Review Article

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Immunological mechanisms of hepatitis B virus persistence in newborns

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Chronic hepatitis B virus (HBV) infection affects millions of people worldwide and about a half million people die every year. India represents the second largest pool of chronic HBV infection worldwide with an estimated 40 million infected people. The prevalence of chronic HBV infection in pregnant women is shown to be 0.82 per cent with the risk of mother-to-child vertical transmission. Hepatitis B e antigen (HBeAg) positivity indicates replicative form of HBV which may play a role in immunotolerance *in utero* by crossing the placenta. In case of HBeAg positivity and high viral load of mother, HBV immunoglobulin is preferably given along with HBV vaccination. Antiviral therapy is recommended for use in the third trimester of pregnancy to reduce the perinatal transmission of HBV, however, use of antiviral therapy should be individualized during pregnancy. Chronic HBV infection in neonates is linked with strong presence of Tregs (T regulatory cells) and defective CD8 T cells pool to produce interferon (IFN)-γ. T cell receptor (TCRζ) chain defects were also associated with decreased CD8 T cell dysfunction. Decreased TCRζ expression could be due to persistent intrauterine exposure of the viral antigens early in embryonic development leading to immune tolerance to HBV antigens in the newborns positive for hepatitis B surface antigen (HBsAg+ve). Therefore, due to HBV infection, T cell tolerance to HBV-antigen may probably leave the newborn as a chronic carrier. However, HBV vaccination may have benefits in restoring acquired immunity and better production of HBV specific antibodies.

Key words Chronic infection - HbeAg - HbsAg - hepatitis B virus (HBV) - perinatal - pregnancy

Hepatitis B virus infection in pregnant mothers and transmission to newborns

Chronic hepatitis B virus (HBV) infection affects over 240 million people worldwide and about a half million people die every year due to the acute or chronic consequences leading to liver failure and liver cancer[1]. HBV is present in blood, saliva, semen, vaginal secretions, and menstrual blood of infected individuals[2].

In Southeast Asia and China, the prevalence of HBV infection among women of child-bearing age is as high as 10-20 per cent[3]. India represents the second largest pool of chronic HBV infection worldwide with an estimated 40 million people. In India, the prevalence of chronic HBV infection in pregnant females is 0.82 per cent[4] and during pregnancy, hepatitis B virus infection presents the risk of mother-to-child (vertical) transmission.

To analyze the source of acquisition of HBV infection in chronic HBV infected patients, mothers of 384 chronic hepatitis B index patients were screened for HBV infection. The mothers of 40.1 per cent index patients were positive for HBsAg. The mothers of 34.1 per cent index patients were positive only for antibodies (total anti-HBc and/or anti-HBe) indicating that the mothers were exposed to HBV infection some time in past[5]. These data provide substantial evidence of present or past HBV infection in mothers of chronic HBV patients, suggesting possible perinatal transmission. It could be possible that one third of mothers, who were initially hepatitis B surface antigen (HBsAg) positive, could have cleared the infection during post-partum period and remained positive only for HBV antibodies[5]. Therefore, vertical transmission of hepatitis B virus could be one of the main causes of chronic HBV infection in our country.

The neonates born to mothers infected with chronic hepatitis B, have 90 per cent risk of acquiring chronic HBV infection and its persistence[6]. In contrast, when HBV is acquired during adulthood, only 5-10 per cent of adults develop persistent chronic HBV infection[7]. Most of the developed countries screen all pregnant women for HBV infection, however, in the developing countries it depends upon the risk factors. In India, there is no consistent policy of screening the pregnant women across the country. A meta-analysis of prevalence of hepatitis B in India showed 2.4 per cent prevalence in general population[8]. However, the prevalence rate of HBsAg positivity in pregnant women varied from 1-9 per cent in different parts of the country and e antigen (HBeAg) positivity rates among them varied from 4.8-68.7 per cent[8].

A large single centre study from north India of 20,104 pregnant women showed a chronic HBsAg positivity rate of 1.1 per cent[9]. Majority of pregnant women with viral hepatitis B are considered as chronic hepatitis B infected but a few may develop acute hepatitis in the third trimester of pregnancy resulting in 1 per cent fulminant hepatitis[10,11]. During pregnancy, acute viral hepatitis involves a particular risk both for the mother and the baby.

