Published in final edited form as:

Pediatr Infect Dis J. 2013 July ; 32(7): 792–794. doi:10.1097/INF.0b013e31828df344.

Decline in Immature Transitional B Cells Following Hepatitis B Vaccination in Hepatitis B Positive Newborns

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Abstract

Humoral immune responses are protective against HBV infection. We characterized B cell phenotypic changes in infants of HBsAg+ve mothers compared with normal and HBsAg–ve infants at birth and one year after HBV immunization. HBsAg+ve infants had higher immature transitional B-cells at birth, which normalized a year after immunization. Immature B-cell response to neonatal HBV exposure is associated with maternal-child transmission of HBV.

Keywords

Transitional B cells; HBV positive newborns; HBV vaccine

INTRODUCTION

Hepatitis B virus (HBV) infection is the most common cause of liver disease. More than 360 million individuals are infected with HBV, which is responsible for 1 million deaths per year, largely linked to serious sequelae such as cirrhosis and liver cancer. [1]. Transmission of hepatitis B virus (HBV) from mother to infant represents one of the most efficient modes of HBV infection. Infants born to HBV positive mothers have a 70%–90% chance of acquiring HBV infection, and 85%–90% of infected infants will become chronic HBV carriers [2,3]. Infection in infancy or early childhood is associated with a high rate of chronicity (25–90%) [4]. Hepatitis B immunoglobulin (HBIG) in combination with three-doses of hepatitis B vaccine is given to the infant to prevent mother-to-child transmission at or around birth [5]. The titer of anti-HBs decreases with age, therefore, active vaccination is required to sustain sufficient levels of anti-HBs to protect young infants from HBV infection. Recent studies showed that children born to HBsAg+ve mothers have a high risk of becoming HBV carriers even after successful administration of immunoprophylaxis [6].

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Financial Disclosure: The authors have no financial relationships relevant to this article to disclose

Conflict of Interest: The authors have no conflicts of interest to disclose

The increased propensity of newborns to become carriers has been largely attributed to defects in both the humoral and cellular arms of the adaptive immune responses [7].

In this regard, abnormalities in B cells such as, increase number of activated and exhausted B cells, increase levels of short lived plasma B cells or immature transitional B cells associated with CD4 T cell lymphopenia or decreased memory B cell response have been demonstrated to be associated with chronic viral infections including chronic HBV infection [8,9]. In order to delineate the role of B cells in newborn infants with vertically acquired HBV infection, we sought to characterize the frequency of various B cell subsets frequency in vertically infected HBsAg+ve newborns compared with HBsAg–ve and healthy newborns at baseline and post HBV vaccination.

SUBJECTS AND METHODS

Subjects

From March 2008 to April 2010, 3156 pregnant women receiving regular antenatal examinations at Lady Hardinge Hospital, New Delhi were screened for hepatitis B surface antigen (HBsAg). A total of 30 mothers positive for HBsAg were recruited in the study. Mothers co-infected with HCV, HIV, premature babies and babies with inborn errors of metabolism were excluded from the study. Of the 30, peripheral and cord blood samples of 22 newborns born to HBsAg+ve mothers who delivered at Lady Hardinge Hospital were collected. The 22 newborns were divided into two groups on the basis of HBV DNA and HBsAg positivity: Group I: (n=12), Newborns were HBsAg+ve, HBV DNA+ve, Group II: (n=10), Newborns were HBsAg–ve and HBV DNA–ve. Newborns born to healthy (HBsAg –ve) mothers were recruited as healthy newborns (n=15). The healthy mothers (n=15) with no previous history or current evidence of liver disease, with normal serum values for transaminases were recruited as controls. In accordance with national recommendations, every newborn received the first dose of hepatitis B vaccine (Energix; GlaxoSmithKline Beecham) within 24 hr of birth followed by doses at 1 and 6 months of age. This study protocol was approved by the Institutional Ethics Committee of the Lady Hardinge Medical College and the Maulana Azad Medical College, University of Delhi, India and written informed consent was obtained from all participant mothers.

Sample Collection

1–3 ml of peripheral blood was collected by peripheral vein puncture from all newborns at birth, before the administration of hepatitis B vaccine and 3–5 ml of peripheral blood was collected at 12 months after the completion of all the three doses of vaccine.

Serologic HBV markers and HBV DNA

The serologic status of every subject with regard to HBsAg was determined by enzyme-linked immunoassays (M/s Ranbaxy Diagnostics, India) in their peripheral blood. All the subjects were evaluated for HBV DNA titer using a quantitative RT-PCR method (Roche, Taqman 48) as per the manufacturer's recommended standard protocols.

Isolation of Peripheral blood mononuclear cells (PBMCs) and Flow Cytometric Analysis

Mononuclear cells were isolated by using Ficoll-Hypaque (Sigma-Aldrich, USA) density gradient centrifugation method. B cells were identified based on CD19 expression and classified by multiparameter flow cytometry based on the expression of defined surface markers as follows: immature transitional (CD19+CD38hiCD24hi), naïve (CD19+CD27–) and resting memory (CD19+CD27+) B cells. PBMCs were taken and surface stained using following antibodies conjugated with different fluorochromes anti-CD19 PE-CY7, anti-CD27 APC, anti-CD24 PE, CD38 FITC (BD-Biosciences) for analysis of the distribution of

different B cell subsets. Cell surface staining was done with fluorochrome conjugated antibodies as per the manufacturer's recommended standard protocol. Cells were acquired on Beckman Coulter Cyan ADP flow cytometer and analysis was done by using Summit v4.3 software.

