FID- ISPAD Research Grant 6-month Progress Report

Timothy P. Foster MD

Project Title: Mechanism of Diabetes Prevention in Combination Proinsulin-GAD RNA

Lipid Particles in NOD mice

Project Summary:

Our overall objective is to advance a safe and efficacious mRNA immunotherapy in type 1 diabetes prevention, while interrogating mechanistic underpinnings of its immunologic effects that can be co-opted as biologic correlates for activity in translational models before human clinical trials. The objective of this study is to determine the differences in immune cell populations in murine pancreata and immune organs that drive the efficacy of anionic proinsulin-GAD mRNA lipid particles for diabetes prevention. We hypothesized that CD4 and CD8 T-cells populations leave the pancreas and pLN and traffic to the spleen for induction of tolerance after administration of anionic proinsulin-GAD RNA lipid particles.

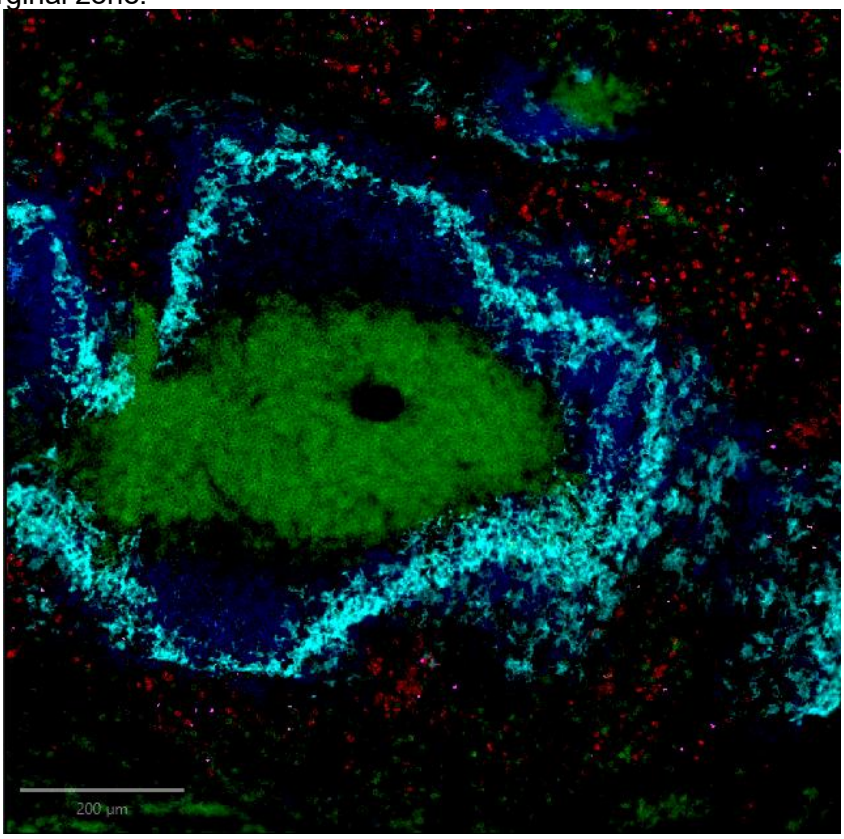
Progress to date:
Thus far, we have completed the treatment of the NOD mice with mRNA lipid particles aggregates (LPAs) and collected tissue (spleen and pancreata) at baseline, 6 hours post dose, 24 hours post dose, and 48 hours post dose to evaluate the differences in immune cells over time. We have visualized the changes in the immune cells in the spleen after mRNA immunotherapy wherein within 6 hours after dosing Ly6g granulocytes increase in the red pulp in the spleen followed by a large increase in CD4 T-cells in red pulp and regulatory T-cells within the white pulp, both in the B-cell zone and T-cell zones (periarteriolar lymphoid sheath). This further hints at our mechanisms of action of preventing diabetes in NOD via induction of immunotolerance and increase in regulatory T-cells.

Plan for the remaining project time:
Unfortunately, our pancreata were sliced too thick and were therefore insufficiently stained. We have since repeated the pancreata slides and are currently re-staining and re-imaging the tissue. We will correlate the immune cells changes in the spleen with those visualized at the same time points in the murine pancreas.

