Demonstration of regulatory CD8 T cell prevalence, phenotype, and functions in autoimmune patients treated with a tolerizing peptide vaccine

MOZART THERAPEUTICS
Orchestrating The Immune System

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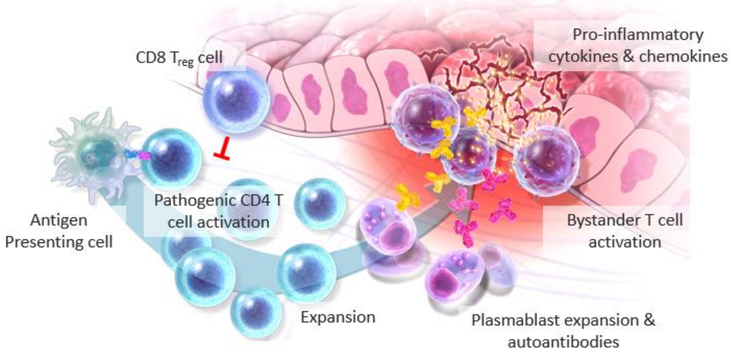
INTRODUCTION:

Others have described a subset of CD8 T cells with immunosuppressive characteristics in inflammatory disease settings. CD8 Treg cell activation, through a canonical T cell receptor, results in oligoclonal expansion and perforin dependent elimination of pathogenic CD4 T cells. We have demonstrated the CD8 Treg network in Celiac patients, and that CD8 Treg can be modulated to enhance their cytolytic elimination of pathogenic CD4 T cells. Here, we evaluate culture conditions to enhance CD8 Treg cell functions and describe the impact of a gluten tolerizing peptide vaccine on CD8 Treg in Celiac disease patients.

METHODS:

We examined the phenotype and function of Celiac patient-derived peripheral blood CD8 Treg cells using flow cytometry, multicolor immunohistochemical staining, supernatant analysis, and TCR sequencing. We then evaluated the impact of a gluten tolerizing peptide vaccine on the CD8 Treg network in Celiac patient derived peripheral blood and tissues.

In autoimmunity, regulatory CD8 T cells fail to control expansion of pathogenic CD4 T cells resulting in downstream inflammation



RESULTS:

Using our previously reported phenotypic and functional readouts to define CD8 Treg activity*, we describe culture conditions that support oligoclonal CD8 Treg cell expansion, plasticity, and cytolytic function. In patients treated with a gluten tolerizing vaccine, we found evidence for CD8 Treg expansion and preferential recruitment to duodenal tissues; however, pathogenic CD4 T cells were still detectable, suggesting insufficient CD8 Treg functional activation.

*Please see Mozart Poster #1090: *"Bispecific CD8 Treg Modulators Regulate A Novel Regulatory CD8 T cell Network And Eliminate Pathogenic CD4 T cells In Live Cell Co-Culture System"*, presented on Monday, May 9, 2022

Celiac patient-derived CD8 Treg cells have a distinct surface phenotype, cytokine secretion profile, and transcription factor profile

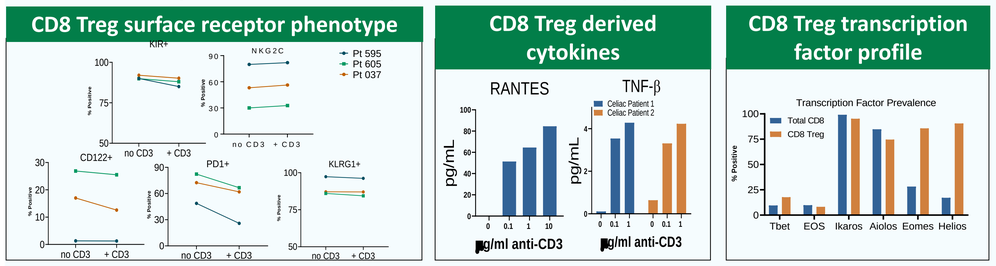


Figure 1 Celiac patient derived CD8 Treg were evaluated by flow cytometry when freshly isolated and following TCR stimulation

- CD8 Treg cells stimulated with increasing doses of TCR agonizing antibody produce inflammatory cytokines in a dose dependent fashion
- CD8 Treg cells express higher levels of transcription factors Helios and Eomes compared to total CD8⁺ T cells

Celiac patient-derived CD8 Treg cells use a cytolytic mechanism in the absence of APCs to specifically eliminate pathogenic CD4 T cells and reduce inflammatory

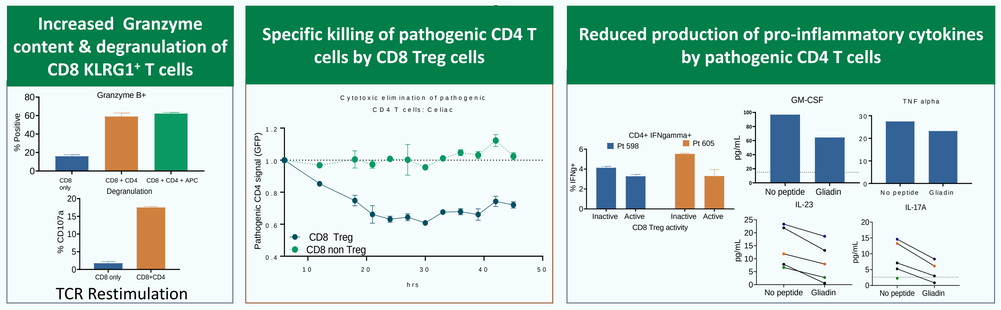


Figure 2 Celiac patient PBMCs were expanded in the presence of cytokines for 12 days. KLRG1⁺ CD8 Treg cells were isolated and cultured with pathogenic CD4s

