ISPAD-JDRF Fellowship Final Report

Project Title: Diabetes Prevention with proinsulin mRNA Vaccines in the NOD Mouse Model

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Background

Type 1 diabetes (T1D) is a T cell-mediated autoimmune disease that targets and destroys the insulin secreting β-cells located in the islets of Langerhans in the pancreas (1). Due to its short- and long-term complications, T1D is both a daily and life-long burden for individuals and their families. Exogenous insulin is needed immediately for those diagnosed with T1D and is required for their lifetime. In the absence of therapy, severe, unsustainable metabolic derangements develop including hyperglycemia and ketoacidosis with associated sequelae including rapid wasting and possible death.

T1D is the result of a multifaceted autoimmune attack on β-cells and develops in genetically predisposed individuals who encounter a stimulus that breaks immune tolerance in β-cell-specific self-reactive T cells (2). Due to the autoimmune etiology of T1D, an immunomodulatory-based therapy is a promising approach to preserve β-cells, which could improve partial endogenous glycemic control. Preventing or stopping β-cell destruction using a disease-modifying therapy, instead of just managing symptoms of hyperglycemia, is a way to lessen the daily burden on those with T1D and reduce the significant disease and therapy-associated morbidity and mortality (3). Many immunotherapies aim to downregulate the immune responses to β-cell antigens in mice and humans by inducing tolerance with antigen administration including GAD alum, nasal/oral insulin, and peptide injections (4). DNA vaccines have also previously been used as immunotherapy in studies and clinical trials with initial success in decreasing rates of diabetes in the nonobese diabetic (NOD) mouse model, but no lasting effects among T1D humans (4,5).

RNA vaccines attained notoriety with the COVID-19 pandemic. However, even before this, RNA nanoparticle vaccines were being studied as a cancer immunotherapy to provide personalized anti-tumor treatment (6). RNA can be loaded into multi-lamellar lipid nanoparticles and when administered intravenously will reliably activate antigen presenting cells throughout organs of the immune system such as spleen, lymph nodes, and bone marrow (7). This may more intensely stimulate an immune response and create lasting immune tolerance, filling the gap of previous immunotherapies including DNA plasmids. Most recently, this technology has expanded to use among autoimmune disease models. In multiple sclerosis mouse models, administration of mRNA vaccines suppressed the disease and aided in the development of regulatory T cells (8). This highlights the need for research utilizing mRNA vaccines in other autoimmune conditions such as T1D to expand the frontier of RNA technology and search for a cure to type 1 diabetes.

Hypothesis and Aim:

We hypothesized that a multi-lamellar liposome vaccine with proinsulin mRNA will prevent diabetes in an at-risk diabetes model by conferring immune tolerance. Our specific aims were to measure the effect of different doses of proinsulin mRNA multi-lamellar liposome vaccine on the time to diabetes development and overall rate of diabetes in the mouse model.

Methods:

Proinsulin II was cloned into plasmid backbones, transcribed into mRNA, and loaded into nanoliposomes composed of a DOTAP lipid backbone (RNA:lipid-particle aggregates (LPA) ratio 1:15). mRNA expression was validated with HEK293 cell culture transfection (Figure 2). NOD/ShiLtJ strain mice were obtained from Jackson Laboratory at 4 weeks of age. Group 1 received four IV tail vein injections of proinsulin mRNA vaccines weekly starting at 8 weeks of age. Group 2 received three IV proinsulin mRNA vaccines at 8 weeks of age every other day plus a single booster dose at 14 weeks of age. Group 3 three IV GFP (green fluorescent protein) mRNA vaccines at 8 weeks of age every other day plus a single booster dose at 14 weeks of age received. Group 4 remained untreated (n=25 mice/group). Mice were monitored after injections to assess for vaccine reactions and checked weekly for diabetes (glucose >250 mg/dl on two consecutive measurements one day apart). Kaplan Meier survival curves, relative risk, and median time to diabetes were used to compare diabetes development between groups (GraphPad Prism 10.0.2). Fisher's exact test was used to compare rate of adverse events between vaccine groups.