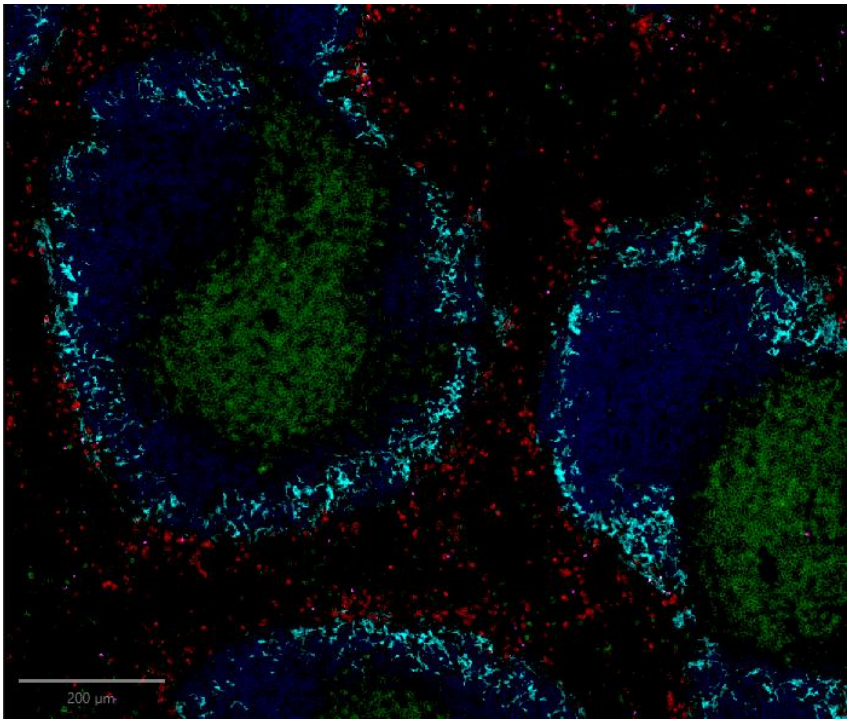
Publications or Presentations:
No publications at this time. Planning to draft after completion.

Figure 1

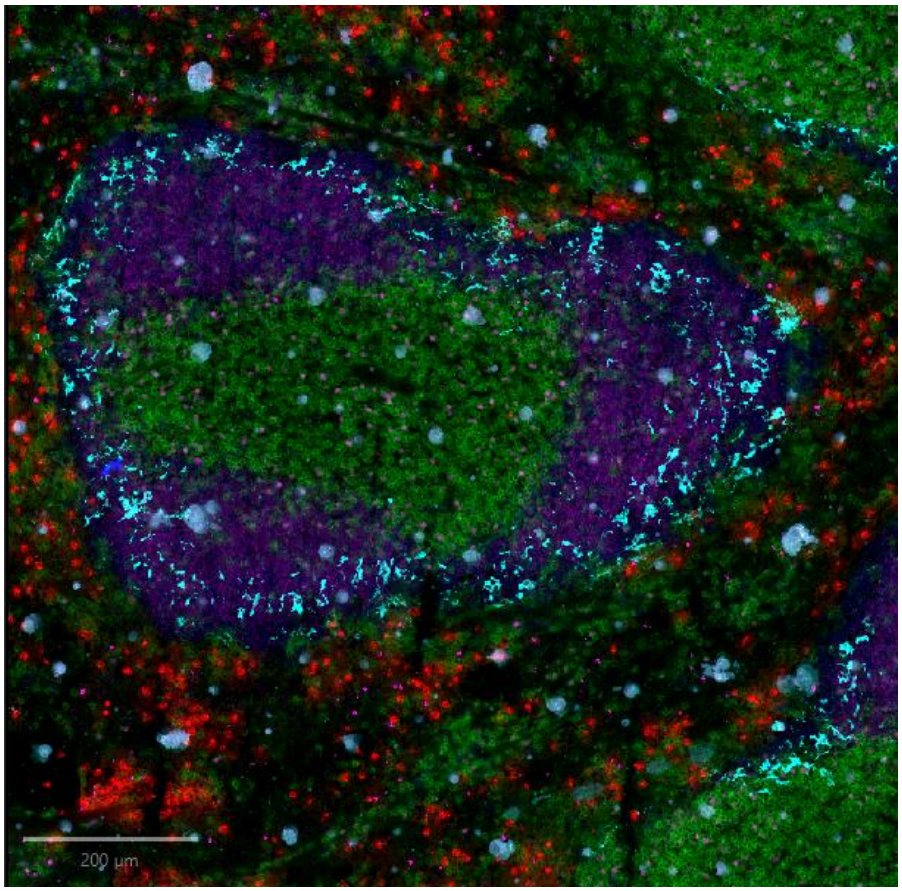
A) Untreated NOD Mouse Spleen: Baseline; Cyan (CD169+ macrophages) demarcates the marginal zone.



B) 6 hours post mRNA LPA NOD Mouse Spleen: Increase in Ly6g+ granulocytes in red pulp



C) 24 hours post mRNA LPA NOD Mouse Spleen: Increase in CD4 T-cells in red pulp (migrating T-cells). Significant increase in FOXP3+ cells in B-cell zones and periarteriolar lymphoid sheath representing follicular regulatory T cells.



Key: Purple=FOXP3; Cyan=CD169; Blue=CD19; Green=CD4, Red=Ly6g