Chapter 1: Introduction

Hepatitis B virus (HBV) infection is a challenging health problem, affecting an estimated 2 billion people worldwide [Ganem D et.al, 2004]. Globally, there are more than 350 million subjects with chronic HBV infection who are at high risk of developing end-stage cirrhosis and hepatocellular carcinoma [Zanetti AR et.al, 2008]. In India, 1-4% of individuals are chronic carriers of Hepatitis B Virus (HBV). Infection with HBV may occur perinatally (vertical transmission), during early childhood (the so-called horizontal spread), through sexual contact or nosocomially. Our group showed that the prevalence of HBsAg positivity among asymptomatic pregnant women in North India is 1.1% with 71% having high HBV DNA levels and having high risk of transmitting infection to their newborns [Pande C and Sarin S.K. et.al, 2011]. Infection in immunocompetent adults results in self limiting illness with high spontaneous viral clearance and only 5-10% rates of chronicity while vertical transmission from mother to newborn leads to chronicity of infection in 90% of cases [McMahon, B.J. et al., 1985, Chang M.H., 2000]. In India and other Asian countries, majority of HBV transmission occurs by vertical transmission from an infected HBV positive mother to the newborn intrapartum or antenatally.

The human fetus and neonates are unusually susceptible to infection with intracellular pathogens resistance to which appears to be mediated by innate and adaptive immune responses. Neonatal life is characterized by heightened sensitivity to infectious agents. Many *in vivo* and *in vitro* studies have described immaturity, deficiencies or immune deviations among T cells, B cells and antigen presenting cells in the newborns [Adkins B et.al. 2004]. Viral clearance and disease pathogenesis are largely mediated by the adaptive immune response in HBV infection

[Zanetti AR et.al, 2008]. HBV persistence is characterized by it either not induce a response or it must overwhelm, evade or counteract it.

Interestingly, HBV "evades" the innate immune response by simply not inducing it, acting as a stealth virus in this regard [Kuss, I. et.al. 2002]. Several viral proteins have been shown to regulate the adaptive immune response to HBV suggesting that HBV may employ active evasion strategies targeting the adaptive immune response. Indeed, it has been shown that antiviral treatment can overcome CD8+ T cell hypo-responsiveness in chronic HBV infection, suggesting that the T cells are present in these subjects but suppressed. Importantly, a recent study suggests induction of an effective HBV specific CD8+ T cell response is dependent on early CD4+ T cell priming which might be regulated by the size of the viral inoculums. In adults clearance of HBV infection is mediated by a vigorous, polyclonal and virus specific CD4 and CD8 antiviral T cell responses while defective CD8 T cells lead to persistent HBV infection [Thimme R. et al. 2003, Bertoletti, A. et.al, 2000].

Very little is known about the persistence of HBV infection in newborns and its interaction with the immune cells after maternal transmission of infection. It is often suggested that HBV is transmitted at birth, perinatally [Maynard JE et.al. 1988, Ghendon Y. et.al. 1987]. The contrary view is that the transmission occurs in utero leading to an immune tolerant state [Pande C et.al 2008]. A study of immune response to HBV infection could help in enhancing our understanding about the mechanisms of HBV transmission to the newborn. Other than naïve and memory CD4/CD8 T cells, regulatory T cells showed the antigen specific or nonspecific suppressive abilities and reduction in IFN-γ, IL-2cytokine-production (I) during persistent HBV infection in adults [TrehanPati, N. et al. 2009, Stoop, J.N. et al., 2005, Franzese, O. et al. 2005].

Therefore, we hypothesized that in newborns these immunosuppressive T regulatory cells in response to chronic exposure to the HBV may play a crucial role in neonatal tolerance contributing to defective T cell mediated adaptive immune responses. In this study, we have compared the cellular immune profiles of CD4, CD8, naive, and specifically FoxP3 expressing regulatory T cells among HBV positive, negative and healthy newborns.

Defects in the phenotype and functions in CD8 T cell population are associated with selective down regulation of CD3ζ chain of T cell Receptor (TCR) in chronic inflammation and high load antigenic persistence like autoimmune disorders, malignancy and chronic viral, and bacterial infections including chronic hepatitis B infection (ref). TCR normally functions in both antigen recognition and signal transduction, which are essential and initial steps of antigen-specific immune responses. TCR mediated signaling events are crucial for the induction of optimal and efficient immune responses against invading pathogens and for the elimination of infected cells. Of the TCR subunits, the CD3ζ-chain plays a rate limiting role in receptor assembly, expression, signaling and determines the T cell functions [Kuss I. et.al. 2002, Baniyash M. et.al 2004, Das A. et al. 2008, Schule J. et.al 2002, Dworacki, G. et al. 2001, Torelli G. F. et al. 2003, Chen, X. et al. 2000, Schmielau J. et.al 2001].

To investigate the possible underlying mechanism of chronicity in newborns we hypothesized that defects in the TCR related to the loss of expression of CD3ζ could lead to T cell dysfunction in HBV positive newborns. Therefore, in the present study, possible association of phenotypic (CD3ζ expression) and functional profiles of CD8 T cells (IFN γ production and CD107a cytotoxicity assay) at birth in HBV positive and negative newborns and compared with healthy newborns.

Despite advances in antiviral therapy, only a minority of patients with chronic hepatitis B will have a sustained response. Thus, primary prevention by vaccination to increase herd immunity remains the main thrust in the control of hepatitis B virus (HBV) infection. A safe and effective vaccine against the hepatitis B virus surface antigen has been available since 1982 [Lavanchy 2004]. Hepatitis B vaccine is effective not only in preventing HBV infection but also in preventing the sequelae of chronic HBV infection. Hepatitis B vaccine is recommended for all neonates of HBsAg positive mothers, and in many countries is also recommended for neonates of HBsAg negative mothers. Vaccination of neonates of HBsAg positive mothers is the most important step toward the eradication of chronic HBV infection.

Thus, to determine the role of HBV vaccine in immune modulation in the newborns we have studied the differences in the cellular immune profiles of CD4, CD8, naive, and FoxP3 expressing regulatory T cells pre and post vaccination. Differences in the Chemokine and Toll like receptor expression on T cells pre and post vaccination in peripheral blood of HBV positive and healthy newborns have also been examined. Importantly we investigated changes in immune profile following vaccination for HBV in the newborns. This information could possibly help to determine the reasons for failure of HBV vaccination in HBV infected newborns.