



## 2011 Research Grant Program Winning Abstract

### Role of microRNAs (miRNA) in Development and Functions of Differentiation of T Helper Cells and Induced Regulatory T Cells

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MicroRNAs (miRNAs), an abundant class of highly conserved small (18–25 nucleotides long) noncoding RNAs, suppress gene expression by binding to the 3-untranslational region (UTR) of target mRNAs. miRNAs are emerging as pivotal regulators of the development and functions of adaptive immunity. Recent studies suggested that Dicer (RNase III endonucleases), which is required for miRNA's maturation, is also essential for T-cell development and effective functions. Dicer deletion at the early double negative stage of thymocyte development reduces both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the periphery, but due to induced cell death in thymocytes, fewer cells reach the periphery. Therefore, miRNA controlled gene expression programmes do not seem to be required at this stage of development. If Dicer is deleted at the double-positive stage of thymocyte development, then it severely reduces natural regulatory T cells (nTregs) in the thymus and periphery. In addition to this, other studies also suggest that Dicer deleted CD4<sup>+</sup> T cells are unable to repress IFN- $\gamma$ . Therefore, they are more prone to differentiate into T helper 1 (Th1) cells. Loss of miR-155 does not affect the nTregs' function in vivo and in vitro. However, Treg numbers are reduced and CD4<sup>+</sup> T cells more prone to differentiate into a Th2 phenotype. Furthermore, miR-326 has been shown to be essential for Th17 development. Thus, these reports suggest that even a single miRNA could alter the phenotype and functions. Several miRNAs are upregulated in nTreg populations, but so far hardly any particular miRNA is known to affect the suppressive function of these cells. Therefore, proper regulation of Th1/Th2/Th7 or induced Treg (iTreg) lineage decisions and cytokine gene activation and silencing are crucial for effective immune function and prevention of autoimmunity, allergy, or infectious diseases. The genes encoding these cytokines (IFN- $\gamma$ , IL-4, IL-17, and TGF- $\beta$ ) are reciprocally regulated during differentiation, being remodeled at the chromatin level for robust transcription in the appropriate lineage but undergoing silencing in the inappropriate lineage.

Therefore, we hypothesise that controlled expression of miRNAs into CD4<sup>+</sup> T cells can differentiate these cells into effector T helper cells or iTregs by changing the transcriptional regulations. To address this question, we have generated 10 different miRNAs' retroviral expressions and decoy lentiviral vectors to explore the effect of differentiation and function on Th cells and iTregs at the molecular level. Some of these miRNAs are highly upregulated in the Tregs and other T helper subsets. Therefore, expression and suppression of these miRNAs should be able to convert CD4<sup>+</sup> T cells into Th effector cells or iTregs in vitro. To check the expression of different miRNAs, expression/decoy vectors (GFP or mCherry), cytokines (BD™ Cytometric Bead Array), chemokines (BD™ antibodies), and transcription factors (BD™ antibodies) using flow cytometry will be employed. In addition to this, we will also try to determine the target sites relevant to cytokines and transcriptional activation and repression genes of Foxp3, GATA3, ROR- $\gamma$ t, and T-bet (BD™ antibodies). This can be achieved with a luciferase expression vector system by generating the construct which contains 3'UTR of various



target genes. Once we have our in vitro study in place, we will make bone-marrow chimera mice transduced with miRNA lentiviral expression or decoy construct, which have roles in differentiation of Th cell or iTreg development to further explore the effect of miRNAs in vivo. The characterisation of T-cell surface markers, intracellular transcription factors, and chemokine and cytokine estimation in these bone-marrow chimera mice will be done with multicolour flow cytometry (BD FACSCanto™ II system) based techniques using BD Pharmingen™ antibodies and BD Cytometric Bead Array kits. Thus, our study will assist in understanding the fundamental biology of miRNAs in T-cell development and functions. Furthermore, we aim to develop miRNA based novel therapeutic targets for autoimmune and infectious diseases.

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