Group 2: Islets/Transplantation/Immunology of T1D. 18: clinical immunology

Dendritic cells from paediatric patients with type 1 diabetes show altered phagocytosis capacity correlating with disease evolution

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Background and aims: Autoimmunity against ß-cells in type 1 diabetes (T1D) is prompted by defective immunological tolerance, an event in which dendritic cells (DCs) play a crucial role as orchestrators of the immune response. Relying on the inherent ability of apoptotic cell clearance to induce tolerance, we designed a liposomal nanotherapy rich in phophatidylserine (PS) –a characteristic signal of the apoptotic cell membrane– and loaded with insulin to mimic apoptotic ß-cells. PS-liposomes administration blunted autoimmunity in experimental T1D through the generation of tolerogenic DCs. Moreover, human DCs from adult patients with T1D were also rendered tolerogenic after PS-liposomes phagocytosis. However, since T1D in children is often more complicated to manage and severe dysglycaemia could impair DCs functionality, our aim was to explore the therapeutic value of PS-liposomes in DCs from paediatric patients (PP) with T1D.

Material and methods: PP with T1D at onset (n=14), with established disease (0.5-11 years of evolution, n=20) and control subjects (CS, n=11) were recruited. Inclusion criteria were 1-18 years and normal BMI; exclusion criteria were being under immunomodulatory treatment or suffering from other autoimmune or other chronic diseases. A 10 mL blood sample was obtained, and DCs were derived *in vitro* from isolated monocytes. PS-liposomes with optimum size and composition for phagocytosis were loaded with human insulin peptides as autoantigen. Therefore, PS-liposomes capture kinetics by DCs, and phenotypic and transcriptional changes in DCs were assessed.

Results: When assessing phagocytosis kinetics, we discovered that DCs from PP with established disease captured PS-liposomes slowlier and less efficiently than DCs from CS (p<0.01, Mann-Whitney test) and PP at onset (p<0.05, Mann-Whitney test), which were also less efficient than DCs from CS (p<0.05, Mann-Whitney test). Phagocytosis kinetics AUC negatively correlated with the time of disease evolution (r=–0.6772, p=0.0139, Spearman's correlation analysis), but not with age, age at onset, BMI, HbA_{1c}, fasting C-peptide nor insulin dose. After PS-liposomes phagocytosis, expression of PS-receptors *CD36* and *MFGE8* genes tended to be upregulated in DCs from CS and PP at onset, and tended to be downregulated in DCs from PP with established disease, agreeing with the trend in phagocytosis kinetics. Nonetheless, membrane expression of immunological markers (HLA class I and II, CD40, CD54, CD86, CCR2, CXCR4) of DCs from PP at onset and with established disease was lower in DCs after PS-liposomes capture than in mature DCs (p<0.05, Mann-Whitney test), consistent with a tolerogenic effect. Interestingly, *TGFB1* gene, involved in immunoregulation, appeared upregulated after PS-liposomes capture only in DCs from PP at onset and with established disease.

Conclusion: These results demonstrate that progression of T1D in PP, and probably temporal accumulation of dysglycaemia, impacts negatively on DCs phagocytic capacity, albeit tolerance induction could still function optimally. This is the first time that this immunological complication of the disease is reported in PP with T1D.

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