Chapter 6: Summary and Conclusions

This thesis describes the potential immune mechanisms of maternal fetal transmission of HBV associated with the underlying defects in the adaptive immune responses in the newborns vertically infected from their mothers (HBsAg positive newborns) in contrast to HBsAg negative newborns born to HBsAg positive mothers who cleared the virus. This section summarizes all the results and the respective concluding facts of the thesis. Lastly, future perspectives and limitations are discussed.

Firstly, comprehensive analysis of immune system of HBV infected and non infected newborns revealed that HBsAg positive newborns have lower proportion of CD3+CD4+ and CD3+CD8+ T cells and CD3+CD4+T cells were enriched in CD45RO+memory rather than CD45RA+ naive phenotype. Moreover, at birth in the HBsAg positive newborns, significantly higher FOXP3+ regulatory T cells were present compared to HBsAg negative and healthy newborns which may facilitate the immune tolerant environment and prevent the development of mature protective immune response in them.

Our data suggest that at birth, HBsAg positive newborns have lower proportion of Chemokine and Toll like receptors expressing CD3+CD4+ and CD3+CD8+T cells specifically CCR1, CCR3, CCR9 and TLR2, TLR4, TLR9 expression were significantly down regulated. Depressed Chemokine and Toll like receptor expression on T cells may suggest defective adaptive immune responses in the newborns with vertically transmitted HBV. In contrast, HBsAg negative newborns have higher expression of Chemokine and Toll like receptor expression which correlated with the viral clearance in them.

Further, we have observed significantly down regulated expression of CD3ζ chain on CD3+CD8+T cells in HBsAg positive newborns defects in TCR signaling. Down regulation of CD3ζ chain directly correlated with decreased IFN gamma production and decreased cytotoxicity (measured by CD107a expression) of CD3+CD8 T cells. This functional skewing of CD8 T cells could be related to the persistent intrauterine exposure of the viral antigens early in embryonic development leading to immune tolerance to HBV antigens in the infected newborns.

HBsAg negative newborns have higher functional CD8 T cells, lower T regulatory cells and higher Chemokine and Toll like receptor expression directly associated with viral clearance and conversely higher Tregs , lower functional CD8 T cells, lower expression of Chemokine and Toll like receptors indicates an ongoing status of established chronic HBV infection and immune tolerant state. HBV vaccination partially restores depressed adaptive immunity against HBV in HBsAg positive newborn through increased expression of CCRs and TLRs whereas sustained expression of T-regulatory cells may play a significant role in the development of chronic HBV infection in these newborns. HBV vaccination is able to only partially restore the host immune response against the infection.

Thus, the concluding facts in the study are, HBsAg positive newborns at birth, display inherent defects in T Cell Receptor related to decreased expression of CD3ζ leading to dysregulated CD8 T cells functions, along with higher T regulatory cells and lower Chemokine and Toll like receptor expression. These observations indicate an ongoing status of established chronic and immune tolerant state in HBsAg positive newborns born to HBsAg positive mothers while in contrast the HBsAg negative newborns born to HBsAg positive mothers have lower T regs, higher functional CD8 T cells and increased expression of Chemokine receptors and toll like receptors who cleared the virus and did not acquire

the infection from their mother. These novel observations add a new perspective to our growing understanding of the mechanisms by which HBV could promote T cell dysfunction related to the loss of CD3ζ chain expression, in addition higher levels of T-regulatory cells support immune tolerance in newborns contributing to the high rate of chronicity in newborns with vertically transmitted HBV infection from their mothers. HBsAg negative newborns born to HBsAg positive mothers New immunomodulatory approaches could be developed to design new vaccination strategies in HBV positive newborns based on these findings.

In addition, our findings suggest the presence of higher levels of transitional B cells (CD19+CD24hiCD38hi) and lower memory B cells in the HBsAg positive newborns compared to the non infected HBsAg negative newborns. Post vaccination decline in immunosuppressive transitional B cells along with elevated memory B cell response in HBsAg positive newborns implicates the beneficial role of vaccine in modulating B cell immunity against HBV. Moreover, after complete vaccination, expansion of CD69+ and CCR5+ activated memory B cells in HBsAg positive newborns might be associated with development of protective B cell response against the virus. These improved B cell responses suggest that HBV vaccination is somehow beneficial for preserving overall immune competency in HBsAg positive newborns but to understand the precise mechanism of disease progression in newborns further larger cohort and long –term population based detailed analysis are needed.