Of the two secretory proteins; HBsAg and HBeAg, HBsAg does not usually cross the placenta, however, small sized HBeAg passes through the placental barrier even with low maternal viral load titre[12,13] (Fig. 1). In newborn, transplacental HBeAg can be detected at one month of age but it would disappear before 4 months of age. However, in a few, infected newborns with HBV viral titres, persistent detection of HBeAg for more than 4 months strongly indicates HBV chronicity[14,15]. It is also observed that anti-HBc (antibodies to core antigen) positivity can be detected more than anti-HBe in the babies born to hepatitis B infected mothers[12,13]. Therefore, anti-HBe till one year of age and anti-HBc till two years of age represent the transplacental maternal antibodies to the virus, and may not be an indicator of present active or past HBV infection in babies born to hepatitis B infected mothers.

Hepatitis B envelope antigen spillage through placenta induces HBV specific T cell tolerance *in utero*[16] and intrauterine infection could be the main cause of the failure of immunoprophylaxis[17-19] (Fig. 1). However, there are several evidences to show that the incidence of intrauterine transmission is rare and only happens in case of placental leakage[20-21].

Infants born to HBeAg positive mothers are likely to be infected and progress to chronicity, however, infants born to HBsAg positive mothers develop acute hepatitis and less likely to progress to chronicity[13]. In north India, HBeAg positivity was 7.8 per cent, and risk of perinatal transmission was 18.6 per cent from HBsAg positive mothers vs. 3 per cent among infants of HBsAg-negative mothers[22,23].

Effect of HBs and HBe antigen on pregnancy

HBV infection does certainly affect the outcome of pregnancy and influence spontaneous abortion, stillbirth, or prematurity. Increased frequencies of reproductive casualties were reported, in pregnant women with acute or chronic hepatitis B infection[24]. With HBV infection, the incidence of preterm birth observed was quite high around 21.9 vs 12.1 per cent in healthy controls[25].

The gestational diabetes and antepartum haemorrhage were also associated with chronic hepatitis B infection[26]. In a case-control study, HBeAg positivity was proved to be more important with high HBV DNA levels in transmission of HBV to infants[27]. HBeAg positivity indicates replicative form of HBV which may play a role in the immunotolerance *in utero* by crossing the placenta[28]. In HBV genotype C, HBeAg seroconversion is longer, which may be the reason for higher perinatal transmissions in this genotype[29]. Therefore, in prenatal screening of pregnant women, it is important to check the HBeAg status along with HBsAg.

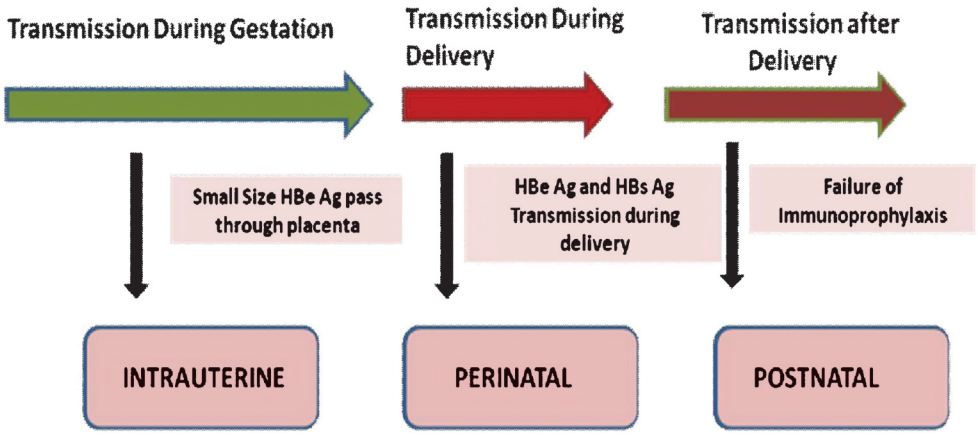

Fig. 1. Secretory proteins crossing the placenta and vertical transmission through during and after delivery.

