

BACKGROUND AND AIMS OF THE PROJECT

Autoimmune diseases are characterized by the breakdown of the immunological tolerance, which leads to a detrimental activation of the immune system resulting in the auto-reactivity against self-antigens. Interleukin 10 (IL-10) is an anti-inflammatory cytokine playing a pivotal role in the establishment of 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-G¹. 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-10². This defect might be ascribed to intrinsic or extrinsic defects of monocytes from patients with autoimmune diseases.

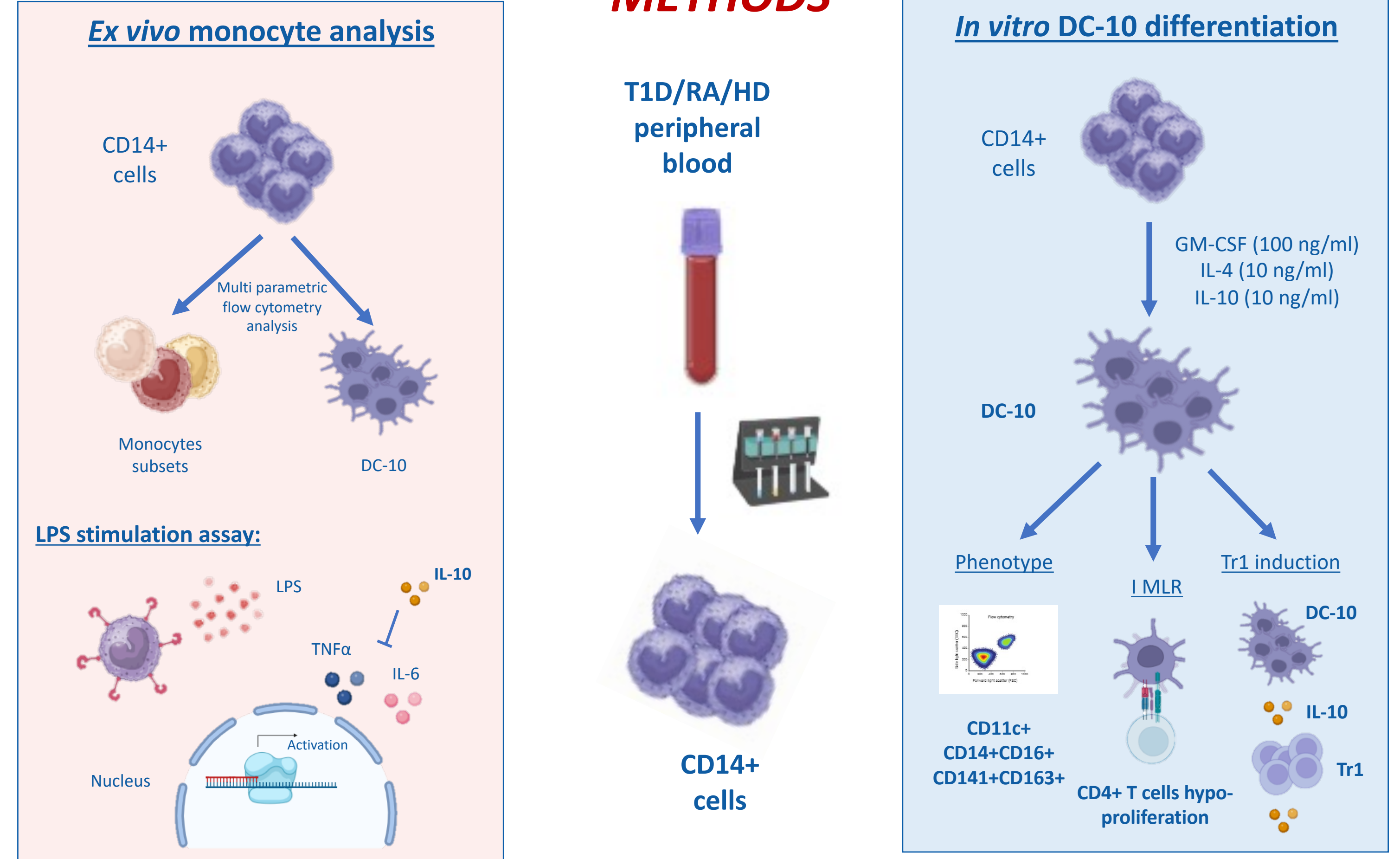
To address this issue, we aimed at:

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 on DC-10 induction and functions in autoimmune patients.

