Characterization of reversible dysfunctions in regulatory CD8 T cell population responsible for pathogenic T cell elimination in T1D patients

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MOZART THERAPEUTICS

Orchestrating The Immune System

BACKGROUND/AIMS:

- Regulatory CD8 T cells (CD8 Treg) have been described as regulators of immune homeostasis and are characterized by oligoclonal T cell receptors (TCRs) directed toward conserved peptide sequences; they appear dysfunctional in patients with autoimmune diseases
- CD8 Treg reduce disease in adoptive transfer studies and are increased in patients with improved outcomes in response to immunotherapies, including in teplizumab and alefacept treated type 1 diabetes (T1D) patients
- CD8 Treg function by selectively killing pathogenic T cells that promote tissue destruction, without broad immunosuppression
- This study sought to define the phenotype and functional characteristics of CD8 Treg isolated from patients with T1D

METHODS

- CD8 Treg from the peripheral blood of T1D patients and from healthy donors were comparatively assessed using flow cytometry
- Functional responses of T1D patient-derived CD8 Treg were evaluated using pan TCR stimulation (anti-CD3) or T1D autoantigen peptide cocktail
- Effects on pathogenic T cells were evaluated using peptide restimulation of peripheral blood mononuclear cells (PBMC)
- Effects on tissue damage were tested using functional human pancreatic islet-derived organoids in the presence of T1D patient PBMC

Model of Dysfunctional CD8 Treg Network

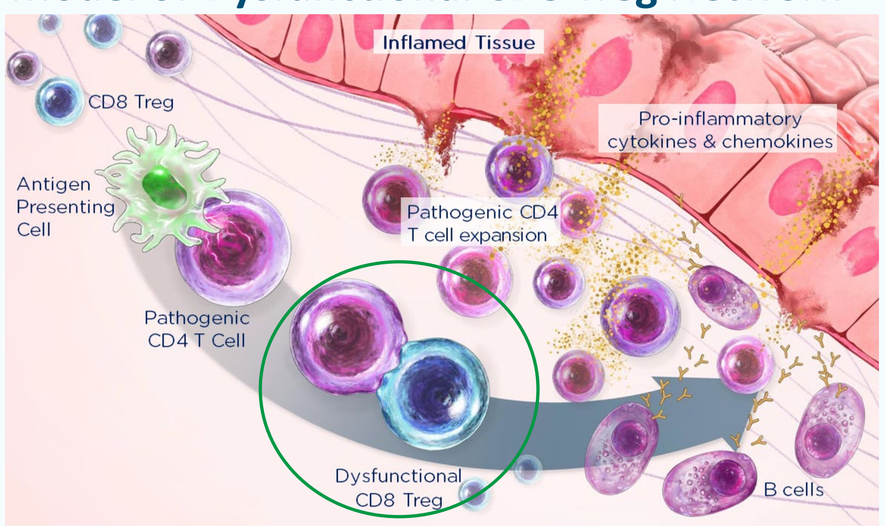

Figure 1. Model of a dysfunctional CD8 Treg network. When CD8 Treg fail to eliminate pathogenic CD4 T cells, CD4 T cells can expand, secrete proinflammatory cytokines, recruit and activate other inflammatory cells (e.g. pathogenic CD8 T cells), and induce autoantibody production. Collectively, this cascade results in the destruction of healthy tissue.

Dysfunctional CD8 Treg are Reduced Yet Present and Highly Responsive to TCR Stimulation in Patients More Than 5 Years After Diagnosis of Type 1 Diabetes