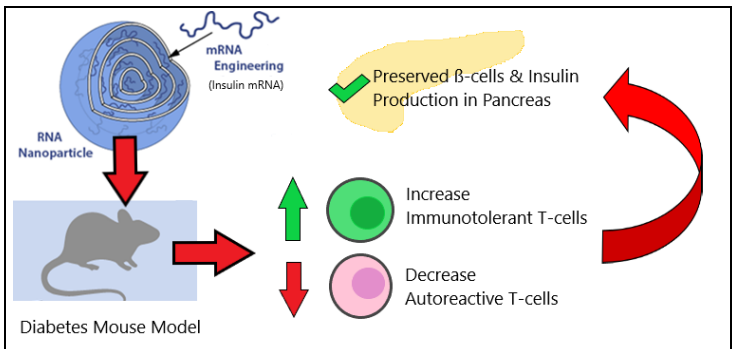

Figure 1: Overall research schema

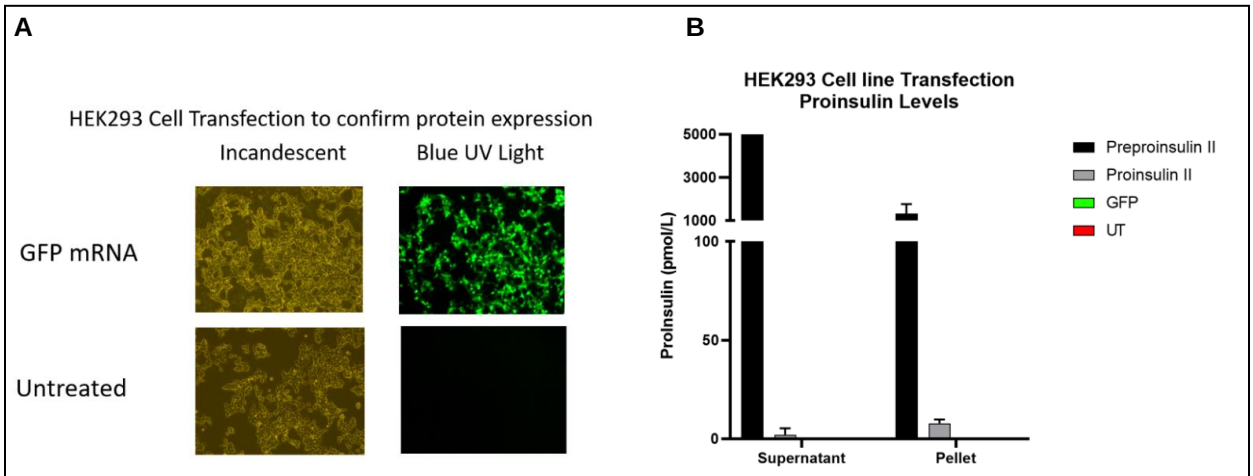

Figure 2: A. Confirmation of vaccine uptake and protein expression in HEK293 cells.
B. Proinsulin II production by HEK293 cells treated with preproinsulin II, proinsulin II, and GFP mRNA LNPs vs untreated (UT) cells. GFP treated and UT cells had no proinsulin translation or secretion.

Results:

Adverse Events: In Group 1, proinsulin mRNA vaccines were well tolerated until the 4th weekly dose when 21% (5/25) of mice experienced an inflammatory response within 30 minutes of vaccination leading to cardiorespiratory collapse. Group 2 experienced this reaction sooner at the 3rd dose in 12% (3/25) of mice; the 4th "booster" dose at week 14 was again well tolerated

without further deaths. Overall vaccine reactions were not significantly different between proinsulin II groups (p=0.703). Group 3 (GFP mRNA) also experienced vaccine reactions at the 4th dose (4/25 deaths), highlighting that the inflammatory effect is not specific to the type of mRNA.