Serovaccination of the newborn

There are chances of vertical transmission and resulting chronic hepatitis B infection in a newborn from a chronic hepatitis B infected mother with HBsAg positivity[30]. Therefore, vaccination against HBV has been implemented recently to prevent HBV infection predominantly acquired perinatally or in childhood[31]. In many countries, immunization programmes for HBV are implemented, despite this HBV prevalence is not decreasing[32]. This may be due to incomplete vaccination or inefficacy of the immunization programme. Screening for HBsAg is essential in all pregnant women. All infants born to mothers who are HBsAg positive must receive a serovaccination against this virus, by intramuscular injection of HBV vaccine and of hepatitis B immune globulin (H-BIG, 100 or 200 IU), in the first 12 hours of birth[9]. Despite improved health policies, there is no national hepatitis B immunization programme in India, thus chronic HBV infection still remains a considerable medical burden, affecting young adults.

HBV vaccine

HBV vaccines are directed against common antigenic epitopes of genotype A and D of HBV surface region, which provide sufficient cross-protection across genotypes to prevent infection[33]. Hepatitis B vaccination alone can prevent transmission in 80-95 per cent of cases, however, in case of HBeAg positivity and high viral load of mother, HBV immunoglobulin is preferrablly given along with HBV vaccination at different sites. Although HBV vaccination along with H-BIG has been reported effective in many studies and transmission rates can be reduced between 0 and 14 per cent[34], the recent data from India showed no significant effect of the combination of vaccination and H-BIG vs. HBV vaccination alone, especially when the viral load is very high during pregnancy[35].

In fact, in many other studies, standard passive-active immunoprophylaxis with hepatitis B immunoglobulin and the hepatitis B vaccine had a failure rate as high as 10 to 15 per cent[36] and these failures are associated with high maternal serum HBV DNA levels[37]. In some cases, vaccine failures are also associated with intrauterine infection in women during pregnancy[38]. Therefore, it is being considered that HBV vaccination alone or along with HBV immunoglobulin is not sufficient and may be prevented by nucleotide analogue therapy. As antiviral therapies are being used to prevent HIV transmission from mother to child, similar strategy could be beneficial in the case of HBV.

Use of antivirals during pregnancy: its safety and concern

Levels of HBV DNA and alamine transaminase (ALT) are highly variable during entire course of pregnancy. In a few cases, HBV DNA levels seemed to rise in the third trimester or in the post-partum period, otherwise for entire duration of pregnancy the levels of HBV DNA remain stable. There are limited data available on anti-viral treatment during pregnancy which show symptomatically or asymptomatically HBV infection clearance during subsequent pregnancies and postpartum[37,38].

Pregnant women with a low HBV viral load do not require immediate treatment, because due to the

passive immunization and active HBV vaccination of the newborn, chances of acquiring infection due to perinatal transmission are negligible. Treatment of the mother can, therefore, be postponed until after the birth. However, with high HBV viral load (>10^5 copies/ml in serum), strategy for treating with antivirals during the last trimester of pregnancy is being considered[39]. Antiviral therapy was also used in pregnant woman with acute exacerbation of hepatitis B, as this was quite effective in reducing possible HBV-associated hepatitis flares or reactivation and made a difference to maternal morbidity and mortality before hepatic decompensation[40,41]. However, vertical transmission has been reported even with the treatment of hepatitis B during the pregnancy and when there was an undetectable viral load at delivery[39].

In antivirals, lamivudine was the first drug which was used to diminish viral load and considered effective in the third trimester of pregnancy and resulted in reduced risk of chronic hepatitis B in the child[41-43]. Oral dose of 150 mg of lamivudine every day during the last month of pregnancy reduced serum HBV DNA concentration and normalised ALT levels till the time of delivery. In the lamivudine-treated group, only 12.5 per cent infants were tested positive for HBsAg in comparison to 28 per cent untreated historical control subjects. Therefore, lamivudine therapy was considered effective in reducing HBV transmission from highly viraemic mothers to their infants who received passive/ active immunization. Despite, the fact that lamivudine therapy leads to suppression of the HBV DNA to undetectable levels in the mother, there is a case report of a newborn with raised ALT levels and positive for HBV DNA at birth, followed by developing chronic hepatitis B virus infection[44].

