Role of microRNAs in effector *versus* regulatory CD4$^+$ T cell differentiation during (auto)immune responses *in vivo*

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Autoimmune diseases are often associated with an imbalance 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). MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level. While individual miRNAs were found to regulate the differentiation of specific CD4$^+$ T cell populations, a holistic approach based on *in vivo* responses is still missing and is key to understand how miRNA networks control this balance in pathophysiology.

To address this, we have established a triple reporter mouse for *Ifng*, *Il17* and *Foxp3,* and subjected it to experimental autoimmune encephalomyelitis (EAE). We performed miRNA-seq analysis on Th1, Th17 and Treg cells isolated from the spleen and lymph nodes (LNs) at peak-plateau stage and found 110 miRNAs to be differentially expressed between the effector and regulatory subsets. We selected 9 candidate miRNAs that were specifically upregulated in one population versus the others. *In vivo* miRNA modulation showed that silencing miR-122 increased the number of IL-17$^+$ cells in the LNs and precipitated the onset of EAE, whereas overexpressing miR-1247 decreased the severity of the disease reducing the number of IFN-γ$^+$ cells in the LNs. Additionally, we identified TGF-β as the key cytokines upstream 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, given that IL-23 and IL-1β are critical to induce Th17-mediated pathogenicity, we have observed a pathogenic gene signature in CNS-derived Th17 cells when compared to peripheral Th17 cells with concomitant decreased levels of miR-122.

Overall, our results suggest that miR-122 regulates IL-17 expression and the pathogenic phenotype of Th17 cells, while miR-1247 maintains the Th1 phenotype in an anti-inflammatory environment. These findings may have important implications for autoimmune diseases, which we are now assessing in samples from Multiple Sclerosis patients.

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