

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

Carolina Cunha^{1†}, Paula Vargas Romero¹, Daniel Inácio¹, Daniel Sobral², Marina Costa¹, Pedro Papotto¹, Francisco Enguita¹, **Anita Quintal Gomes^{1,3*}** and Bruno Silva-Santos^{1†}



¹Instituto de Medicina Molecular - João Lobo Antunes (IMM-JLA), Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal
²Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal
³H&TRC Health & Technology Research Center, ESTeSL - Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal
*anitagomes@medicina.ulisboa.pt



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Introduction

MicroRNAs are an abundant class of evolutionarily conserved small non-coding RNA species that control gene expression at the post-transcriptional level and are estimated to regulate most of developmental and physiological processes¹. They are involved in many aspects of immunity, notably, in the differentiation and function of CD4⁺ T cell subsets². CD4⁺ T cells play critical roles in the host defense against pathogens, but they are also responsible for the generation of immune-mediated diseases³. This arises from an incorrect balance between the various CD4⁺ T cell subsets, namely pro-inflammatory effector T cells, including the IFN- γ -producers T helper 1 (Th1) cells and the IL-17-producers Th17 cells, and anti-inflammatory regulatory T cells (FoxP3⁺ subset)⁴. Although various individual miRNAs have been implicated in CD4⁺ T cell biology, data is missing on how miRNA regulation networks may control the balance between effector and regulatory T cells in pathophysiological conditions *in vivo*.

Aim

To dissect the role of miRNAs in the post-transcriptional regulation of effector and regulatory CD4⁺ T cells differentiation *in vivo*.

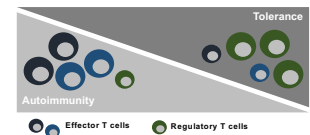


Figure 1: Schematic representation of the immunological balance between effector and regulatory CD4⁺ T cells. Excessive immune responses and/or deficiency in tolerogenic mechanisms can lead to autoimmune diseases and chronic inflammation. On the other hand, unbalanced induction of regulatory T cells may result in immunodeficiency and persistent infections.

Experimental model and methods

1. Establishment of triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice

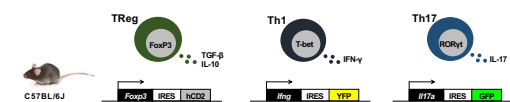


Figure 2: Schematic representation of regulatory (TReg) and effector (Th1 and Th17) populations in triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice.

2. EAE induction in triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice

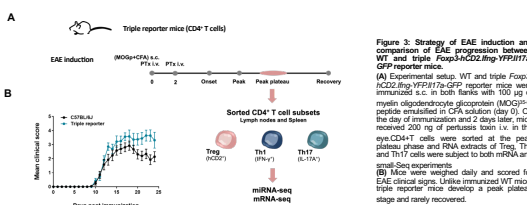


Figure 3: Strategy of EAE induction and comparison of EAE progression between WT and triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice. (A) Experimental setup. WT and triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice were immunized s.c. in both flanks with 100 μ g of myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ peptide emulsified in CFA solution (day 0). On the day of immunization and 2 days later, mice received 200 ng of pertussis toxin i.v. in the eye. CD4⁺ T cells were sorted at the peak disease phase and RNA extracts of Treg, Th1 and Th17 cells were subjected to both mRNA and small RNA sequencing. (B) Mice were weighed daily and scored for EAE clinical signs. Unlike immunized WT mice, triple reporter mice develop a peak plateau stage and rarely recovered.

2. Treatments with anti-miR-122 and miR-1247 impact EAE progression

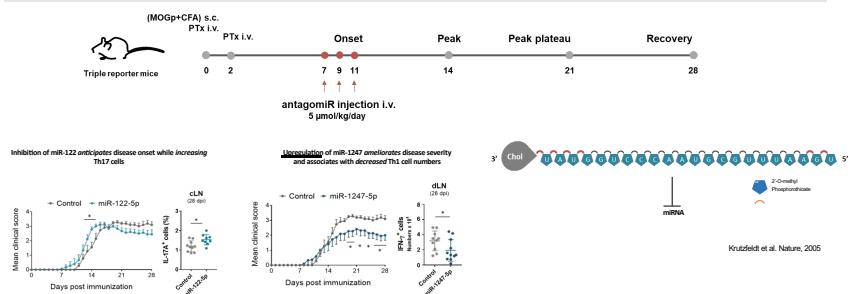


Figure 4: Treatments with anti-miR-122 and miR-1247 impact EAE progression. (A) Experimental timeline. Triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice were immunized s.c. in both flanks with 100 μ g of (MOG)₃₅₋₅₅ peptide. On the day of immunization and 2 days later, mice received 200 ng of pertussis toxin i.v. in the eye. At days 7, 9 and 11, mice received 5 μ mol/kg of specific anti-miR-122 i.v. in the eye. Cell-miR-67-3p was used as control. (B) Mice were scored for EAE clinical signs for 28 days. (C) FACS plots analysis of IFN- γ and IL-17 expression levels in either cervical or draining cLN or dLN of mice subject to miR-1247 and miR-122 anti-miRs.