Recently, telbivudine was evaluated for its efficacy and safety in the third trimester of pregnant women in one of the clinical trials and also compared with lamivudine[41,44]. Both antivirals, showed reduction in HBV DNA levels in mothers from log 8 to log 2. Newborns were given hepatitis B vaccination as well as immunoglobulin within 24 h of birth and completed vaccine schedule. After one year of birth, 18 per cent of children in lamivudine group showed HBsAg positivity, however, in telbivudine group only 2 per cent children showed HBsAg positivity. Therfore, telbevudine was considered to be better antiviral than lamivudine[41]. Use of antivirals from the first trimester showed more birth defects than their use in third trimester. Usage of recent antivirals in the first trimester, including emtricitabine, tenofovir, lamivudine, telbivudine, and adefovir showed more than 1.5 fold increase in overall birth defects[41].

Most of the antiviral data support lamivudine and tenofovir usage in the pregnancy than adefovir and entecavir, as safety of entecavir is questionable. The global recommendations are to use tenofovir, lamivudine, and telbivudine during pregnancy and substantial registry evidence positively supports the use of tenofovir, which is a potent inhibitor of HBV[44]. However, in the case of lamivudine or telbivudine antiviral therapy, genotypic resistance should be assessed during treatment[45].

Antiviral therapy is recommended to continue in post-partum period but the safety of anti-viral therapy during lactation period is a concern. Though HBsAg has been detected in the breast milk, but globally breast feeding has not been contraindicated in HBsAg positive mothers[46]. There are not many studies discussing the effects of antiviral therapy during lactation period[47,48], however, a study on lamivudine treated pregnant women showed that infant received only 2 per cent of recommended antiviral dose through breast milk and the tenofovir treated HIV group showed only 0.03 per cent release of recommended dose in breast milk[49].

Antiviral therapy might not prevent perinatal transmission of HBV infection in every newborn, therefore, use of antivirals during pregnancy need to be individualized and as the evaluation and management of abnormal liver tests in the pregnant women is challenging, importance of understanding case by case natural history of chronic HBV infection in the peri-partum period is extremely vital.

After birth, HBsAg positivity in children varies. In India, children below 15 yr have 1.3-12.7 per cent HBsAg positivity, whereas in other countries it ranges from 0-7.8 per cent[3-5]. Ultimately children after perenatal transmission with detectable HBV DNA levels are being treated with antivirals and interferon[50], however, the success rate and adverse effects need to be determined.

Role of human leukocyte antigens (HLA)

The implantation of a fertilized ovum followed by placental and foetal development can be compared to a transplanted graft having both maternal and paternal HLA molecules. The foetal derived placental trophoblasts ensure survival by avoiding rejection from the maternal immune system and evading infection.

The trophoblast cells lack expression of classical HLA class I and II molecules, express HLA-G, which is instrumental in preventing placental cell death by maternal NK cells in maternal deciduas[51].

Pregnancy supports HBV infection

The liver also plays a crucial role in the metabolism of different hormones, including estrogens and progesterone. The normal course of pregnancy is bound to have a number of physiological changes and these changes may affect the normal course of chronic hepatitis B infection in infected women[52-54]. During pregnancy, successful foetal development is necessary by eliciting poor immune response against foetal antigens. Therefore, weak immunity of the mother might allow HBV viral replication and increases the chances of perinatal transmission of HBV infection in children.

Sex hormones such as androgens, estrogens and progestrone can directly interact with the cells of the immune system, thus impacting the development of immune responses. The female sex hormones estrogen and progesterone have been implicated as playing a role in modulating the local immune system and altering cytokines during pregnancy.

Progesterone, a hormone associated with the maintenance of pregnancy, is immunosuppressive and decreases NK cell cytotoxicity, inhibits nitric oxide (NO) and tumour necrosis factor (TNF)-α production. Progesterone induced binding factor inhibits the activity of dendritic cells (DCs) that generate proinflammatory responses and favour the induction of tolerogenic DCs. It also controls the activity of natural killer (NK) cells and the differentiation of T cells into T helper cell type 2 (Th2) like clones (Fig. 2). Therefore, progesterone mediates the immunological effects, and induces the production of Th2 dominant cytokines like IL-3, IL-4, and IL-10[55] (Fig. 2). The Th2 phenotype induced by progesterone is a prerequisite for the maintenance of pregnancy, which is associated with the susceptibility and the existing disease exacerbation[56,57]. The anti-inflammatory properties of progesterone prevent the development of Th1 responses that could result in foetal abortion[58].

