Jeremy Racine Ph.D. Updates

Update on 3-14-18

In part through the contributions of the DRC, I have now published a manuscript in Diabetes https://doi.org/10.2337/db17-1467 detailing the creation of several new mouse models for type I diabetes (T1D) research and therapy development. Genes encoding what are called major histocompatibility complex (MHC) proteins (designated HLA in humans) are the primary contributors to T1D development in both humans and the NOD mouse model. Using a genetic engineering technique known as CRISPR/Cas9, I have eliminated the murine MHC encoding genes in NOD mice and replaced them with human counterparts called HLA-A2 and B39 hypothesized to be important in T1D development. The HLA-A2 genetic variant is found in a sizeable portion of Caucasian type I diabetic patients. HLA-B39, while being a rare variant, has been associated with patients that have extreme early onset T1D. Production of these genetically engineered NOD mice allows them to be used to develop and therapies tailored specifically to patients expressing these HLA molecules. Work is currently ongoing using these mice to test a therapy tailored to HLA-A2 since this molecule characterizes a preponderance of T1D patients.

Another CRISPR generated ND mouse detailed in the above manuscript will allow for the expression in the absence of murine counterparts of combinations of HLA molecules contributing to T1D development in humans. Additionally, these models are being further engineered to allow the future transplantation of immune cells from diabetic patients in order to test drugs in mouse-"test-tubes" before attempting their use in patient populations.

Update on 10-10-17

Just prior to initiation of this project, I successfully removed the two MHC Class I genes found in NOD mice. I proposed to target the Class II variant found in these NOD.cMHC1$^{-/-}$ mice in order to generate a new "blank slate" mouse model (NOD.cMHC$^{-/-}$) to allow the introduction of Class I or Class II HLA combinations prevalent in diabetic patient populations. I have now successfully generated this mouse and am in the final process of characterizing it and plan on submitting a manuscript in the coming month(s) so that the entire diabetic research field has access to this new model. Additionally, I have shared this mouse with a collaborator who is in the process of moving these new mutations into an immunodeficient mouse model (NSG mice) so that human blood cell populations can be transferred into these mice and potential immune therapies can be tested. This novel immunodeficient version will have utility far beyond the field of diabetes. The next step in this project is to introduce two HLA Class I variants found in subsets of diabetic patients, either HLA-A2.1 or our newly characterized-in-mice HLA-B*39:06. Once these genes have been introduced, HLA Class II variants will then be introduced into these two new models.

While working on developing this next generation mouse model, I proposed to begin testing a "vaccination-like" therapy in which insulin and IGRP peptides are coupled to small "microspheres." Recently completed analyses in the first-generation mouse model (NOD.β2m$^{-/-}$.HLA-A2.1) indicates that when injections are initiated just prior to diabetes onset, the combination therapy with all four peptides drastically reduced progression to diabetes. Treatment

with the two insulin peptides was partially effective, and treatment with the two IGRP peptides was no better than controls. Additionally, preliminary experiments indicate that short-term therapy (3 injections) is close to providing some protection compared to controls, and additional cohorts are now being followed to determine if there is in fact a statistical significance in protection. Studies using the next generation mouse model NOD.cMHC1$^{-/-}$.HLA-2.1 are now underway to compare the findings to the results with the first-generation model. This next generation model provides us the opportunity to determine whether this therapy shows efficacy after the appearance of anti-insulin autoantibodies, or whether it has to be initiated in as-risk individuals prior to the appearance of these antibodies in circulation. The next step in this project is to characterize the mechanisms of disease protection and to determine whether anti-insulin autoantibody status prior to initiation of treatment affects efficacy of the therapy.

Update on 5/19/17

This project has two aims, both of which have been initiated. The first aim of this project is to create new models that carry specific genetic traits found in the type 1 diabetes patient population so that therapies are designed with more specificity. To accomplish this, the mouse model currently used in research has to be prepared to receive these new genetic traits by first removing the mouse genes for these traits. In particular, there are three genes that have to be removed before we can start to introduce some of these genetic traits from diabetic patient populations. Two of those genes have already been removed, and work on the 3rd gene has been initiated and several candidate models are currently being tested. The next step of this project will be to determine the best of these new models with which to start introducing traits from diabetic patient populations.

The second aim of this project is to develop a therapy designed specifically for one trait found in a sizable portion of diabetic patients, specifically those patients carrying the genetic allele HLA-A*0201. This therapy, which is a vaccine-like approach, has already shown promise in a proof-of-principle 1st generation mouse model carrying this genetic trait. Work has been initiated to repeat these results in a 2nd generation model carrying this trait. This 2nd generation model will allow the fine tuning (based on specific blood markers) the necessary timing of the first dose of this “vaccine”. Additionally, I plan on determining whether this therapy can be used over a short time frame (<3 months), or if it will require periodic injections to maintain protection for diabetes development. Finally, I will be determining how specifically this therapy is re-educating the immune system in order to make improvements for this therapy, and to educate the future design of similar therapies for other genetic traits.