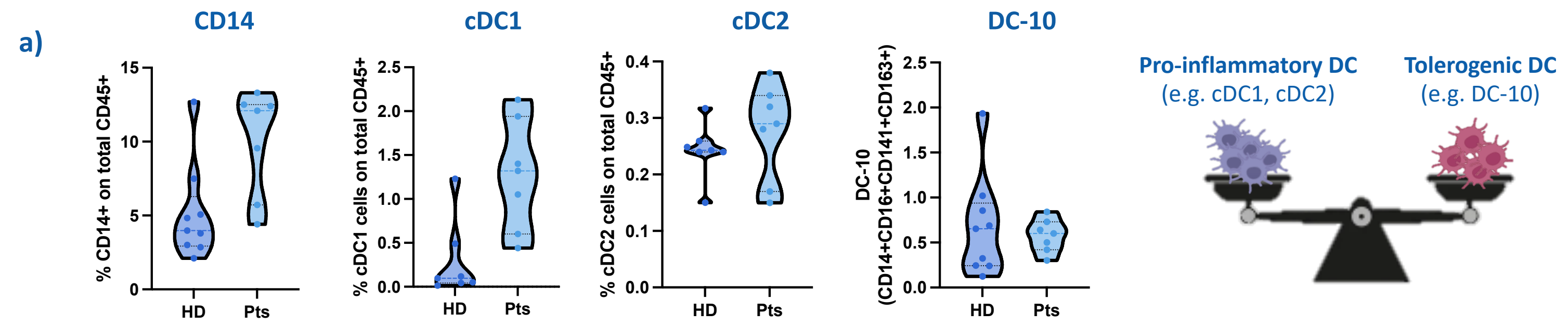
References 1. Gregori et al., *Blood* (2010) 2. Avancini et al., *Cell Reports* (2023)

