CN-NFAT pathway blockade increase the proliferative and metabolic activity of dendritic cells and induces a tolerogenic phenotype

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Abstract:

The Nuclear Factors of Activated T cell (NFAT) is a group of transcription factors activated by the calcium-calmodulin dependent phosphatase Calcineurin (CN). Initially identified in T lymphocytes, these factors play pivotal roles in T cell activation and proliferation. Moreover, as described in our previous articles, NFATs are also activated in dendritic cells (DCs) through CD14 signaling upon lipopolysaccharide stimulation, leading to differentiation and apoptosis. Given this foundation and emerging evidence emphasizing the immunosuppressive role played in different pathological contexts by specific myeloid cell types such as Myeloid-Derived Suppressor Cells, our project aims to investigate the involvement of the NFATs in early DC differentiation and its potential contribution to the development of an immunosuppressive phenotype.

To explore this, we transfected a splenic dendritic cell line called D1 with CN inhibitor peptide VIVIT using a lentiviral vector, generating DC-iCN. To assess the impact of CN-dependent pathway inhibition in DC-iCN, we conducted a proteomic analysis, along with functional and metabolic assays. The immunosuppressive activity of DC-iCN was determined by a Mixed Leukocyte Reaction (MLR) assay. Lastly, to assess whether the inhibition of NFATs affected DC hematopoiesis, we intravenously injected nanoparticles carrying VIVIT in an *in vivo* murine model.

Inhibiting the CN-dependent pathway in DCs increased their growth rate and disrupted the cell cycle, prolonging the G2/M phase and shortening the G1 phase. Metabolically, CN-dependent pathway inhibition induced a marked Warburg effect, characteristic of rapidly proliferating cells. Furthermore, DC-iCN demonstrated a highly immunosuppressive phenotype, differentiating naïve T cells into FOXP3+ regulatory T cells in MLR assays. Interestingly, inhibiting the CN-dependent pathway *in vivo* expand the granulocyte-macrophage progenitor involved in DC ontogeny. These findings suggest that blocking the CN-dependent pathway alters early DC differentiation, that in this conditions acquire characteristics of undifferentiated cells and a highly tolerogenic phenotype. Further analyses in humans will complement these discoveries.