MicroRNA-146a controls IFN-γ production and functional plasticity in γδ T cells by targeting Nod1

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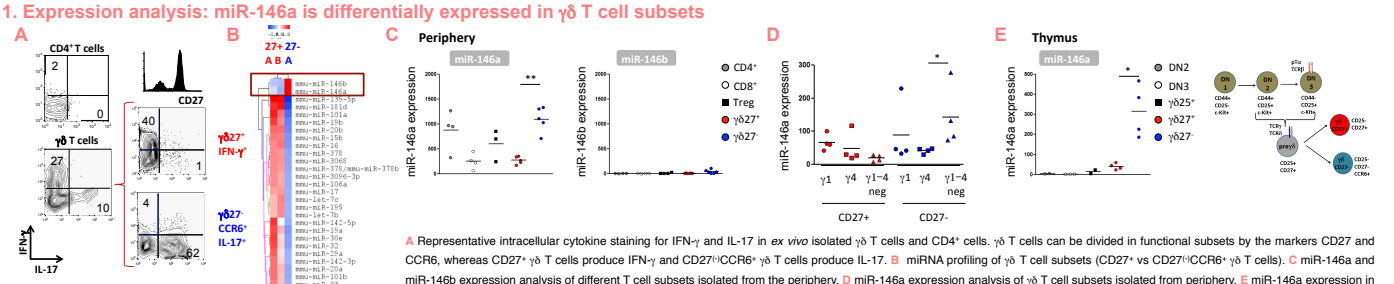
BACKGROUND AND OBJECTIVES

γδ T cells have emerged as key providers of the proinflammatory cytokines interleukin 17 (IL-17) and interferon-γ (IFN-γ) in various models of infection, inflammation and autoimmunity. Our previous epigenetic and transcriptional analyses have shown that whereas CD27⁺ γδ T cells are committed to IFN-γ expression, the IL-17 producing CD27- subset has limited plasticity to co-express both cytokines under inflammatory conditions (Schmolka et al. *Nat Immunol* 2013). To further understand the molecular control of this plasticity we now investigated the potential role of microRNA (miRNA)-mediated post-transcriptional regulation.

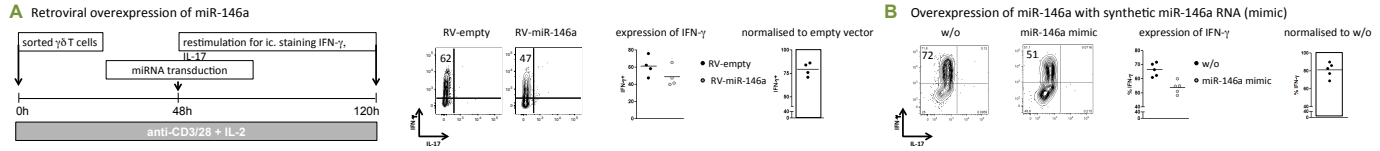
RESULTS

1. Expression analysis: miR-146a is differentially expressed in γδ T cell subsets



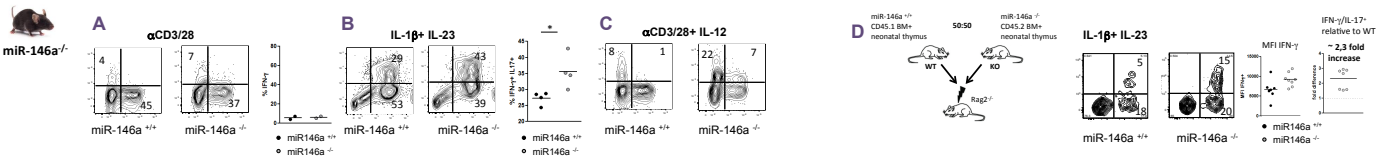
A Representative intracellular cytokine staining for IFN-γ and IL-17 in *ex vivo* isolated γδ T cells and CD4⁺ cells. γδ T cells can be divided in functional subsets by the markers CD27 and CCR6, whereas CD27⁺ γδ T cells produce IFN-γ and CD27⁽⁾CCR6⁺ γδ T cells produce IL-17. B miRNA profiling of γδ T cell subsets (CD27⁺ vs CD27⁽⁾CCR6⁺ γδ T cells). C miR-146a and miR-146b expression analysis of different T cell subsets isolated from the periphery. D miR-146a expression analysis of γδ T cell subsets isolated from periphery. E miR-146a expression in thymic γδ T cell subsets. Scheme of γδ T cell development in the thymus (DN, double negative for CD4 and CD8 markers). (C-E) Expression levels normalised to miR-423-3p.