METHODS



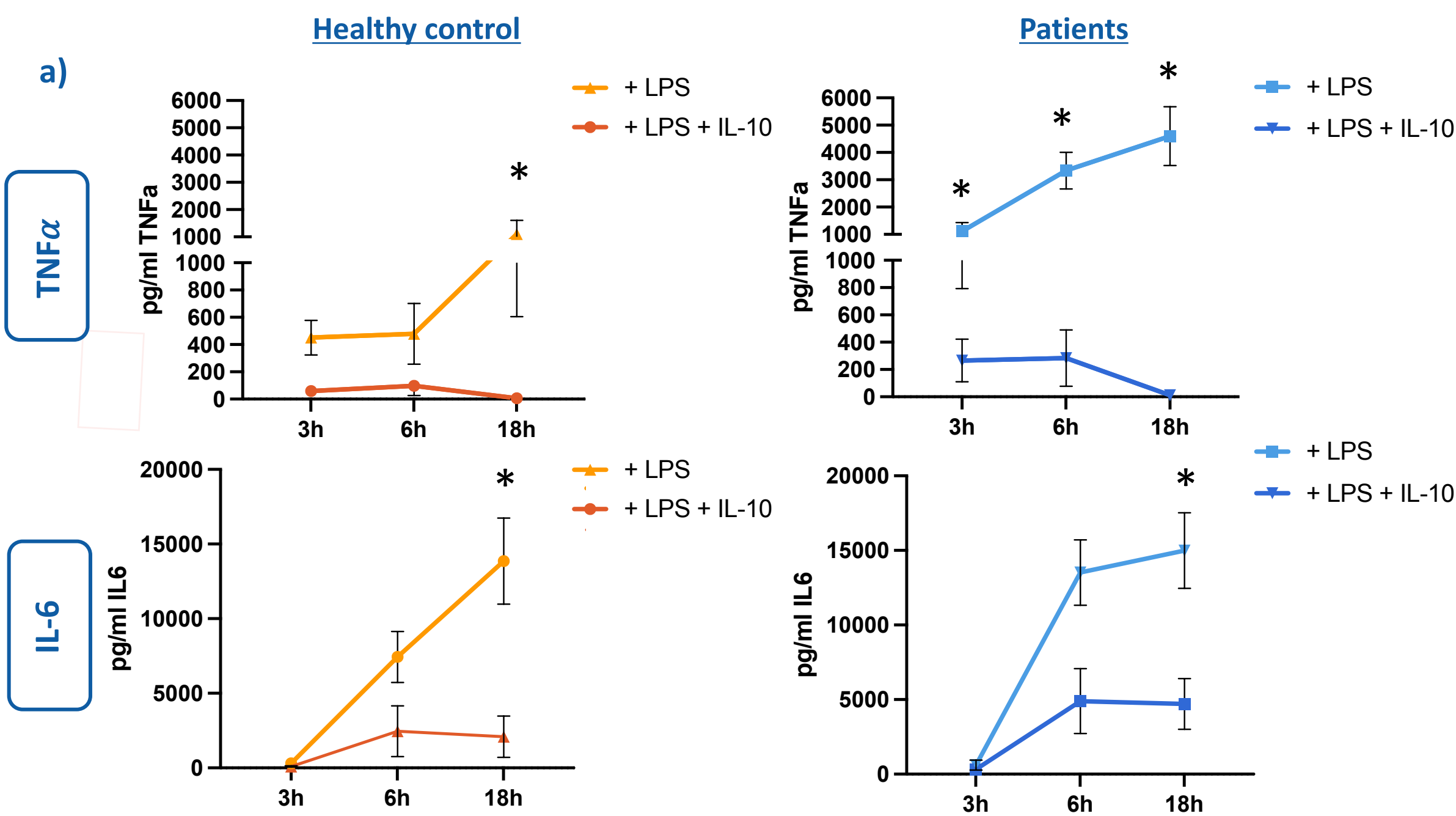
RESULTS

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



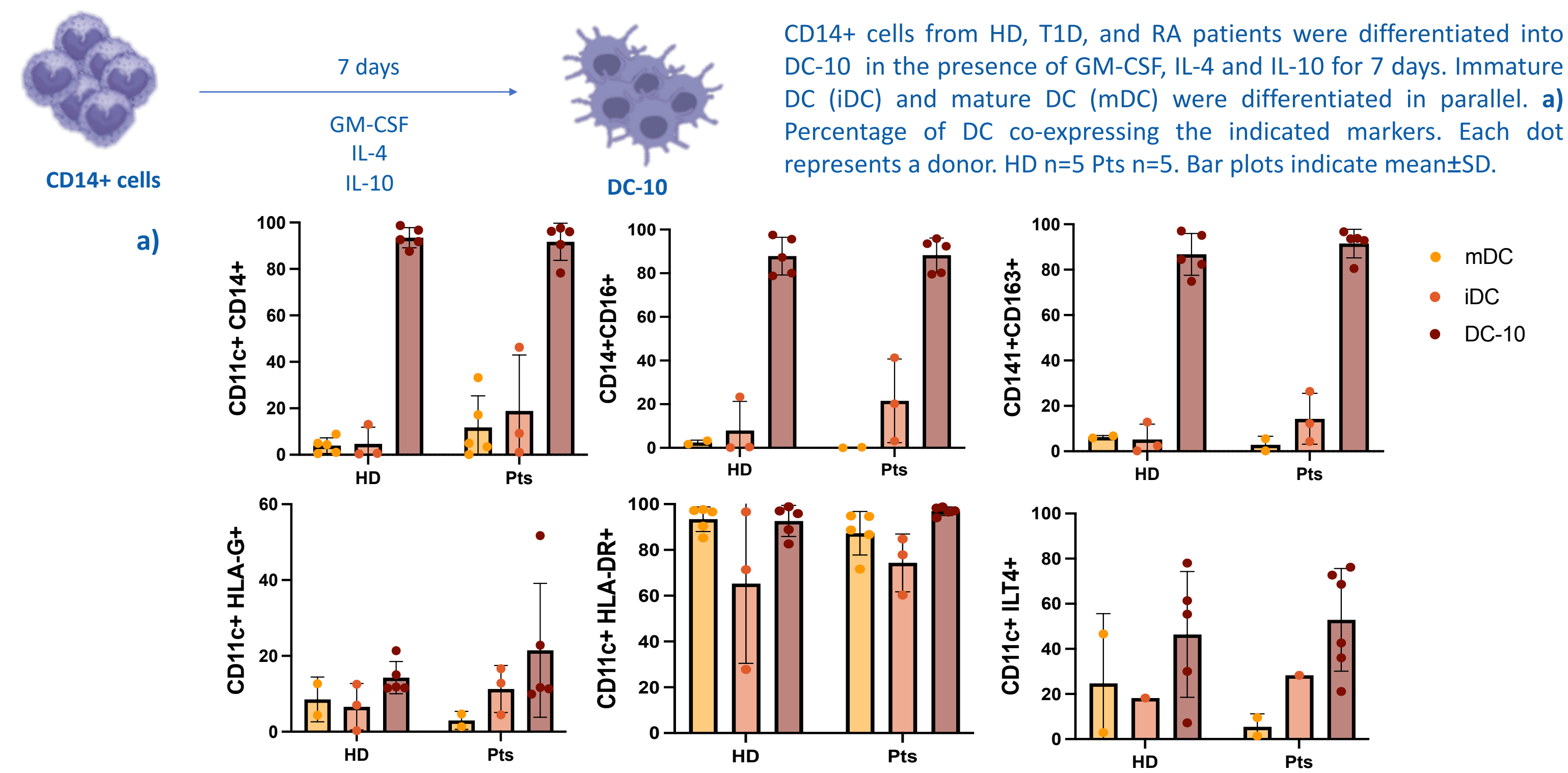
The frequencies of CD14⁺ cells, cDC1, cDC2 and DC-10 were evaluated on whole blood from T1D, RA and HD. a) Violin plot indicating the frequency of CD14⁺ cells, cDC1, cDC2 and DC-10 on the total CD45⁺ cells. cDC1 = co-expression of CD11c+CD141+ in the CD14⁺ fraction; cDC2 = co-expression of CD11c+CD141+ on total CD45⁺ cells; DC-10 = co-expression of CD14+CD16+CD141+CD163+ within the CD11c+ compartment. Each dot represents a donor. HD n=9, HD; Pts= 7 (T1D n=4, and RA n=3).

2. Autoimmune monocytes secrete higher levels of pro-inflammatory cytokines compared to healthy monocytes that are inhibited by IL-10



