A Newly Discovered Regulatory CD8 T Cell Network Has The Potential To Regulate And Eliminate Pathogenic CD4 T Cells In Autoimmune Mediated Disease Of The Gut

Crane, CA; Bowser, J; Fasnacht, R; Gardell, JL; Maurer, M; Julien, S; Templeton, ML; Therriault J; Swiderek, KM

MOZART THERAPEUTICS

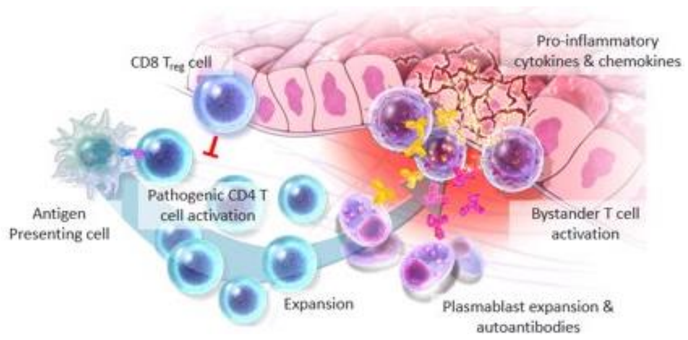
Orchestrating The Immune System

Introduction

Several groups have described a subset of immunosuppressive CD8 T cells in inflammatory disease settings, suggesting a protective role for select CD8 T cells in autoimmunity. We hypothesized that a dysregulated regulatory CD8 T cell (CD8 Treg) network was similarly involved in the pathology of autoimmune diseases of the gut, including Celiac disease, Crohn's, and Ulcerative Colitis. Here, we have demonstrated the presence of this CD8 Treg cell network in Celiac patients and its potential to reduce pathogenic CD4 T cell prevalence.

We characterized a surface marker and functional phenotype associated with CD8 Treg prevalence and consequences of TCR-mediated activation. Using this phenotype, we then confirmed the presence and prevalence in Celiac patient PBMCs and duodenal tissues (Fig.1). Activation resulted in a rapid and specific cytolytic mechanism of action CD8 Treg cells. Using Celiac disease as platform to introduce a known antigenic trigger for autoimmunity, we defined CD8 Tregs and their activity in patients with other autoimmune diseases, including Crohn's disease and Ulcerative Colitis.

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



Methods

- Using antigen induced animal models of experimental autoimmune encephalomyelitis (EAE), as well as Celiac patient-derived PBMCs and tissues, we evaluate CD8 Treg functions
- Methods include flow cytometry, soluble analyte analysis, and immunohistochemistry.
- We describe co-culture assays to define the phenotype, relevant mechanisms of action, dosing, and kinetics of CD8 Treg functions after exposure to gluten peptides

CD8 Treg cells are mobilized by surrogate peptide cocktail and reduce pathogenic CD4 T cells

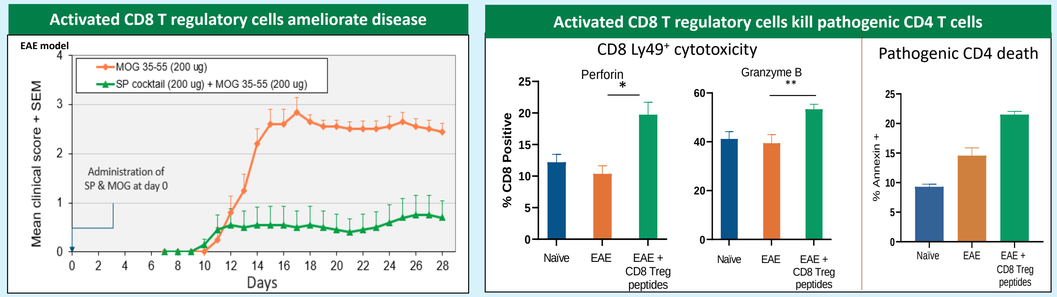


Figure 1 EAE was induced in C57/BL6 mice by MOG peptide and assessed for disease onset and severity. One cohort (green) was simultaneously injected with a surrogate peptide cocktail that has been demonstrated to suppress disease.

