ROLE OF MICRORNAS ON T CELL DIFFERENTIATION DURING IMMUNE RESPONSES IN VIVO

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CD4$^+$ T cells are key players in host defense against pathogens, but an incorrect balance between CD4$^+$ T cell subsets, namely pro-inflammatory effector cells, including T helper 1 (Th)1 and Th17 cells (IFN-γ- and IL-17-producers, respectively), and anti-inflammatory regulatory cells (Treg; Foxp3$^+$ subset), can lead to immune-mediated diseases. MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. While individual miRNAs were shown to regulate the differentiation of specific CD4$^+$ T cell populations, a holistic approach based on *in vivo* responses is missing and is critical to understand how miRNA networks control this balance under physiological conditions.

To address this, we have established a triple reporter mouse for *Ifng*, *Il17* and *Foxp3,* and subject it to experimental autoimmune encephalomyelitis (EAE). We perform miRNA-seq analysis on Th1, Th17 and Treg cells isolated from the spleen (SPL) and lymph nodes (LNs) at peak-plateau stage and found that 110 miRNAs are differentially expressed between effector and regulatory subsets. We further selected 8 candidate miRNAs that were specifically upregulated in one population versus the others. Both overexpression and inhibition studies showed that miR-126a limits IL-17$^+$ expression in Th17 cells *in vitro*. Treatment with antagomiRs *in vivo* showed that silencing miR-122 increased the number of IL-17$^+$ cells in the LNs and precipitated the onset of EAE, whereas inhibition of miR-1247 decreased the severity of the disease by reducing the number of IFN-γ$^+$ cells, also in the LNs. Additionally, we identified IL-6 and TGF-β as the key cytokines upstream miR-126a and miR-1247 expression, respectively. While both IL-6 and TGF-β also induce miR-122 expression, we found that IL-23 and IL-1β repress its expression. Interestingly, and given that IL-23 and IL-1β are critical to induce Th17-mediated pathogenicity, we have consistently observed a pathogenic gene signature in CNS-derived Th17 cells when compared to peripheral Th17 cells with concomitant decreased levels of miR-126a and miR-122.

Overall, our results suggest that miR-126a and miR-122 regulate IL-17 expression and the pathogenic phenotype of Th17 cells to prevent excessive inflammation in the periphery while miR-1247 maintains the inflammatory phenotype of Th1 cells in an anti-inflammatory environment.