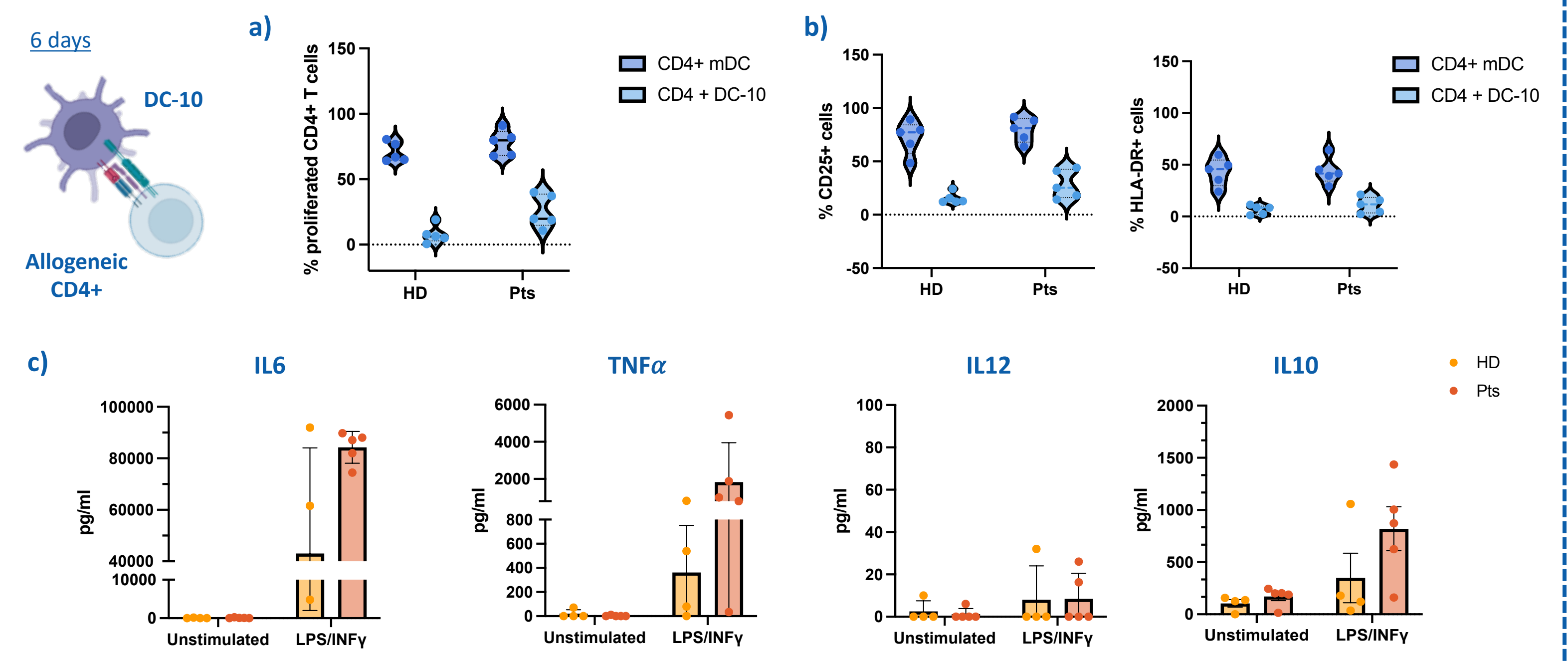
CD14⁺ cells isolated from PBMCs were stimulated with LPS alone or in combination with IL-10. Culture supernatants were harvested after 3h, 6h or 18h of stimulation and TNF-α and IL-6 concentrations were measured by ELISA. a) Levels of IL-6 and TNFα at the indicated time points are presented. Mean ± SEM, n= 6 HD and n=6 Pts. P value by Wilcoxon paired test LPS vs LPS + IL-10 condition., * P < 0.5. Only significant differences are shown.