- CD8 Treg cells showed increased cytolytic activity as measured by flow cytometry when co-cultured with pathogenic CD4s even in the absence of APCs
- Pathogenic CD4 T cells are reduced when co-cultured with CD8 Tregs
- Pro-inflammatory cytokines produced by pathogenic CD4 T cells are reduced by specific killing by CD8 Treg cells

CD8 Treg cells are present in blood and affected tissues of autoimmune patients and increase following gluten exposure

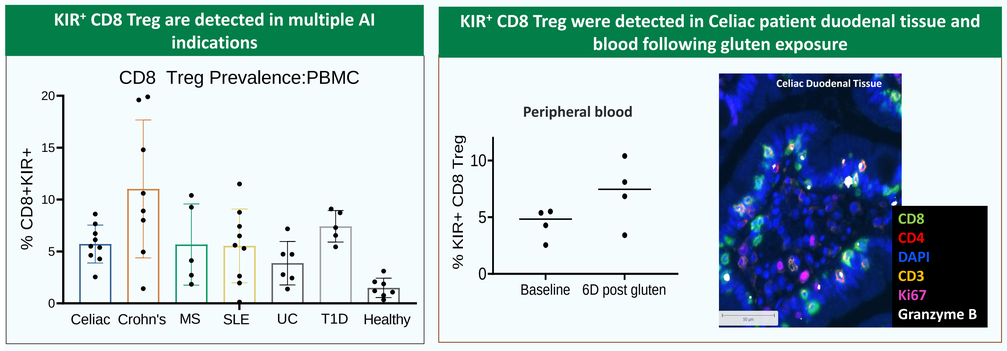


Figure 3 CD8 T reg cell network presence was evaluated in multiple AI indications, including in PBMC derived from Celiac patients before and after gluten consumption

- CD8 Treg cells are more prevalent in PBMCs from donors with autoimmune diseases [Celiac, Crohn's, Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE), Ulcerative Colitis (UC) and Type 1 Diabetes (T1D)] compared to healthy donors
- CD8 Treg cells are detected in duodenal tissues from Celiac patients following gluten consumption
- Gluten exposure causes a similar increase in KIR⁺ CD8 Treg cells in the peripheral blood of Celiac patients

Cytokine conditions alter CD8 Treg phenotype

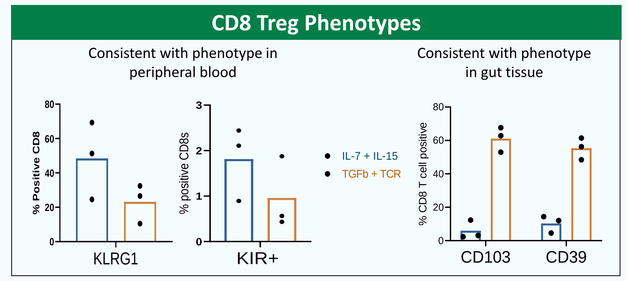


Figure 4 Celiac patient derived CD8 T cells were cultured in varying cytokine conditions before surface makers were evaluated by flow cytometry

- Expansion in IL-7 and IL-15 results in higher expression of KLRG1⁺ and KIR⁺ CD8 T cells, consistent with a peripheral Treg population
- Expansion in TGFβ and TCR stimulating antibodies increased levels of CD103⁺ and CD39⁺ CD8 T cells, consistent with a tissue-resident population

Treatment with a gluten-tolerizing vaccine increases CD8 Treg cells in blood of Celiac patients

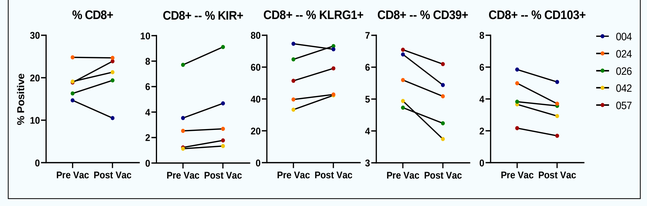


Figure 5 Celiac patient derived CD8 Treg were evaluated by flow cytometry before and after treatment with a tolerizing gluten peptide

- KIR⁺/KLRG1⁺ CD8 T reg cells were more prevalent in blood post vaccination, indicating the vaccine increased circulating number of CD8 T regulatory cells
- A reduction in CD39⁺/CD103⁺ CD8 Treg cells in the periphery might signify their recruitment to the gut post vaccination

CONCLUSIONS:

- We have demonstrated the presence of distinct CD8 Treg in peripheral blood and tissue in multiple autoimmune indications
- CD8 Treg cells have been demonstrated to possess a cytolytic mechanism of action
- Cytolytic elimination of pathogenic CD4 T cells can occur in the absence of APCs
- CD8 Tregs respond favorably yet insufficiently to treatment with a gluten-tolerizing peptide
- CD8 Treg directed therapies may have therapeutic effects in autoimmune disease

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Contact:
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References:

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