In contrast to progesterone, estrogen is considered a proinflammatory mediator. Estrogen has been shown to stimulate the production of the proinflammatory cytokine TNF[59,60], which is known to directly interact with the interferon (IFN)-γ promoter[61], and further has been shown to enhance antigen-specific CD4+ T cell responses[62]. The ability of estrogen to drive proinflammatory, Th1-associated immune responses induces higher concentrations of the proinflammatory cytokines, IL-6 and IFN-γ. Th1 responses are associated with protective immunity and favour disease resolution. Therefore, progesterone favours Th2 response which protects foetal development and estrogen leads to Th1 response, which favours HBV disease resolution.

Pregnancy being a relatively immunosuppressed state, some of the chronic hepatitis B infected mothers may develop hepatitis flare or fulminant hepatic failure (FHF) due to immune restoration during the peri-partum period[63-65]. Generally, after delivery a significant increase in liver disease activity is being observed. , Overt liver dysfunction was maximum, observed in 43 per cent of mothers who were HBeAg positive within the first post-partum month[66]. These robust immune responses have cleared HBeAg in 12.5 per cent of mothers during the first month of post-partum period[67].

HBV infection and T cell immunity

HBV is not cytopathogenic, still HBV infection carries a significant risk of developing severe liver diseases, including chronic hepatitis B (CHB), cirrhosis, and hepatocellular carcinoma (HCC). HBV specific T cells mediated host immune response plays an important role in viral clearance as well as viral persistence which leads to chronicity as well as HCC[67]. Different HBV genotypes have been found in different ethnicity and global locations. Mechanism of immune tolerance may be influenced by different HBV markers and it is observed that exposure of the immature immune system in uterus to transplacental HBeAg induces immunotolerance leading to low HBeAg sero-conversion rates in children[68,69].

The constant immunologic changes that occur during pregnancy appear to mostly occur locally, however, these changes alter the maternal immune system grossly during gestation. Increase in systemic inflammation also results in different complications related to pregnancy and delivery[70]. Infiltrations of virus- non specific inflammatory cells in the liver also participate actively in HBV-associated liver pathogenesis. The downregulation of Th1 cytokines such as TNF-α and IFN-γ was found in early pregnancy[71]. Therefore, a delicate balance of cytokine effects is necessary because overproduction of IFN- γ during the first trimester of human pregnancy leads to