- Surrogate peptides (SP) activate CD8 Ly49$^+$ T cells and prevent disease
- ↑ CD8 Ly49$^+$ T cells ↓ pathogenic CD4 T cells
- CD8 Ly49$^+$ T cell mobilization increases cytolysis & pathogenic CD4 T cell killing

Celiac patient-derived CD8 Treg cells use a cytolytic mechanism to specifically eliminate pathogenic CD4 T cells and reduce inflammatory cytokines

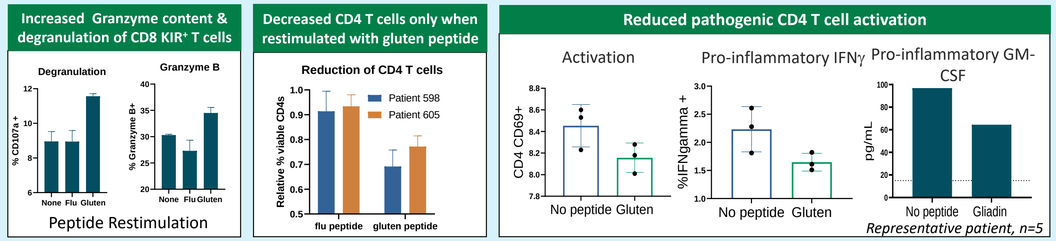


Figure 2 Celiac patient PBMCs were expanded in the presence of cytokines for 12 days. CD8 Treg and CD4 T cells were isolated and cultured with freshly isolated autologous antigen presenting cells pulsed with gluten or flu peptide cocktails for 48 hr and analyzed by flow and ELISA.

- Pathogenic CD4 T cells are reduced following gluten, but there was no change following flu peptide restimulation
- CD8 Treg in Celiac patients are not inherently dysfunctional when ratio of activated CD8 Treg to pathogenic CD4 T cells are increased

Celiac patient-derived CD8 Treg cells have a distinct surface phenotype, cytokine secretion profile, and impact on pathogenic CD4 T cell-derived inflammatory cytokines

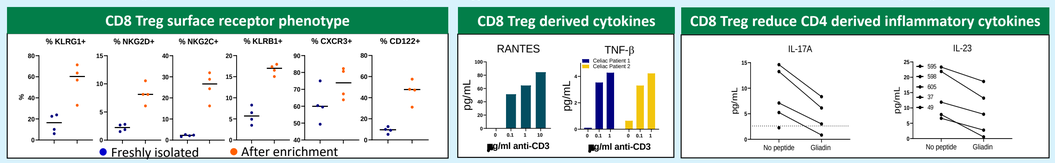


Figure 3 Celiac patient derived CD8 Treg were evaluated by flow cytometry when freshly isolated and following cytokine enrichment and activation. CD8 Treg stimulated with increasing doses of TCR agonizing antibody produce inflammatory cytokines in a dose dependent fashion and reduce pro-inflammatory cytokine production in coculture following gliadin peptide restimulation.

CD8 Treg cells are present in blood and affected tissues, and increase following exposure to gluten

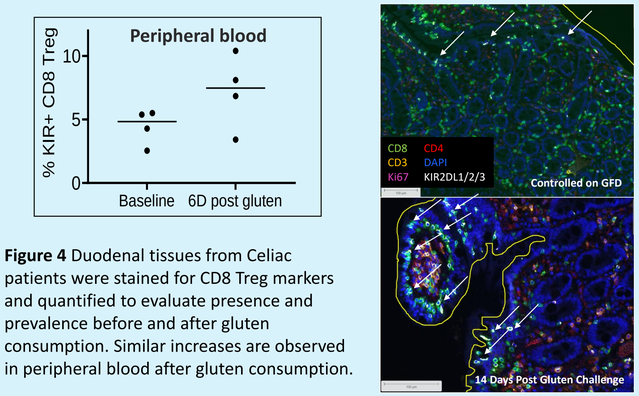


Figure 4 Duodenal tissues from Celiac patients were stained for CD8 Treg markers and quantified to evaluate presence and prevalence before and after gluten consumption. Similar increases are observed in peripheral blood after gluten consumption.

CD8 Treg cells are elevated in other autoimmune diseases

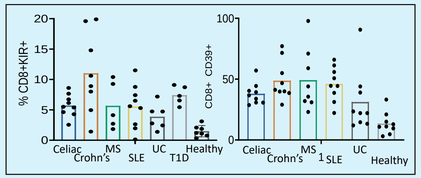


Figure 5 PBMCs from healthy donors, patients with Type 1 Diabetes (T1D), Ulcerative Colitis (UC), Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), Crohn's, or Celiac were evaluated for CD8 Treg prevalence

Conclusions

- We describe a novel CD8 T regulatory cell network present in autoimmune mediated gut disorders and other inflammatory disease.
- CD8 T regulatory cells use a cytolytic mechanism to specifically eliminate pathogenic CD4 T cells
- Recovery of CD8 T regulatory cell functions may suppress pathogenic T cells and reduce severity of inflammatory disease
- This network may be targeted by immune-modulating biologics

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Contact:
Follow up questions can be directed to Kristine Swiderek, Chief Scientific Officer, Mozart Therapeutics kswiderek@mozart-tx.com or by visiting the website at https://www.mozart-tx.com/

References:
Saligrama et al. Opposing T cell responses in experimental autoimmune encephalomyelitis. *Nature*. 2019 doi: 10.1038/s41586-019-1467-x