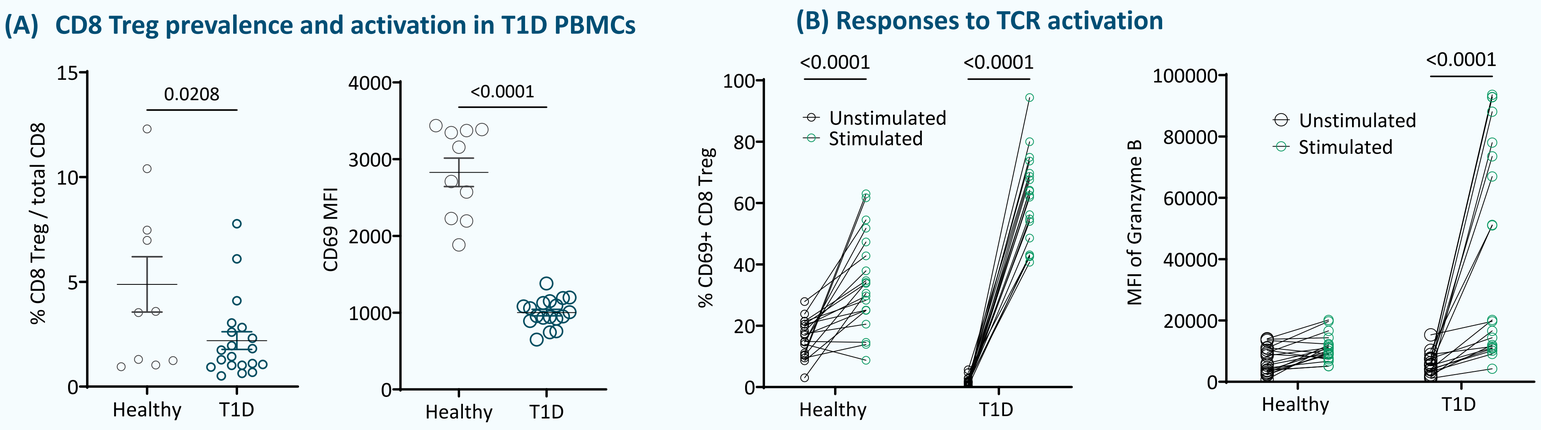

Figure 2: CD8 Treg in T1D patient PBMC collected more than 5 years since diagnosis are dysfunctional, but remain highly responsive to TCR stimulation (A) Proportion of CD8 Treg out of the total CD8 T cell population, and their activation status using flow cytometry analysis of prevalence and activation status (CD69). (B) PBMC from healthy (n=10) or T1D donors (n=20) were cultured for 24 hours in anti-CD3 and activation status and cytolytic capacity (Granzyme B) were assessed.

Antigenic Peptides Induce CD4 T Cell Activation in T1D PBMC

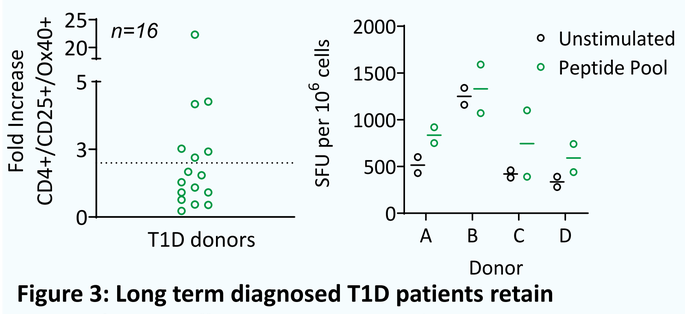

Figure 3: Long term diagnosed T1D patients retain autoantigen recall responses. T1D patient PBMC were stimulated with a pool of 11 peptides derived from GAD65, IA2 and PPI. (A) Increase in proportion of $CD4^+CD25^+Ox40^+$ cells relative to unstimulated. PBMC showing >2x fold increase were judged to be responders. (B) Number of IFNγ spot forming units (SFU) in 4 of the responding patients from (A).

Human Pancreatic Organoids Contain Relevant Cell Populations with Expected Localization

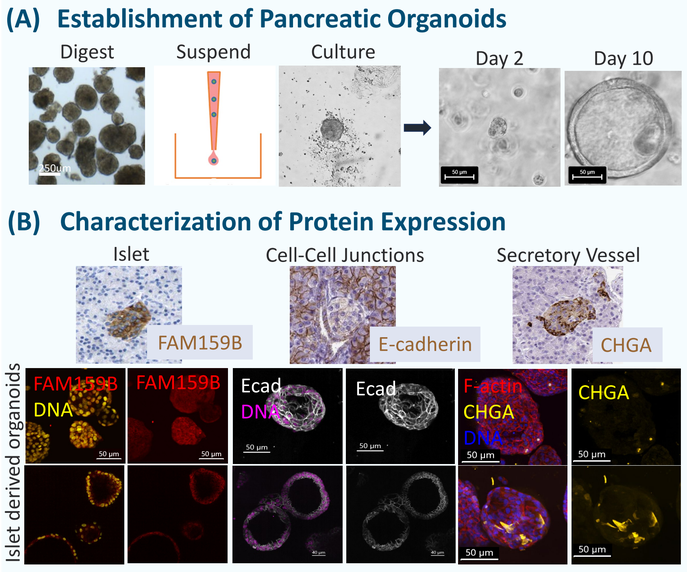

Figure 4: Pancreatic organoids were generated from freshly isolated human islets (A) and expression of islet markers was observed via immunocytochemistry (B). Islet marker FAM159B (red, left) and cell junction marker E-cadherin (white, middle) were expressed throughout pancreatic organoids. Chromogranin A (CHGA, yellow, left) was expressed in discrete regions, supporting a secretory phenotype.

