Restoration of Immune Imbalance in Type 1 Diabetes with Simultaneous Notch and eIF5a Inhibition

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Immune cell plasticity is the ability of immune cells to switch between functional states in response to cytokine milieu. In Type 1 diabetes (T1D), T effector cells attack pancreatic β-cells while T regulatory cells fail to contain this immune attack. The present study explores immune cell plasticity in response to simultaneous treatment with eIF5a (eukaryotic translation initiation factor 5A) inhibitor N1-Guanyl-1-7-diaminoheptane (GC7) and Notch inhibitor anti-DLL4 in human peripheral blood. Delta-like-ligand-4 (DLL4), in the Notch signaling pathway, is a key regulator of cell fate decisions and modulates immune cell behavior, while eIF5a regulates gene expression. Peripheral blood mononuclear cells (PBMCs) were isolated from patients with T1D (n=3-4) and healthy controls. To evaluate the plasticization of these cells into Tregs, Treg deficient CD4 (CD4+CD25-) cells were cultured with GC7(100µM) + anti-DLL4(10µg/ml) + rhGAD65(4µg/ml) for 7 days. Cells were quantified using flow cytometry and compared with conventional stimulation by anti-CD3/CD28 dyna beads. We observed that 60-70% of CD4+CD25- cells were plasticized into T regulatory cells (CD4+CD25+). We also investigated the functional stability of plasticized Tregs compared to freshly isolated naïve T regulatory cells from the same patient. The plasticized T regulatory cells were co-cultured with T-effector cells in Treg: T-effector ratios of 0:1, 1:1, 1:2, and 1:0, and suppression/proliferation was accessed after 5 days. Flow cytometry revealed that plasticized cells expressed regulatory phenotype (CD4+CD25+FoxP3+) and suppressed T-effector cells. We further evaluated GC7+ anti-DLL4 for adverse effects on cell viability for 7 days, demonstrating no significant difference between control and GC7+anti-DLL4 treated groups in terms of live, dead, and apoptotic cells until 48 hrs. However, a significant increase in dead cells post 48 hours in the treated group was observed, and the cellular signature of cells confirmed increased plasticized Tregs (CD4+CD25+FoxP3+) (2-fold) killing T effectors. This experiment provides a means by which previously committed CD4 T cells or intermediate subsets can be pushed to acquire a T regulatory cell phenotype to restore immune imbalance in autoimmune disorders, particularly T1D. This approach of immunomodulation is novel and may, in the future, find its way to clinical trials once confirmed with a larger patient sample dataset.

