Characterization of Autoimmune Monocytes to Develop Effective Tolerogenic Dendritic Cells

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BACKGROUND AND AIMS OF THE PROJECT

Autoimmune diseases are characterized by the breakdown of immunological tolerance, leading to a detrimental immune system activation, resulting in auto-reactivity against self-antigens. Interleukin 10 (IL-10) is an anti-inflammatory cytokine that plays a pivotal role in establishing peripheral tolerance. IL-10-mediated modulation of human monocytes during dendritic cell (DC) differentiation promotes the *in vitro* generation of a subset of tolerogenic DC, known as DC-10, that efficiently induce antigen-specific Type 1 regulatory (Tr1) cells via secretion of IL-10 and expression of the tolerogenic molecules ILT-4 and HLA-G[1]. We have recently reported that monocytes from multiple sclerosis (MS) patients are less effective than those isolated from healthy donors (HD) in differentiating into functional DC-10[2]. This defect might be ascribed to intrinsic or extrinsic defects of monocytes from patients with autoimmune diseases.

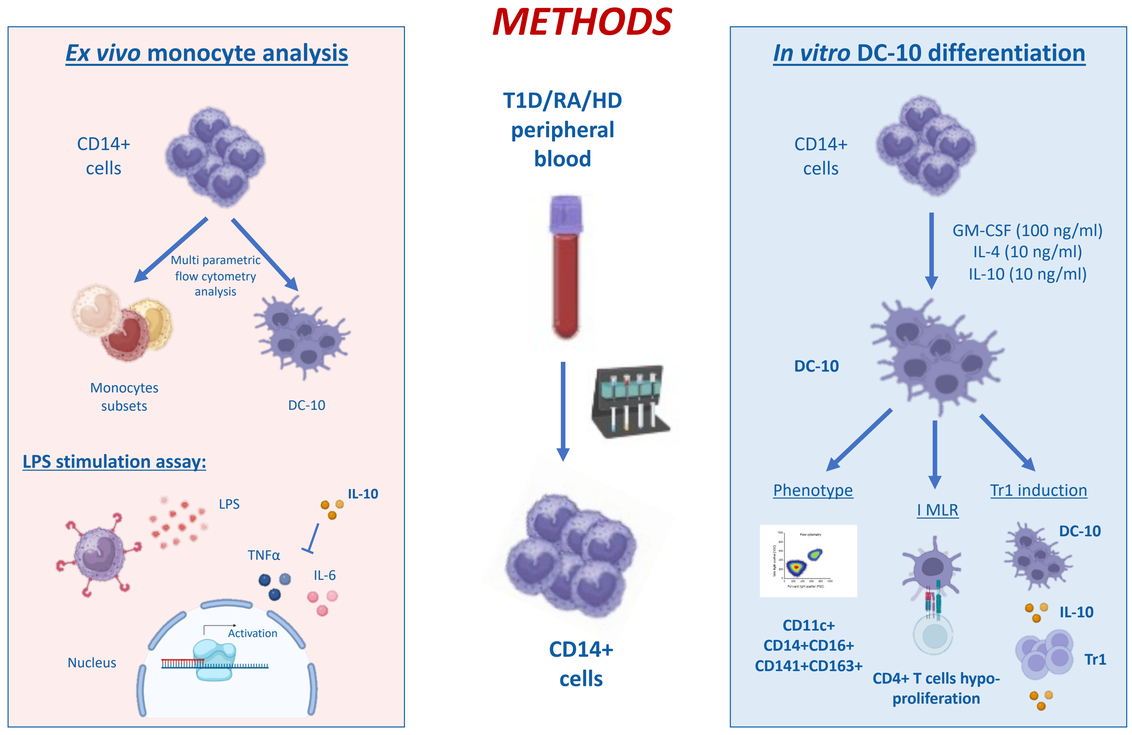
To address this issue, we aimed at:

1. performing phenotypic, molecular, and functional characterization of peripheral blood monocytes of autoimmune patients (Type 1 Diabetes and Rheumatoid Arthritis);
2. characterizing the ability of these monocytes to differentiate into DC-10;
3. evaluating the functionality of patient's derived DC-10 in MLR assays (i.e. , proliferative response, expression of activation markers on allogeneic CD4+ T cells, and induction of Tr1 cells).