3. Autoimmune monocytes can efficiently differentiate into DC-10



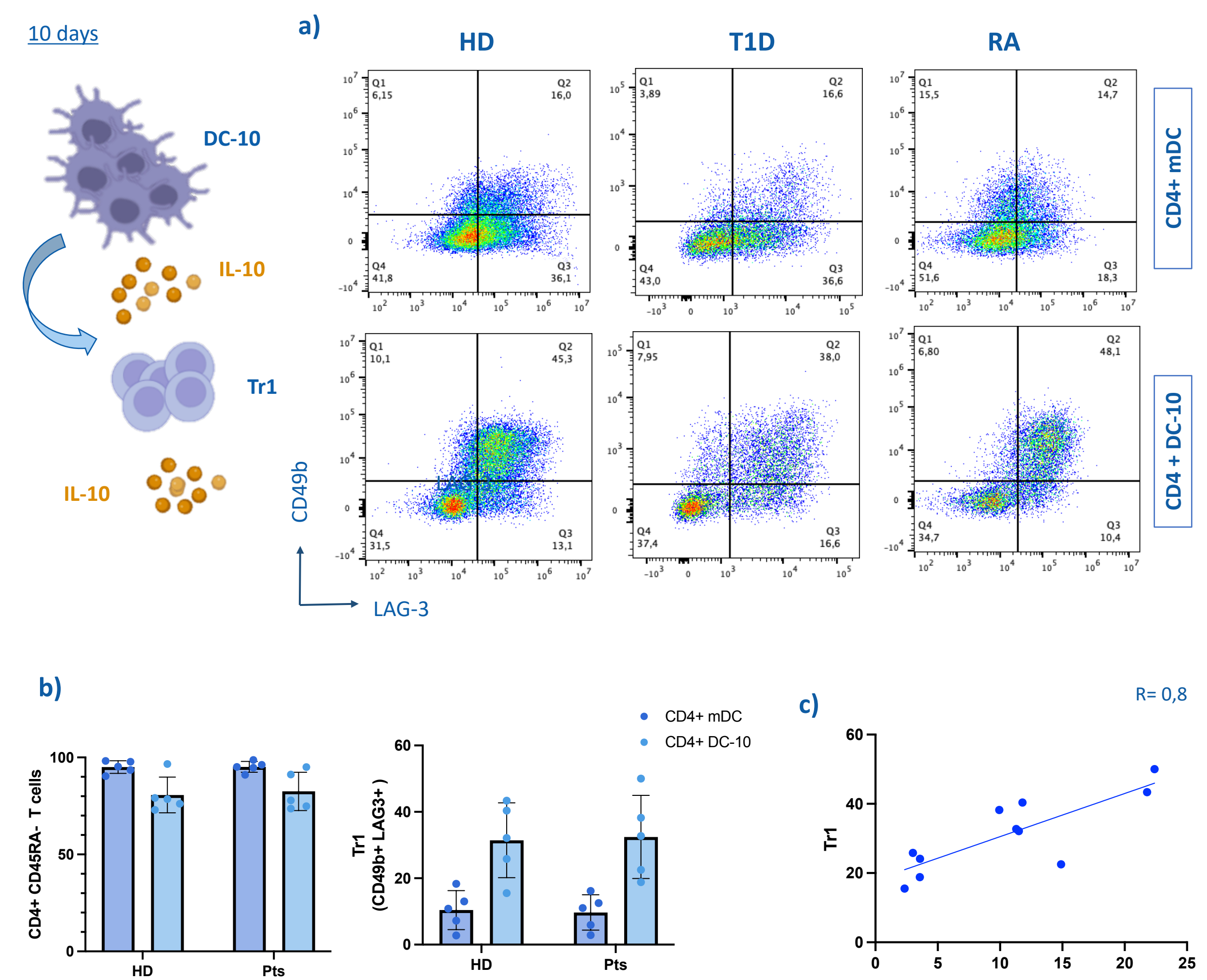
CD14⁺ cells from HD, T1D, and RA patients were differentiated into DC-10 in the presence of GM-CSF, IL-4 and IL-10 for 7 days. Immature DC (iDC) and mature DC (mDC) were differentiated in parallel. a) Percentage of DC co-expressing the indicated markers. Each dot represents a donor. HD n=5 Pts n=5. Bar 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. 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. HD n=5, Pts n=5. At the end of DC differentiation, cells were left unstimulated or stimulated with LPS and IFNγ for 24h or 48h. The concentration levels of IL-10, IL-6, TNFα, and IL-12 were assessed in the culture supernatants by ELISA. IL-6, IL-10 and TNFα levels were measured after 48h of stimulation, only IL-12 concentration was evaluated after 24h of stimulation. c) Levels of the indicated cytokines secreted by unstimulated (left) or LPS/IFNγ stimulated cells (right) are shown.

5. Autoimmune DC-10 promote Tr1 cells



DC-10 and mDC were co-cultured with allogeneic 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 co-expressing CD49b and LAG-3 (right panel). Bars indicate mean ± StD, n=5 for HD, and n=5 for pts. Each dot represents a single donor. 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 lower in peripheral blood of autoimmune patients compared to healthy subjects.
- Monocytes isolated from peripheral blood of autoimmune patients secrete higher levels of pro-inflammatory cytokines (IL-6 and TNF-α) compared to monocytes from HD.
- T1D and RA monocytes efficiently differentiate into DC-10, but 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 evaluate the expression of co-stimulatory and tolerogenic molecules (e.g. CD86, HLA-G and ILT4) on DC-10 in peripheral blood of autoimmune patients (phenotype and gene expression).
- To investigate the mechanisms underlying the low hypo-proliferation induction by autoimmune DC-10.
- To assess the suppressive function of Tr1 cells induced by autoimmune DC-10.
- To define the ability of autoimmune CD4⁺ T cells to differentiate into Tr1 cells.