countries.

TDF is the treatment of choice for prevention of MTCT. The impact of short-term TDF use in multiple gestations is unclear because most studies (especially in China) were conducted in women with single pregnancies. Recently, TDF-resistant mutants were identified in a treatment-naive Chinese patient with CHB.[123] The potential risk of reselection of resistant variants by repeat TDF exposure is unknown but predicted to be extremely rare.

There are evolving data on enhanced prophylaxis strategies with the current HBV vaccine, recently approved adjuvanted HBV vaccines, novel viral biomarkers, and new anti-HBV therapies that may have future applications in management of HBV in pregnancy and prevention of HBV MTCT.

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