Statistical Analysis

Frequencies of B cell subpopulations were calculated using Summit v 4.3 software. Differences in frequencies of different B cell subsets between HBsAg+ve, HBsAg-ve and HC newborns nonparametric Mann-Whitney-U test or one-way ANOVA (Kruskal Wallis test with Dunn's multiple comparison tests). Post vaccination immune response was studied by comparing the frequencies at different time points using non parametric Wilcoxon Signed Ranks test. P values < 0.05 were considered to be statistically significant. Data was analyzed using the Graph Pad Prism.

RESULTS

Decline in Immature Transitional B cells and increased memory B cells following vaccination in HBsAg+ve positive newborns

To characterize B cells in newborns in the setting of chronic HBV infection, we compared peripheral blood samples of newborns with HBsAg+ve, HBsAg-ve from HBsAg+ve mothers and healthy newborns from HBsAg-ve mothers. All groups showed comparable levels of total B cells, naïve B cells ($P = 0.05$). However, at birth, HBsAg+ve newborns showed significantly higher proportion of transitional B cells and lower proportion of resting memory B cells as compared with HBsAg-ve infants and HC ($P = 0.05$) [Fig.1, SDC Fig.1]. Further, to investigate the effect of vaccine on B cell immune response in the newborns during HBV infection, we aimed to compare pre and post vaccination B cell response in the HBsAg+ve and HBsAg-ve newborns from HBsAg+ve mothers. At 12 months post-vaccination HBsAg+ve newborns showed a significant decline in transitional B cell population (5.01% Vs. 1.09%; $P=0.001$) and increase in resting memory B cells as compared with at birth before vaccination [Fig.1]. After vaccination, at 12 months, no significant difference was observed in transitional B cell population (1.59 % Vs. 2.2% $P=NS$) among HBsAg+ve and HBsAg-ve newborns.

DISCUSSION

In this study, we have demonstrated higher levels of immature transitional B cells in HBsAg+ve newborns as compared with HBsAg-ve infants born to HBsAg+ve positive mothers at birth before vaccination. However, the frequency of immature transitional B cells declined at 12 months post vaccination in HBsAg+ve newborns, while no changes were observed in HBsAg-ve and HC newborns.

Infants born to HBsAg+ve mothers must receive universal immunization; both HBV vaccination (active immunization) and hepatitis B immune globulin (passive immunization) within the first 12 hours of life. However, no studies have characterized completely the effectiveness of HBV vaccination on B cells from infants receiving this vaccine. Evaluating changes in humoral immunity induced by HBV vaccine is critical in our understanding of the role of active immunization for HBV exposed infants to prevent development of chronic HBV infection. Such evaluations will provide valuable insights into the mechanisms of

SDC Fig.1: Transitional B cells in HBsAg+ve, HBsAg-ve and healthy newborns. FACS dot plot showing higher immature transitional B cells in HBsAg+ve newborns compared with HBsAg-ve and healthy newborns. Representative FACS dot plot showing the gating strategy used for analysis of percentage of immature transitional (CD19+CD38hiCD24hi) B cells.

developing protective immunity against HBV in this vulnerable population. In the present study, we demonstrate the long-term benefit of less expensive active immunization by administering 3-dose series of Hepatitis B vaccine to the HBsAg+ve newborns born to HBsAg+ve positive mothers.

We showed higher levels of circulating immature transitional B cells in HBsAg+ve newborns as compared to HBsAg–ve newborns born to HBsAg+ve positive mothers at birth before vaccination. The expansion of transitional B cells that are immature, resting and relatively resistant to activation and proliferation stimuli has also been shown in chronic HBV and HIV infected individuals with advanced disease [9,10]. Thus, at birth predominance of immature transitional B cells may be due to the effect of in utero HBV infection which could explain the vulnerability of these newborns to the progression of chronic HBV infection. Further our data showed post-vaccination decline in transitional B cell population. Following vaccination marked decline in the transitional B cell population in HBsAg+ve may implicate the normalization of the B cell phenotype. Conceivably, this may be due to an indirect effect of vaccination on B cells leading to normalization as the vaccine itself does not alter B cell phenotype in HBsAg–ve and HC newborns. The improved B cell responses suggest that HBV vaccination is somehow beneficial for improving the overall immune competency in HBsAg+ve infants but to understand the precise mechanism of disease progression further larger cohort and long –term population based detailed analysis is needed.

A potential weakness of our study was the unavailability of non-vaccinated HBsAg+ve newborns to compare with vaccinated HBsAg+ve newborn. However, inclusion of this rare group was not ethically feasible because of the standard of care of providing mandatory HBV vaccination program of Government of India and also to avoid any risk to the newborns babies. Functional characterization of B cells would have been ideal however, this was limited due the scarcity of samples obtainable for newborn infants.

Acknowledgments

We express our special thanks to the parents of newborns who enthusiastically participated in the study.

Funding Sources: This study was supported by the grant from Department of Biotechnology (sanction letter no. BT/PR7647/MED/14/1053/2006) and first author was awarded with Senior Research fellowship for 3/1/2/26/2010-RHN conducting this work from Reproductive Health and Nutrition Department, Indian Council of Medical Research, India.

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Fig. 1. Decline in Immature Transitional B cells following vaccination in HBsAg+ve positive newborns

At birth before vaccination, HBsAg+ve newborns showed significantly higher proportion of transitional B cells as compared to HBsAg-ve newborns and HC ($P < 0.05$) whereas all the groups showed comparable levels of circulating total, naive and resting memory B cells ($P > 0.05$). At 12 months post vaccination, a decline in transitional B cell population was observed in HBsAg+ve newborns. Representative bar graphs showing the differences in the frequencies of B cell subsets.