

Enhancement of Treg-mediated Suppression by NKT Cells in T1D

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Purpose: Type 1 Diabetes (T1D) is an organ-specific autoimmune disease characterized by the autoreactive T cell-mediated destruction of insulin-producing beta cells of the pancreas. T cells with regulatory functions, including CD4+CD25++Foxp3+ regulatory T cells (Tregs) and invariant natural killer T cells (iNKT) are important in controlling pathogenic autoreactivity. Both populations are reduced in numbers or have compromised functions in several human autoimmune diseases, including T1D. Previous data from our laboratory have described a differential distribution of iNKTs and Treg cell populations, in the pancreas of a T1D patient at disease onset. Namely, all Treg cells were found inside the islets together with a small number of NKTs, whereas the remaining NKT cells occupied the periinsular area. A very similar distribution pattern was found by immunofluorescence analysis on pancreas from diabetic NOD mice (20 weeks), that was not evident in prediabetic (9 weeks) or disease onset (14 weeks) mice, where the NKT cells were well outside the insular area. We have proposed an active cross-talk between these two types of regulatory T cells in the target organ of autoimmune disease. Our aim is to analyze their functional interaction in the pancreas of T1D patients.

Methods: To study the possible interaction between these two populations of immunoregulatory T cells, we selected iNKT and Treg cells from healthy donors PBMCs, based on the expression of CD3 and CD56 and expanded *in vitro* with CD1d+ APCs pulsed with α GalactosylCeramide (α GalCer) obtaining a population of 99,6 % of $V\alpha 24J\alpha 18CD3$ iNKTs cells. Tregs were selected by their expression of CD4 and CD25hi and were expanded *in vitro* with anti-CD3 and anti-CD28 coated beads using the Rapid Expansion Method (REM). With this method, we obtained 99% pure CD25++CD3+CD4+Foxp3+ Tregs cells. We then analyzed the capacity of the iNKTs to modify the suppression of T effector cells (Tef) *in vitro* proliferation by Tregs, by measuring H3-thymidine incorporation after 4 days of culture, using different ratios of Treg:Teff cells. After that, we analyzed the capacity of the iNKTs to modify the suppressor effect of Treg cells by adding different numbers of iNKT cells to the same cultures.

Summary of Results: The results from NOD mice showed that both cell types are present at the diabetic pancreas but they localize inside the islets following a sequential pattern: Tregs associate preferentially to pancreatic islets even in non-diabetic pancreas while NKT cells enter the islets later in the development of the autoimmune response. iNKTs and Tregs can be expanded *in vitro* maintaining their phenotype and functionality. Treg cells with the capacity to suppress T effector cells by an average of 36% increased the effect to a 60% after the addition of NKT cells to the assay. We are investigating the mechanism by which NKT cells enhance the suppressor effect by addressing the requirement of cellular contact and the production of inhibitory soluble factors.

Conclusions: NKT cells have an adjuvant effect on Tregs by increasing their capacity of inhibiting Tef proliferation *in vitro*. The co-localization of these two cell populations in the diabetic pancreas suggest that this effect could be taking place *in vivo*. If confirmed in human, this adjuvant effect could represent a tool to overcome the proposed resistance of diabetic Tef cells to suppression by Tregs.