Diabetes Development: The overall rate of diabetes by 32 weeks of age in all proinsulin II mRNA vaccinated mice was 70.6% compared to 95.2% of untreated mice (relative risk 0.741 95% CI 0.56-0.955), with no significant difference in diabetes development between proinsulin vaccine groups (p=0.968). The diabetes rate of mice receiving GFP mRNA vaccines was similar to proinsulin vaccines at 70.5%. Median time to diabetes was 19 weeks in Group 1, 21 weeks in Group 2, 18 weeks in Group 3, and 16 weeks in untreated control (Figure 3).

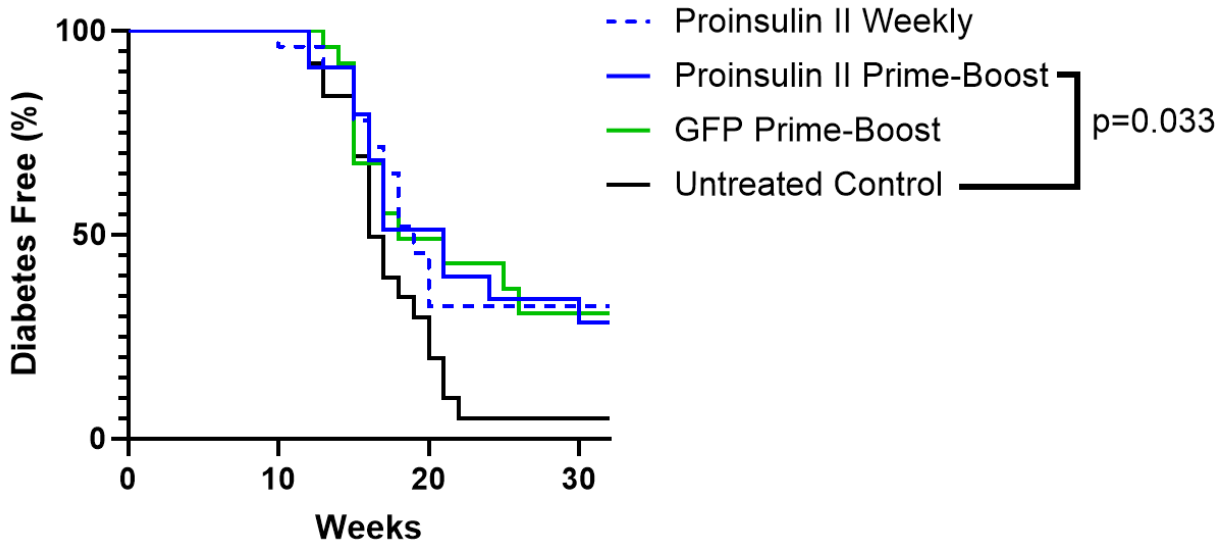

Figure 3: Diabetes rate of female NOD mice treated with four IV doses of cationic mRNA vaccines at 8 weeks of age

Conclusions:

Cationic proinsulin mRNA multi-lamellar liposome vaccines may prevent diabetes development in NOD mice. However, GFP vaccines also significantly decrease the rate of diabetes in mice, emphasizing a significant nonspecific, immunomodulatory effect of mRNA lipid nanoparticles. The rate of vaccine reactions and diabetes development were not significantly different with a weekly vaccine dose schedule compared to a prime-boost approach. Reactions occur with both endogenous and foreign mRNA. However, increased time between treatment doses delays the timing of adverse reactions.

Future Directions:

Further mechanistic studies are needed to delineate whether vaccine effects are from true immunotolerance or altered cell trafficking (or both) in proinsulin versus GFP mRNA vaccines. Future experiments investigating vaccine modifications to reduce side effects and improve efficacy are additionally required to facilitate the development of a translatable therapy for human type 1 diabetes.

References:

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