3. Cytokines modulate miR-122 and miR-1247 expression levels

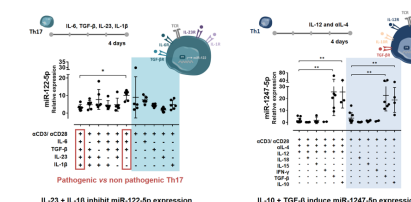


Figure 6: Expression profile of miR-122 and miR-1247 in CD4⁺ T cells stimulated with different cytokine cocktails. RT-qPCR analysis of miR-122 (A) and miR-1247 (B) expression in CD4⁺ T cells cultured *in vitro* for 4 days in the presence of indicated cytokines and antibodies.

4. miR-122 and miR-1247 are downregulated in Th17 or Th1 cells, respectively, isolated from the CNS

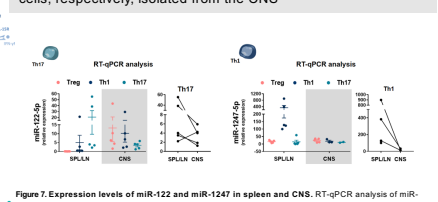


Figure 7: Expression levels of miR-122 and miR-1247 in spleen and CNS. RT-qPCR analysis of miR-122 (A) and miR-1247 (B) expression in CD4⁺ T cells isolated from the spleen and lymph nodes (SPLN) and the central nervous system (CNS) of triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice challenged with EAE.

Results

1. A set of 110 miRNAs are differentially expressed between effector and regulatory T cells *in vivo*

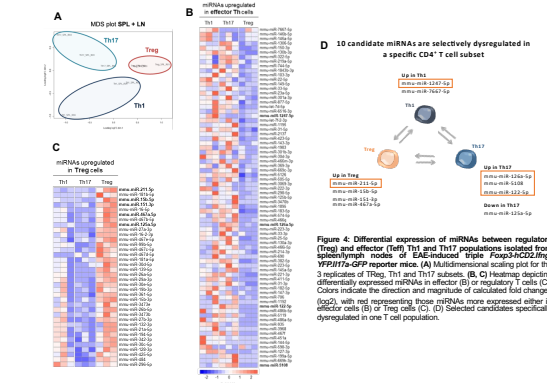
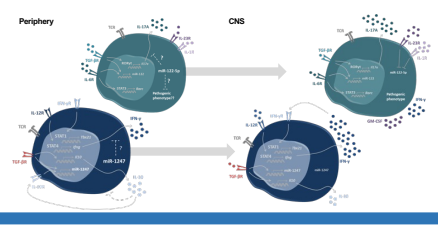


Figure 4: Differential expression of miRNAs between regulatory (Treg) and effector (Th1 and Th17) populations isolated from spleen lymph nodes of EAE-induced triple *Foxp3-hCD2.lfng-YFP.II17a-GFP* reporter mice. (A) Multidimensional scaling plot for the 3 replicates of Treg, Th1 and Th17 subsets. (B, C) Heatmap depicting differentially expressed miRNAs in effector (B) or regulatory (C) T cells. Colors indicate the direction and magnitude of calculated fold changes (log2), with red representing those miRNAs more expressed either in effector cells (B) or Treg cells (C). (D) Selected candidates specifically dysregulated in one T cell population.

Summary

miR-122 and miR-1247 control the pathogenic phenotype of effector Th subsets during CNS autoimmunity

Figure 8: Schematic representation of the working hypothesis. In the periphery, miR-122 is highly expressed in Th17 cells and negatively regulates IL-17A levels and their pathogenic phenotype. Once Th17 cells invade the CNS and from the inflammatory environment, IL-23 and IL-18 inhibit the expression of miR-122, expression and the negative regulation is subverted allowing Th17 cells to acquire pathogenic properties characterized by the production of IFN- γ and GM-CSF. Regarding Th1 cells, we hypothesized that miR-1247 is induced by anti-inflammatory cytokines to limit the expression of IFN- γ and prevent excessive inflammation in the periphery. In the CNS, this self-regulation mechanism is no longer in place, miR-1247 levels decrease and Th1 cells produce higher levels of IFN- γ contributing to damage the CNS.



Future work

- Identification of miR-122 and miR-1247 target mRNAs via differential Ago2-IP followed by RNA-seq
- Assessment of miR-122 and miR-1247 expression in Multiple Sclerosis patients samples

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