6. Summary

T cell dependent B cell responses depend on an intricate interplay of dendritic cells, CD4 T cells and B cells within the secondary lymphoid organs. CD4 T cells essentially mediate directing cognate responses derived from dendritic cells to B cell in the form of B cell help. This enables activated B cells to differentiate into antibody producing plasma cells.

Recirculating Naïve T cells get activated upon recognizing the antigen on the surface of dendritic cells and transiently upregulate chemokine receptor CXCR5. Expression of CXCR5 enables these cells to respond to CXCL13 and relocate to B cell follicles for cognate interactions with B cells. Activated CD4 T cells and B cells recognizing the same antigen interact at the border between T cell zone and B cell follicle. This results in the formation of germinal centers, which supports B cell affinity maturation, isotype switching and formation of plasma cells. CXCR5 expressing CD4 T cells in germinal centers are known as follicular B helper T (T_{FH}) cells, which were earlier thought to represent T helper 2 (Th2) cells. Present study aims to identify the actual follicular B helper T cells by investigating their functional and molecular aspects.

Approximately 60% of the CD4 T cells express CXCR5 in tonsils, which is a representative secondary lymphoid organ. These cells also express classical memory marker CD45RO and varying levels of costimulatory molecule ICOS. A subset of these cells also expresses CD57. Based on the co-expression of CXCR5 and ICOS, CD4 T cells from tonsils were subdivided into $CXCR5^{-}ICOS^{-/lo}$, $CXCR5^{lo}ICOS^{int}$ and $CXCR5^{hi}ICOS^{hi}$ subsets and used for downstream *in vitro* experiments. $CXCR5^{lo}ICOS^{int}$ and $CXCR5^{hi}ICOS^{hi}$ cells did not differ in chemotatic response to CXCL13. Tonsillar CD4 T cell subsets restrict expression of chemokine receptors as compared to peripheral blood subsets namely, Naïve, T_{CM1}, T_{CM} and T_{EM}. Follicular B cell help is tested using cocultures of tonsillar CD4 T cell subsets and autologous B cells and assessing the immunoglobulin production in the culture supernatants by using ELISA. $CXCR5^{hi}ICOS^{hi}$ cells were observed to be the most potent B cell stimulators. These cells also secreted highest levels of CXCL13 in cocultures. $CXCR5^{hi}ICOS^{hi}$ cells showed reduced proliferative potential and enhanced susceptibility to apoptosis as compared to $CXCR5^{lo}ICOS^{int}$ and $CXCR5^{-}ICOS^{-/lo}$ cells, which might indicate a state of terminal differentiation. Previous reports have shown that subset expressing CD57 might be the actual subset involved in B cell help. Using the cocultures of $CD57^{+}$ and $CD57^{-}$ subsets within $CXCR5^{lo}ICOS^{int}$ and $CXCR5^{hi}ICOS^{hi}$ cells, it was observed

that B cell stimulation and CXCL13 secretion was independent of CD57 expression. Based on these observations we propose that $CXCR5^{hi}ICOS^{hi}$ cells represent follicular B helper T cells. $CXCR5^{-}ICOS^{-/lo}$ might represent antigen inexperienced cells and $CXCR5^{lo}ICOS^{int}$ could be the precursors of $CXCR5^{hi}ICOS^{hi}$ cells.

To characterize tonsillar CD4 T cell subsets at molecular level and determine their relationship with peripheral blood subsets, large-scale gene expressing profiling was carried out. It was evident that $CXCR5^{hi}ICOS^{hi}$ cells constitute a distinct subset and distantly related to other effector cells. Gene expression data from tonsillar subsets indicates that $CXCR5^{hi}ICOS^{hi}$ cells are derived from $CXCR5^{-}ICOS^{-/lo}$ cells via $CXCR5^{lo}ICOS^{int}$ cells. In addition, gene expression data also suggests that follicular help might be a non-Th1/Th2 entity. Analysis of differential expression of genes in various functional classes indicated previously unknown genes and pathways laying foundations for future work.