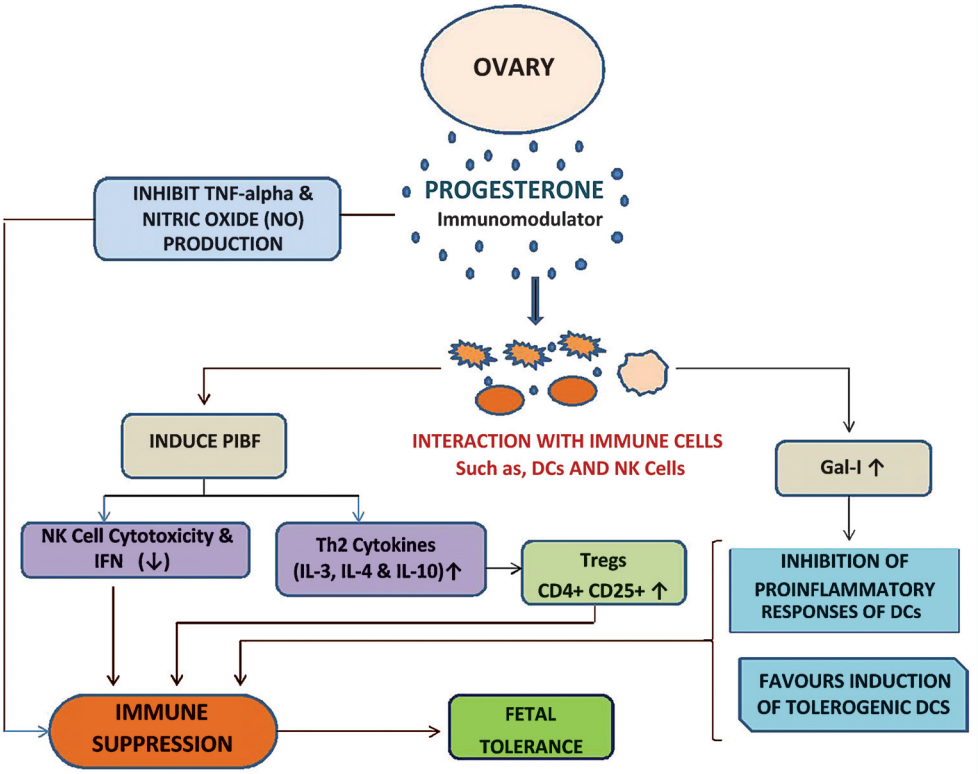

Fig. 2. Release of hormones induce immune suppression and fetal tolerance during pregnancy.

foetal rejection. As pregnancy progresses, the most compatible tilt occurs towards the Th2-type cytokine profile such as IL-4, IL-5, IL-9, and IL-25 and a T repressor type 1 (Tr1) or T-helper type 3 (Th3) such as TGF-β and IL-10 and away from Th1-type cytokines[72,73]. When Th2-like activity is increased during pregnancy, the elevated immune suppressive CD4+ CD25+ Treg cells suppress both Th1-like and Th2-like immunity against paternal/foetal alloantigens and checks IFN-γ reactions during pregnancy[74,75] (Fig. 3).

These alterations in the immune system during pregnancy induces a surge in cell mediated immunity cytokines such as TNF-α and IFN-γ, which could play a significant role in clearing the underlying HBV infection in HBsAg positive mothers during pregnancy and thereby help to tailor therapy after delivery to enhance the clearance of HBV[72].

During pregnancy, the regulatory T cells suppress Th1 response and induce Th2 type immunity, contributing to an inadequate immune response against the virus with rise in viral load and decline in ALT levels[76]. A retrospective study has observed that in nearly 50 per cent of HBsAg positive pregnant mothers, after delivery there is increase in ALT when the immune status recovers which also influences with more maternal morbidity[77,78].

The precise roles of the hormones and cytokines alterations happening during "immunological orchestra" of pregnancy have not been clearly studied in HBsAg positive mothers. A study focused on the immune cells in HBsAg positive and negative mothers and their newborns. This study revealed that there was no difference in the frequencies or percentage of CD4, CD8 T cells in HBsAg+ve mothers and their newborns compared to HBsAg-ve and healthy mothers[79]. Then question is how the HBsAg+ve newborns develop chronic liver disease at a later stage of life. Shrivastava *et al*[76] have analysed the immune cells functionality. When functional assays were performed for CD8 T cells, functionality was compromised at birth in HBsAg+ve newborns. Along with non functional CD8 T cells, presence of higher levels of immunosuppressive regulatory T cells was also observed, which probably was instrumental in establishing chronic infection in neonates (Fig. 4).

