
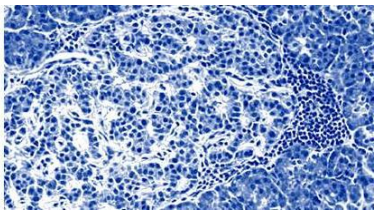

Metabolic Regulation of Autoreactive CD4+ T cells Prevents Autoimmune Diabetes

AUTHORS

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PURPOSE

Type 1 Diabetes (T1D) is a chronic inflammatory disease that results from immune-mediated destruction of the insulin- secreting, pancreatic β cell, with CD4+ T cells largely mediating pathogenesis. Over time, this destruction results in loss of β cell mass and function, reduced insulin- secretion, and a failure to control blood glucose levels; ultimately culminating in hyperglycemia. Current treatment regimens call for the daily administration of exogenous insulin to maintain euglycemia, **however insulin is not a cure**. The goal of our work is to take advantage of the metabolic changes occurring as T cells move through the various stages of their lifecycle. Specifically, CD4+ T cells undergo metabolic reprogramming upon activation, and switch to a glycolytic profile, similarly to cancer cells. Inhibitors of the glycolytic pathway, including the drug PFK15, a competitive inhibitor of a key rate limiting enzyme in glycolysis, have shown great promise in FDA clinical trials. *We hypothesize that administration of the anti- glycolytic PFK15 will inhibit the activation and clonal expansion of diabetogenic CD4+ T effectors, thereby preventing disease onset.*

METHODS

To determine the ability of PFK15 to inhibit CD4+ T cell responses to diabetes relevant antigens *in vitro*, we used the NOD.BDC.2.5.TCR-Tg mouse (BDC2.5), which recognize a β cell protein and can transfer diabetes. Splenocytes from these mice were stimulated *in vitro* for 24- 72 hours with their cognate peptide +/- PFK15. Supernatants were collected for lactate and effector cytokine production, and T cells were stained for activation markers and analyzed by flow cytometry. To evaluate the ability of PFK15 to prevent T1D *in vivo*, we used an adoptive transfer model where BDC2.5 splenocytes are transferred into NOD.*scid* recipients. This induces diabetes in approximately 14 days. A cohort of animals received PFK15 soluble drug i.p. (10 mg/kg) or PFK15 microparticles (MP) ($1/4^{th}$ the dose of soluble drug) subcutaneously at a site in close approximation to the pancreas. Animals receiving blank MPs served as controls. Animals were treated and monitored every 3 days for onset of

hyperglycemia and body weights were measured to assess toxicity of the drug. Pancreata were harvested at sacrifice for histological analyses and immunofluorescence staining.

SUMMARY OF RESULTS

Our *in vitro* results indicate that PFK15 soluble and MP formulations are capable of inhibiting CD4+ T cell activation, proliferation, and effector function. In adoptive transfer studies, PFK15 proved to prevent or delay disease onset, with no apparent toxicity. Histological analysis revealed invasive insulitis in blank MP treated animals while peri- islet insulitis was observed in PFK15 treated groups, correlating with preservation of β cell mass as measured by insulin staining by immunofluorescence. Lastly, we found that there was increased expression of the master regulatory T cell transcription factor FoxP3 in islets treated with PFK15, indicating increased Treg control within the islet.

CONCLUSIONS

The results suggest that targeting glycolysis as a means to control self-reactive T cells is a novel and effective approach to prevent T1D. Further, we have shown that MP formulations of PFK15, even when delivering a lesser dose, is able to delay adoptive transfer of disease at the site of inflammation. It is our hope to optimize MP delivery in an effort to limit systemic off target effects of soluble drug. Further, these studies will provide information on the substrates and metabolites that drive CD4+ T cell differentiation that can be used to better understand how to manipulate T cell responses in a number of different settings, including the tumor microenvironment and chronic infection.