- **Tiitle:** *"Systematic Characterization of Autoimmune Monocytes to Develop Effective Tolerogenic Dendritic Cell-Based Immunotherapies"*

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Introduction: Tolerogenic dendritic cells (tolDC) are a promising approach to modulate antigen-specific immune response in autoimmune diseases. IL-10 conditioning is a promising approach to generate effective tolDC (known as DC-10). Previous data showed that monocytes from multiple sclerosis patients are less efficient in differentiating into functional DC-10 compared to healthy controls (HC). This defect might be ascribed to intrinsic or extrinsic features of monocytes from autoimmune patients. To delve into this aspect, we aim to characterize the monocytic compartment of T1D, RA, and NMOSD patients.

Methods: We assessed the frequencies of immune cell subsets in HC, T1D, RA and NMOSD patients by multiparametric flow cytometry. CD14+ cells were stimulated with LPS alone or together with IL-10 and cytokines levels were measured by ELISA. Moreover, CD14+ cells from HC, T1D, and RA patients were *in vitro* differentiated into DC-10, alongside with mature DC (mDC). DC-10 and mDC were co-cultured with CD4+ T cells to assess their ability to induce inhibition of I MLR and Tr1 induction in an allogeneic setting.

Results:

Autoimmune patients with T1D, RA, and NMOSD displayed increased frequency of pro-inflammatory cDC1, cDC2 but similar DC-10 levels compared to HC. LPS-stimulated monocytes from T1D and RA patients exhibited increased secretion of pro-inflammatory cytokines. While IL-10 modulation effectively dampened TNF-α production, its impact on IL-6 was less pronounced compared to HC monocytes. In parallel, autoimmune monocytes were efficiently differentiated into DC-10, but the resulting cells were only partially effective in inhibiting proliferation of allogeneic T cells compared to DC-10 differentiated from HC monocytes ($p<0,01$). Nevertheless, autoimmune DC-10 promoted Tr1 cell differentiation at comparable levels of HC DC-10. To expand our study, we are currently characterizing NMOSD patients as potential target suitable for Ag-specific tolDC cell therapy. Preliminary data showed that PBMCs from NMOSD patients exhibited variable T cell responses to ACQP4 antigen (n=5).

Conclusions

Our data showed that autoimmune monocytes are skewed toward a pro-inflammatory phenotype but can be effectively differentiated into partially functional DC-10. We will extend our analysis to NMOSD patients, focusing on monocyte and B cell compartment. Additionally, we will conduct transcriptomic analysis on autoimmune scRNA-seq datasets to investigate tolerogenic signatures.

WORD count: 2447 ch.
LIMIT: 2500 ch.