2. Gain-of-function: miR-146a reduces IFN-γ production by γδ T cells



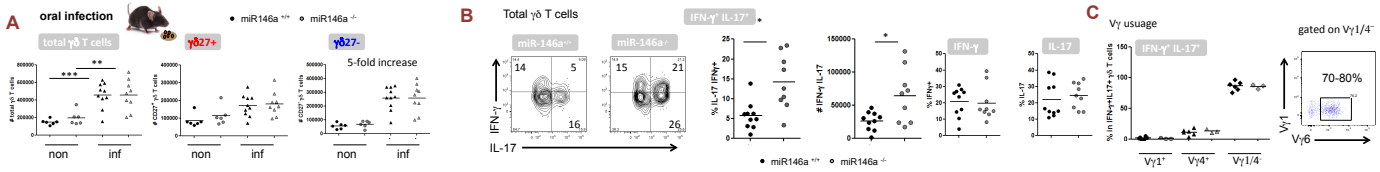
A Workflow of miR-146a retroviral overexpression strategy. Representative intracellular staining of peripheral γδ T cells expressing either retroviral GFP-control vector (RV-empty) or the retroviral vector containing the native stem loop of miR-146a (RV-miR-146a) (A) or miR-146a mimic versus a synthetic RNA control (w/o) (B). Graphs depict the frequency of IFN-γ⁺ γδ T cells or frequency of IFN-γ⁺ γδ T cells normalised to either empty vector (A) or to synthetic RNA (B). Each dot represents one individual experiment.

3. Loss-of-function: Loss of miR-146a increases IFN-γ⁺ and IFN-γ⁺ IL-17⁺ γδ27⁻ T cells



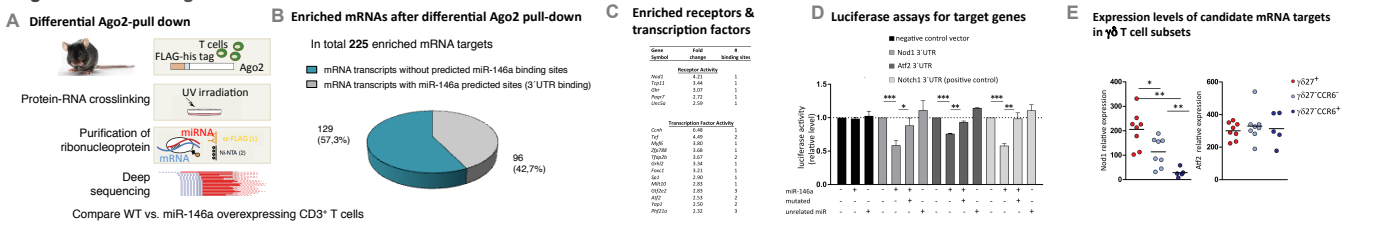
In vitro cultures using CD27⁻ γδ T cells either isolated from miR-146a⁻/⁻ mice or WT littermate controls, TCR activated (A), incubated with IL-1β+ IL-23 (B) or TCR activation + IL-12 (C), or CD27⁻ γδ T cells isolated from 50:50 mixed chimeras incubated with with IL-1β+ IL-23 (D). Graphs depict the frequency of IFN-γ⁺ or IFN-γ/IL-17⁺ γδ T cells or frequency of IFN-γ⁺ γδ T cells normalised to WT . Each dot represents one individual experiment.