Results will improve the knowledge of DC-10 induction and functions in autoimmune patients.

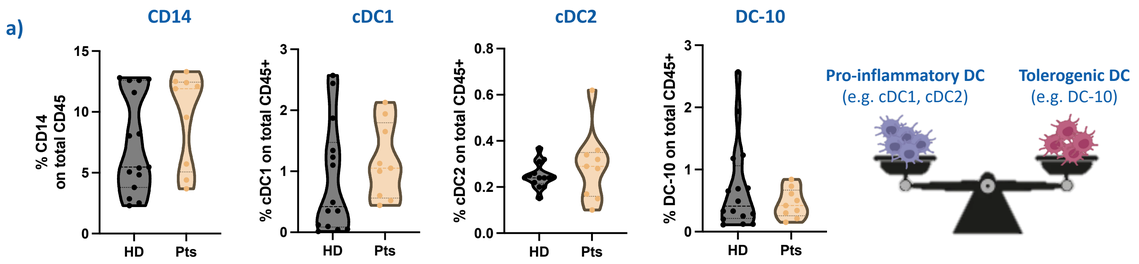
References *1. Gregori et al., Blood (2010) 2. Avancini et al., Cell Reports (2023)*

METHODS



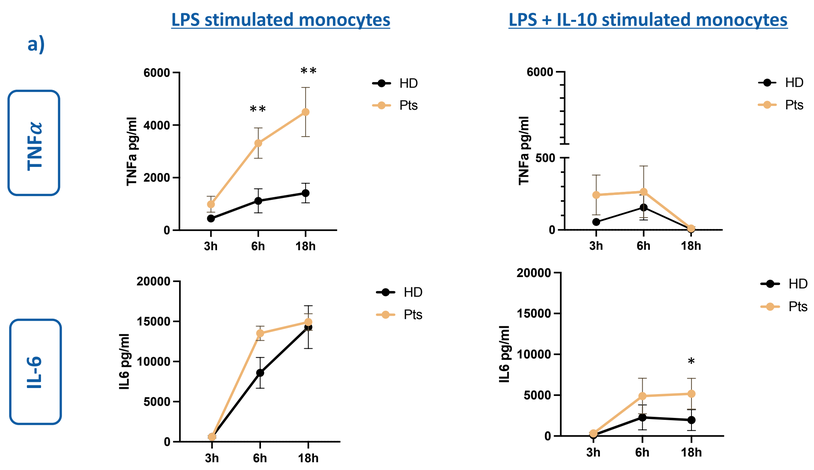
RESULTS

1. Comparable frequency of DC subsets in autoimmune patients and healthy controls



The frequencies of CD14+ cells, cDC1, cDC2 and DC-10 evaluated on whole blood from T1D, RA and HD. **a)** Violin plot indicating the frequency of CD14+ cells, cDC1, cDC2 and DC-10 on total CD45+ cells. cDC1 = co-expression of CD11c+CD141+ in the CD14- fraction; cDC2 = co-expression of CD11c+CD1c+ on total CD45+ cells; DC-10 = co-expression of CD14+CD16+CD141+CD163+ within the CD11c+ compartment. Each dot represents a donor. HD n=14, HD; Pts= 9 (T1D n=5, and RA n=4).