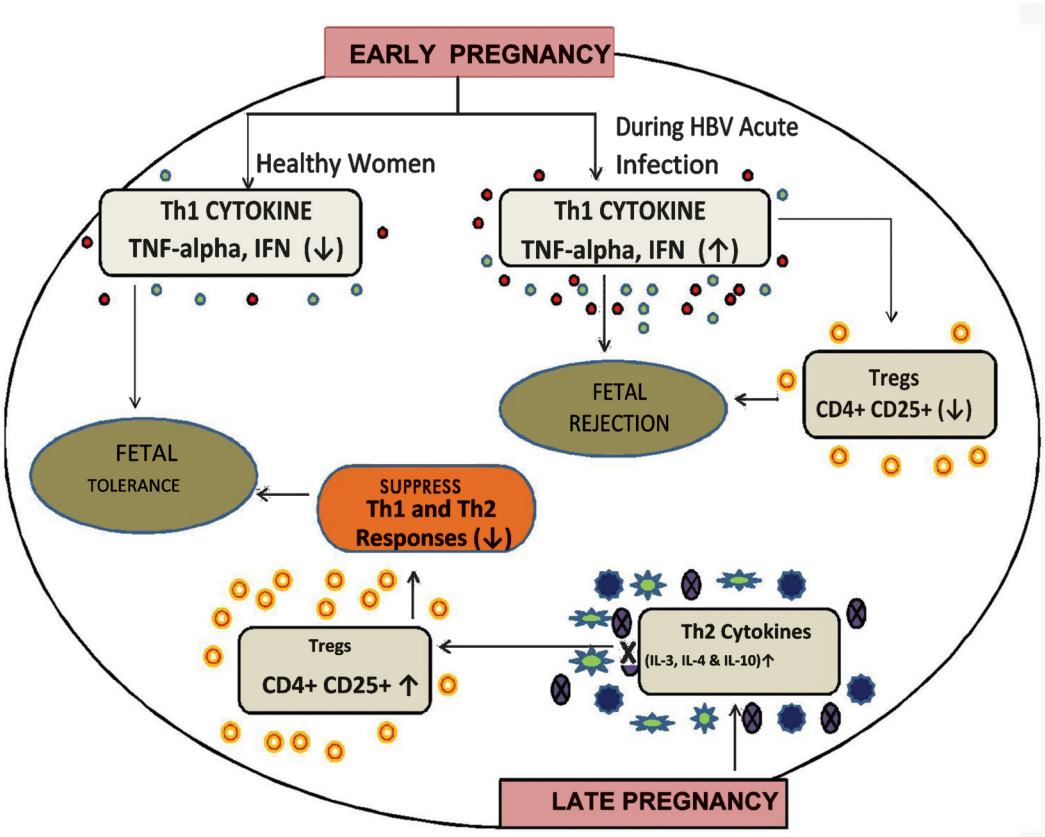

Fig. 3. Role of regulatory T-cells in regulating Th1/Th2 response leading to fetal rejection of fetal tolerance.

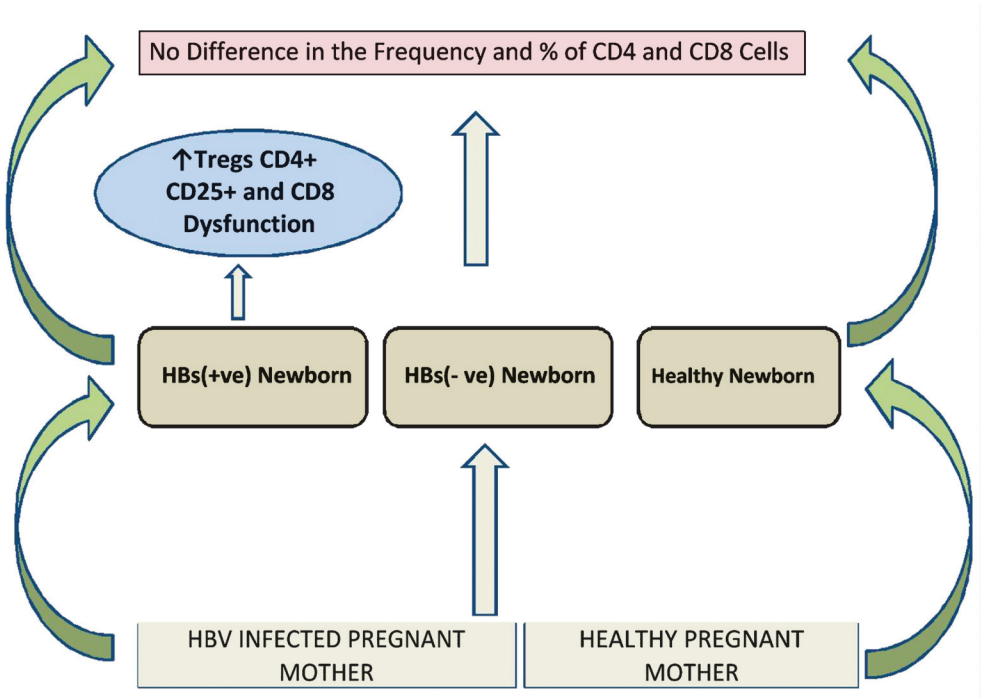

Fig. 4. Immune response in HBV infected pregnant mothers and its outcomes.