4. *Listeria* infection: increased IFN-γ⁺ IL-17⁺ γδ T cells in miR-146a⁻/⁻ mice



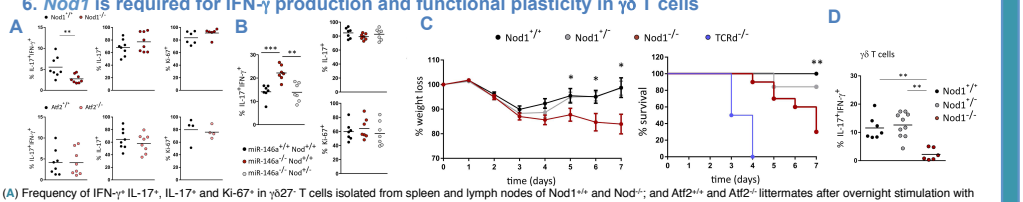
Oral *Listeria monocytogenes* infection of miR-146a⁻/⁻ mice A Number of total γδ T cells, CD27+ γδ T cells and CD27- γδ T cells in miR-146a⁺/⁺ and miR-146a⁻/⁻ mice isolated from naive or Listeria infected mice (7DPI). B Representative intracellular staining of γδ T cells isolated from the spleen of *Listeria m.* infected miR-146a⁺/⁺ and miR-146a⁻/⁻ mice. Graphs depict the frequency of IL-17⁺, IFN-γ⁺ or IFN-γ/IL-17⁺ γδ T cells or number of IFN-γ/IL-17⁺ γδ T cells. C Graph represents the frequency of Vγ γδ T cell subsets of IFN-γ/IL-17⁺ γδ T cells. Representative FACS blot for Vγ6 staining.

5. Ago2 IP: mRNA targets of miR146a



(A) Scheme of Ago2 pull down experiment. (B) Enriched mRNAs after differential Ago-2 pull down experiment. (C) Receptors and transcription factors enriched among Ago-2 IP mRNAs by miR-146a overexpression. (D) Confirmation of target genes identification by dual luciferase assays. (E) Expression analysis of Atf2 and Nod1 in γδ T cell subsets. Each dot represents on individual mouse.

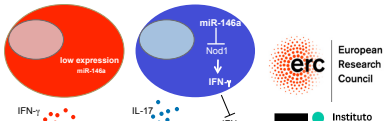
6. *Nod1* is required for IFN-γ production and functional plasticity in γδ T cells



(A) Frequency of IFN-γ⁺ IL-17⁺, IL-17⁺ and Ki-67⁺ in γδ27⁻ T cells isolated from spleen and lymph nodes of Nod1⁺/⁺ and Nod⁻/⁻; and Atf2⁺/⁺ and Atf2⁻/⁻ littermates after overnight stimulation with IL-1β plus IL-23. (B) Frequency of IFN-γ⁺ IL-17⁺, Ki-67⁺ and IL-17⁺ in γδ27⁻ T cells isolated from spleen and lymph nodes of miR-146a⁺/⁺Nod1⁺/⁺ and miR-146a⁻/⁻Nod1⁺/⁺ and miR-146a⁻/⁻Nod1⁺/⁻ littermates stimulated with IL-1β plus IL-23. (C) Weight loss and survival of Nod1⁺/⁺, Nod⁺/⁻, Nod⁻/⁻ and TCRd⁻/⁻ mice infected with *Listeria* (D) Frequency of IFN-γ⁺ IL-17⁺ within γδ T cells isolated from spleen of Nod1⁺/⁺, Nod⁺/⁻ and Nod⁻/⁻, 7d after infection with *Listeria monocytogenes*.

TAKE-HOME MESSAGE

(Schmolka et al. Sci Immunol: in press)



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MM Instituto de Medicina Molecular Lisboa