2. Autoimmune monocytes secrete higher levels of pro-inflammatory cytokines compared to healthy monocytes

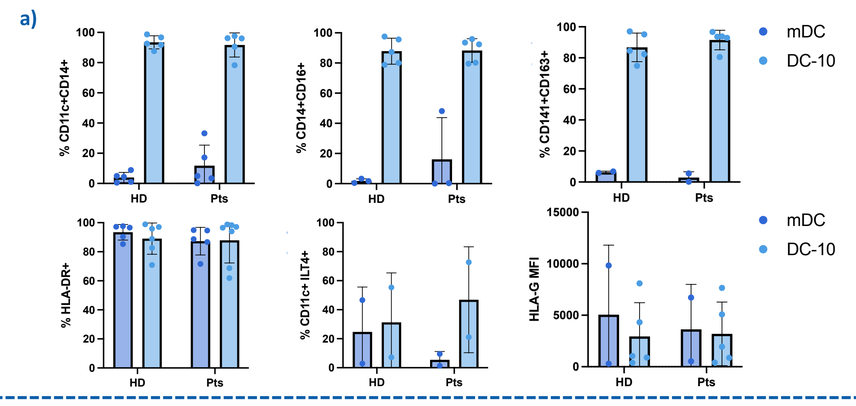


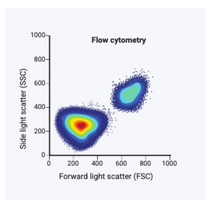
CD14+ cells isolated from PBMCs were stimulated with LPS alone or in combination with IL-10. Culture supernatants were harvested after 3h, 6h or 18h of stimulation and TNF-α and IL-6 concentrations were measured by ELISA. **a)** Levels of IL-6 and TNFα at the indicated time points are presented. Mean +/- SEM, n= 12 HD and n=8 Pts. P value by Mann-Whitney unpaired test HD vs pts. *= P <0.05. ** = P < 0.01 Only significant differences are shown.

3. Autoimmune monocytes can efficiently differentiate into DC-10

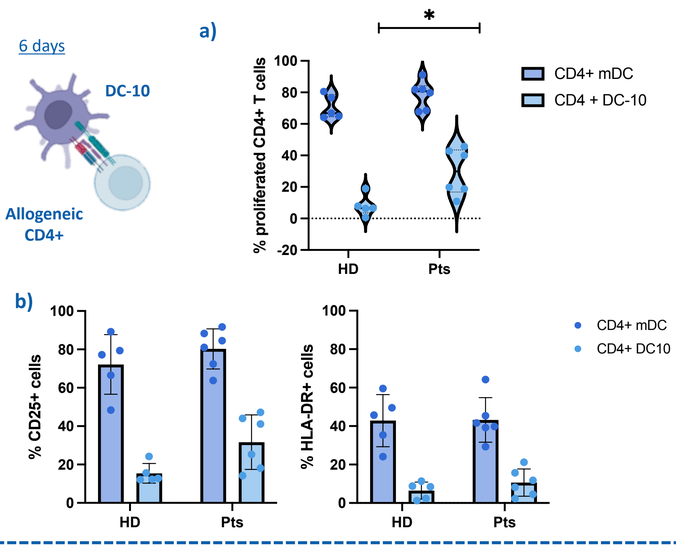


CD14+ cells from HD, T1D, and RA patients were differentiated into DC-10 in the presence of GM-CSF, IL-4 and IL-10 for 7 days. Mature DC (mDC) were differentiated in parallel. **a)** Percentage of DC co-expressing the indicated markers. Each dot represents a donor. HD n=5 Pts n=5. Bar plots indicate mean±SD.