It is known that the regulatory T cells suppress the proliferation, cytokine-production (IFN-γ, IL-2), cytolytic activity of naïve and antigen specific CD4 and CD8 T cells, functions of antigen presenting cells (APCs) and B cells through secretion of anti-inflammatory cytokines such as IL-10 or TGF-β[80]. These specialized regulatory T cells could possibly facilitate immune tolerant environment of newborns preventing the development of mature protective immune response and also support Medawar hypothesis; "Antigen encountered during fetal life induces a state of acquired immunological tolerance and mammals exposed to foreign homologous tissue cells during fetal life never react immunologically, or react to a limited degree only"[81]. Initially immune tolerance was also considered due to deletion or inactivation of T cells[82] and, therefore, neonatal period has been viewed as a 'window of opportunity' for inducing tolerance to specific antigen[83]. At the time of birth with HBV infection, T cell tolerance to HBV-Ag may probably leave the newborn as a chronic carrier. Therefore, one aspect of chronic HBV infection in neonates is linked with strong presence of Tregs (regulatory T cells) and another aspect is why CD8 T cells capacity to produce IFN-γ and CD107a cytotoxicity decreased. In the literature, TCR signaling defects were significantly linked with functionality of T cells, in many diseases, downregulated expression of TCRζ chain on CD8 T cells have been observed, TCRζ chain defects eventually lead to decreased TCRζ expression positively correlated with decreased CD8 T cell dysfunction[84,85]. Decreased TCRζ expression could be due to persistent intrauterine exposure of the viral antigens early in embryonic development leading to immune tolerance to HBV antigens in the HBsAg+ve newborns.

Shrivastava *et al*[78] also observed that HBsAg+ve newborns had lower expression of chemokine receptors CCR1, CCR3 and CCR5 on CD4+ and CD8+T cells at birth. In many chronic diseases, presence of chemokines and toll like receptor was also not observed on leuckocytes, which may eventually contribute to infection persistence[86,87].

Till date, there are only phenotypic studies which suggested possible defects in cell setting and mechanisms, in which diminished expression of TCRζ chain associated with CD8 T cell dysfunction in HBsAg+ve newborns was observed compared to HBsAg-ve and healthy uninfected newborns[78]. These observations add a new perspective to our growing understanding of the key mechanisms by which HBV could promote T cell dysfunction related to the loss of TCRζ chain expression. Additionally, we also speculate the role of T regs in the setting of immune tolerance.

The predisposition of newborns to infections has been attributed to defects in both the humoral and cellular arms of the adaptive immune responses. Infants with a diminished pool of B and T lymphocytes show lower serum complement levels and impaired antigen presenting ability of DCs and eventually reduced immunoglobulin production of B cells even after regular vaccination[88-90]. Development of mature B cells from naïve population is very important and HBV viral infection in adults are characterized by increased number of activated and exhausted B cells, increased levels of short lived plasma B cells or immature transitional B cells or decreased memory B cell response[91-93].

There was a general tendency of higher levels of transitional B cells and lower memory B cells in HBsAg+ve newborns as compared to HBsAg-ve newborns immediately at birth. After 12 months post HBV vaccination, immature transitional B cells were declined and there was a rise in memory B cell with increased frequencies of CD69+ and CCR5+ activated memory B cell subpopulation[93]. The improved B cell responses suggest that HBV vaccination is somehow beneficial for improving the overall immune competency in HBsAg +ve newborns, but to understand the precise mechanism of disease progression further, larger cohort and long-term studies are needed.

In summary, higher levels of immunosuppressive T regulatory cells and CD8+ T cell dysfunction in HBsAg +ve newborns are suggestive of already established chronic and immune tolerant state of HBV infection at birth during vertical transmission. However, HBV vaccination may have benefits in restoring acquired immunity and better production of HBV specific antibodies.

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