Organoids Produce Functional β-Cell Markers: Pro-Insulin, C-Peptide, and Insulin

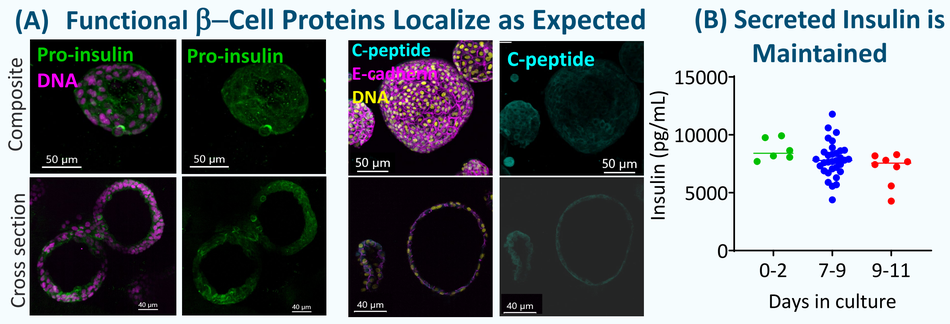

Figure 5: Pancreatic organoids have islet specific functional markers. (A) Organoids expressed β-Cell-specific markers Proinsulin (green, upper left) and C-peptide (cyan, upper middle), as detected by immunocytochemistry. (B) β-Cell secreted insulin was detected in pancreatic organoid culture supernatants for the duration of the experiment by immunoassay. (C) FAM159B (green) or NKx6.1 (cyan) positive islet cells also expressed the β-Cell functional markers C-peptide (black) and Pro-insulin (purple) as determined by flow cytometry. FMO and negative controls are shown in red.

Autoantigenic Peptide Stimulated T1D Patient PBMC Increase β-Cell Death in Pancreatic Organoids

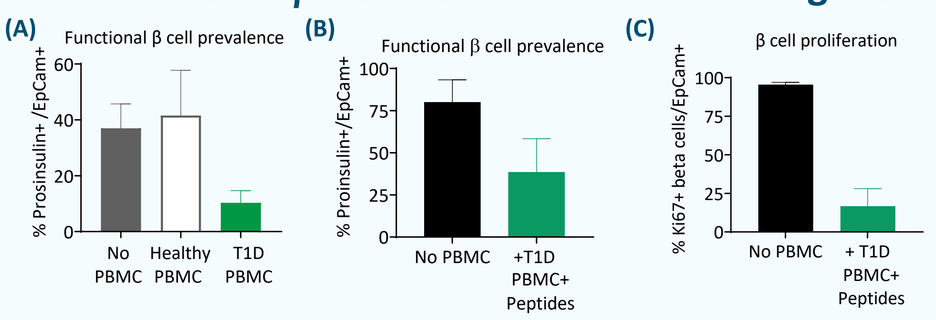

Figure 6: Assessment of pancreatic islet tissue destruction. (A) Pancreatic organoids co-cultured with PBMC from T1D patients had reduced functional β-Cells compared to healthy donor PBMC. (B) Autoantigenic peptides increased β-Cell death compared to organoids cultured in the absence of PBMC and (C) reduced β-Cell proliferation

T1D PBMC + Autoantigenic Peptides Induce β-Cell Death and Diabetogenic Cytokines

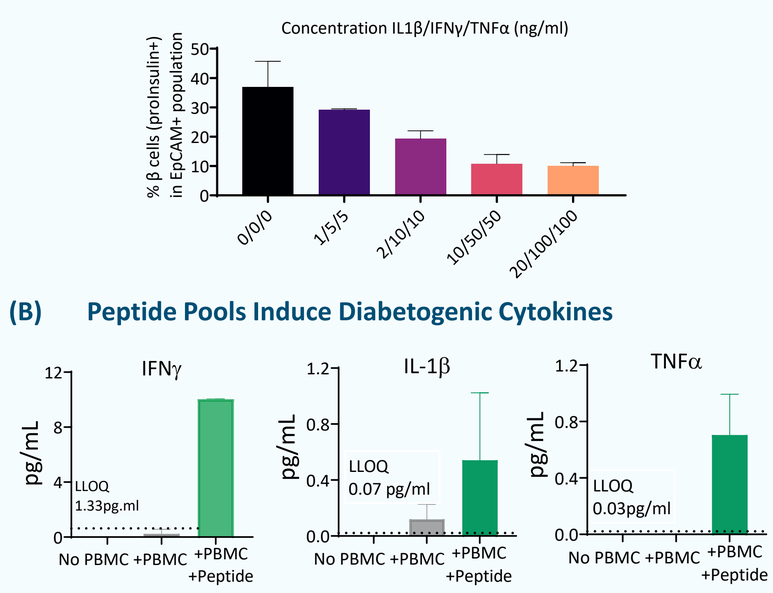

Figure 7: **Diabetogenic cytokines as a possible driver for β-Cell death.** (A) Pancreatic organoids cultured with increasing concentrations of diabetogenic proinflammatory cytokines caused a loss of viable Proinsulin+ β-Cells. (B) T1D PBMC+ organoid cocultures were stimulated with autoantigenic peptides, and increased secretion of IFNγ, IL1β, and TNFα, a possible driver for β-Cell death in Fig.6.

RESULTS AND CONCLUSIONS

- CD8 Treg from T1D patients appear reduced in prevalence and dysfunctional
- Ex vivo T1D organoid cultures allow complex assessment of in tissue immune responses
- CD8 Treg fail to control pathogenic T cell activation and cytokine production in T1D PBMC, and β–Cell damage in pancreatic organoids
- CD8 Treg functions in T1D PBMC can be restored
- Our data support the development of therapeutics designed to restore CD8 Treg functions in patients with T1D

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References: Li, et al. KIR + CD8 + T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. *Science,* 2022. DOI: 10.1126/science.abi9591