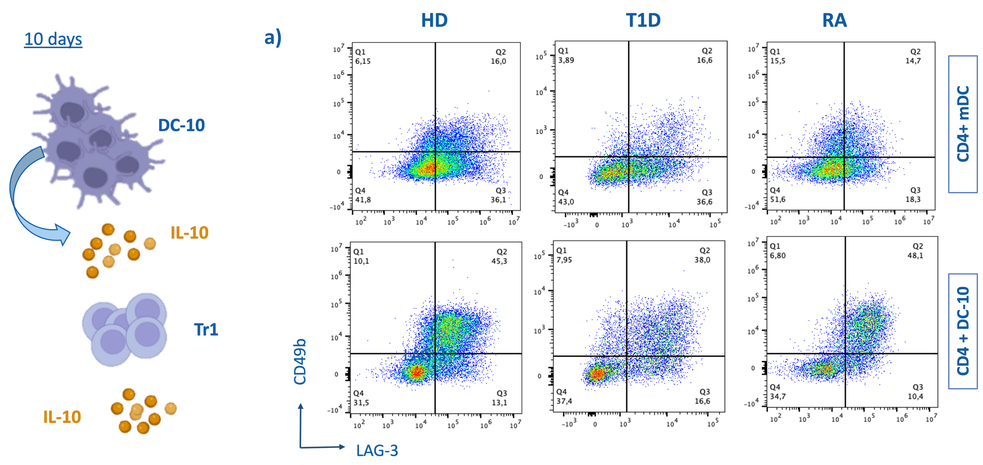


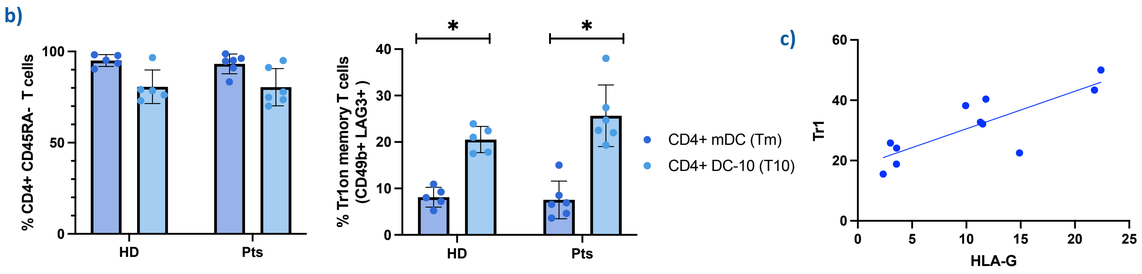
4. Lower inhibitory activity of DC-10 differentiated from autoimmune monocytes



DC-10 and mDC were co-cultured with allogeneic CD4+ T cells stained with a proliferation dye at [1:10] ratio for 6 days, and proliferation was evaluated as proliferation dye dilution. At the end of the co-culture, CD4+ T cell proliferation and expression of activation markers (HLA-DR and CD25) were analysed by flow cytometry. **a)** Violin plot indicating proliferated cells expressed as percentage of cells that have diluted the dye. Mean +/- SD, HD n=5 and pts n=6. P value by Mann-Whitney unpaired t test. *= P <0.05. **b)** Violin plot indicating the frequency of CD4+ cells expressing the activation markers CD25 (left panel) and HLA-DR (right panel). Each dot represents a donor.

5. Autoimmune DC-10 promote Tr1 cells





DC-10 and mDC were co-cultured with allogenic CD4+ T cells at [1:10] ratio for 10 days. At the end of the culture the frequency of Tr1 cells was assessed by the co-expression of CD49b and LAG-3 by flow cytometry. **a)** Representative plots showing the percentage of Tr1 cells co-expressing CD49b and LAG-3 in the memory compartment. **b**) Bar plots showing the percentage of memory T cells CD4+CD45RA- (left panel) and of Tr1 cells within memory T cells compartment (right panel) co-expressing CD49b and LAG-3. Each dot represents a single donor. Bars indicate mean +/- StD, HD n=5, pts n=6. P value by Wilcoxon paired t test. *= P <0.05. **c**) Pearson correlation plot between the frequency of Tr1 cells induced in the T-DC10 co-culture and HLA-G expression on the respective DC-10.

CONCLUSIONS

- DC-10 frequency is comparable in the peripheral blood of autoimmune patients and healthy controls.
- Monocytes isolated from the peripheral blood of autoimmune patients secrete higher levels of pro-inflammatory cytokines than monocytes from HD.
- T1D and RA monocytes efficiently differentiate into DC-10 but resulting DC-10 are less effective in inhibiting I MLR.
- DC-10 from autoimmune patients promote Tr1 cells at levels comparable to those obtained with DC-10 from HD.

FUTURE STEPS

- To investigate the mechanisms underlying the low hypo-proliferation induction by autoimmune DC-10.
- To assess the function of Tr1 cells induced by autoimmune DC-10.
- To define the ability of autoimmune CD4+ T cells to differentiate into Tr1 cells.
- To evaluate the transcriptomic profile of autoimmune monocytes and DC subsets.

"All these notes and proceedings are confidential. You are bound not to communicate or disclose these information and results to any third party".

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