Conclusions

At Birth (Pre-vaccination)

HBsAg positive newborns have:

- Higher T regulatory cells and lower Chemokine and Toll like Receptors expression, indicative of ongoing status of established chronic HBV state.
- Significantly down regulated expression of CD3ζ on CD8+T cells resulted in phenotypically and functionally skewed T cell phenotype and functions.
- Functional impairment observed in the IFN gamma production capacity of CD8 T cells related to persistent exposure of the virus *in utero* in HBsAg positive newborns and attributable to loss of CD3ζ expression on CD8 T cells.
- Higher levels of transitional B cells (CD19+CD24hiCD38hi) and lower memory B cell response in the HBsAg positive newborns compared to the HBsAg negative newborns

Post Vaccination

- HBV vaccination partially facilitates the host immune response by partial restoration of depressed adaptive immunity, through increased expression of Chemokine and Toll like Receptors on T cells.
- Persistently higher levels of T regulatory cells could be the possible underlying mechanism of existing immune tolerant environment of the newborns resulting in chronic HBV infection.
- Post vaccination decline in immunosuppressive transitional B cells along with elevated memory B cell response in infected newborns implicates the beneficial role of vaccine in modulating B cell immunity against HBV

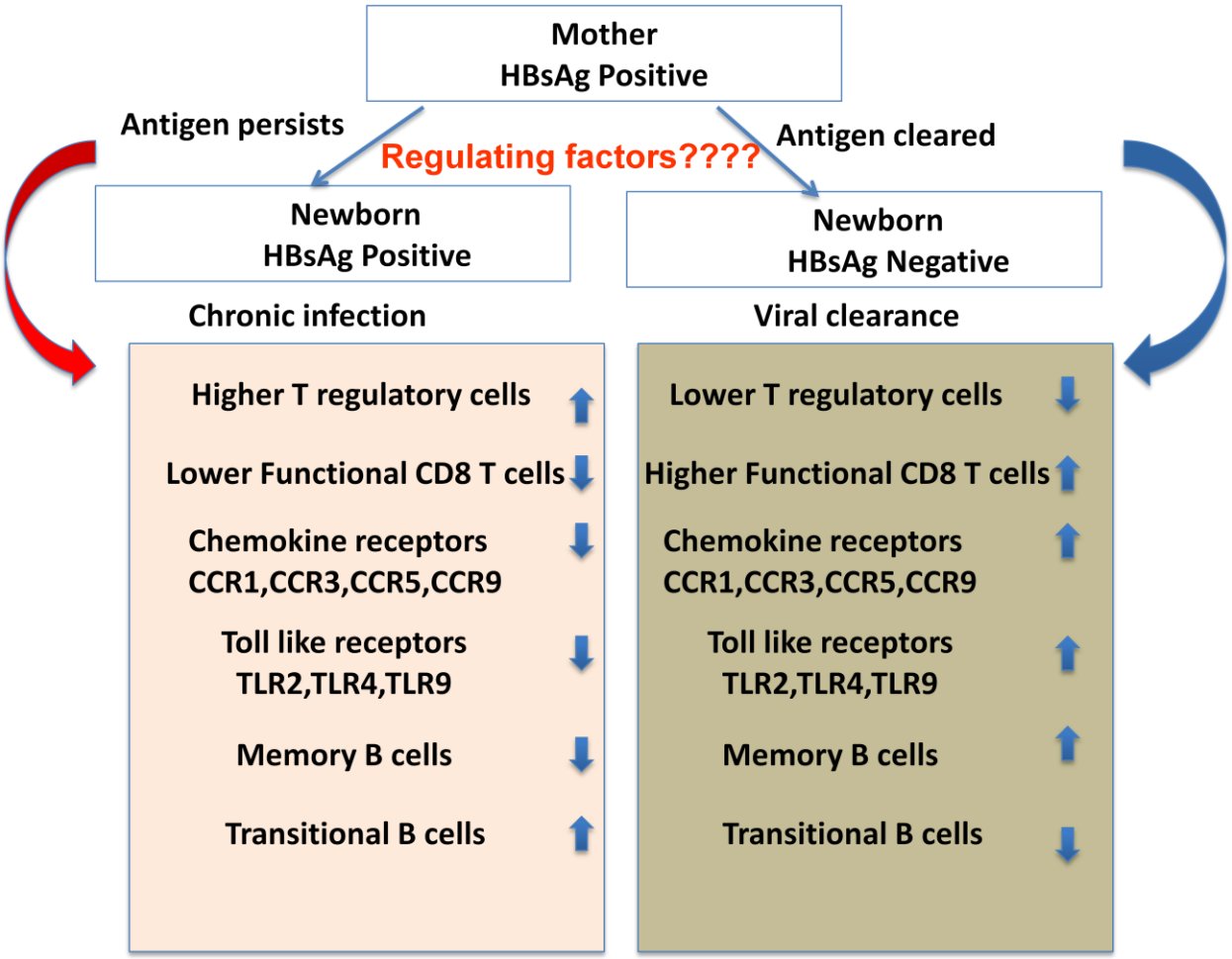

Fig.6.1 Conclusion of the study

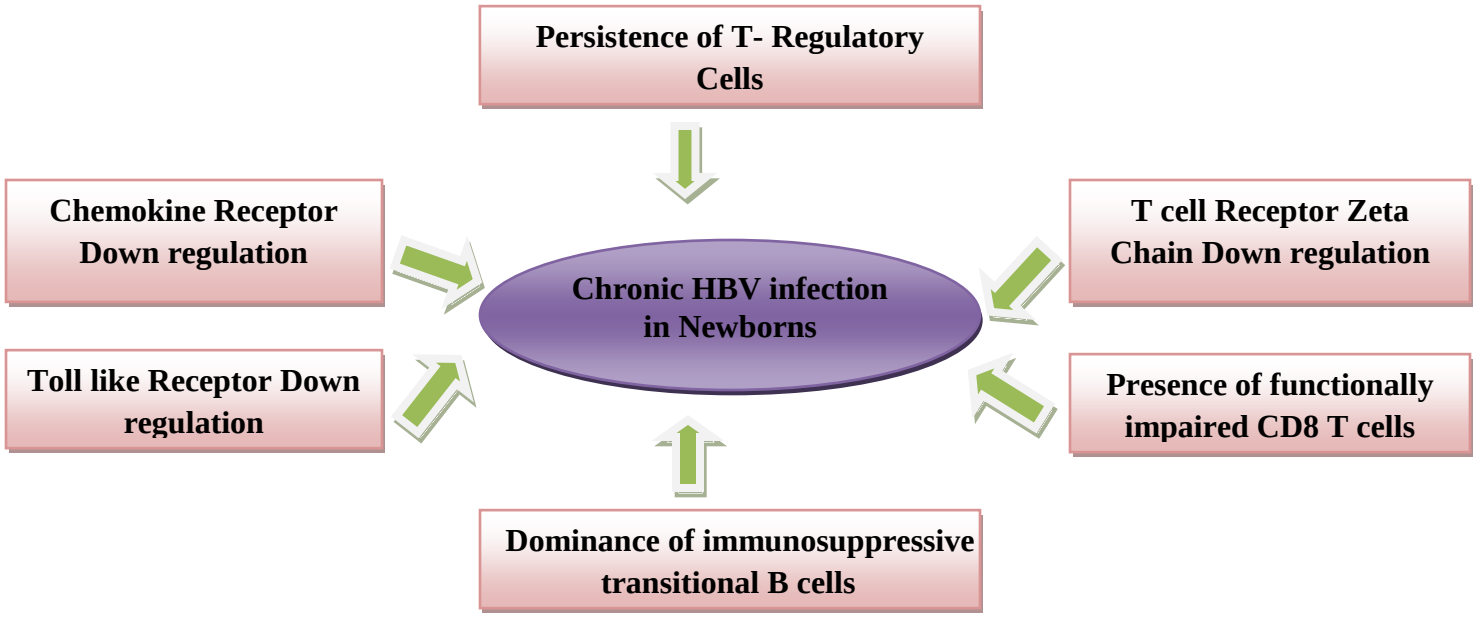

Fig. 6.2 Schematic model: Possible immune mechanisms leading to chronic HBV infection in newborns

Limitations of the study or Future work

- To functionally characterize the role of the increased T regulatory cells, and their antigen-specific and non-specific regulatory potential that has not been analyzed.

- Virus specific responses need to be examined.

- Study is quite stimulating and important as very few studies are done addressing this hypothesis but the results are somewhat preliminary and thus need to be analyzed in depth.