I. Over the summer, I had the opportunity to participate in the Medical Student Summer Research Program. I performed basic science research in the laboratory of of the Division of Endocrinology in the Department of Medicine. His laboratory is working to improve the efficacy of islet cell transplantation as a treatment for type 1 diabetes. Because my time spent in the lab was both enriching and enjoyable, I hope to continue my work over the next several years through the Research Thesis Program.

A portion of the research being performed in Dr. 's lab is devoted to looking at several factors or molecules that may increase the likelihood of islet cell survival once transplantation has occurred. One of these molecules is insulin-like growth factor I (IGF-I), a peptide synthesized and secreted predominantly by the liver but also produced locally by practically every tissue in the body. Recent studies have shown that this molecule plays an important role in the development and maintenance of the beta cell mass. Because it possesses these interesting qualities, I plan to examine the role that IGF-I might have in increasing the efficacy of islet cell transplantation.

My summer work focused primarily on discovering the pathways by which IGF-I exerts its mitogenic effects on pancreatic beta cells. I obtained my results through cell culture, Western blot analyses, and flow cytometry. I had the opportunity to share my progress with my colleagues at the poster session. The next logical step in my research is the *in vivo* application of IGF-I, which represents a challenge I have yet to experience but am ready to undertake. Through the Research Thesis Program, I hope to continue to learn more about endocrinology from a variety of perspectives, both in the laboratory and possibly in a clinical setting. My ultimate goal is to produce a paper on the overall results of my research. Most importantly, my participation in the Research Thesis Program will further my insight into a career in academic medicine and continue my exposure to the research mission of the Feinberg School of Medicine.

II.

Specific Aims

The purpose of this project is to assess whether IGF-I has the ability to increase the efficacy of pancreatic islet cell transplantation. We hypothesize that the delivery of IGF-I from a microporous scaffold used as a platform for pancreatic islet transplantation will enhance islet function through its effects on cellular apoptosis and proliferation. The specific aims of this project are as follows.

1. To determine if IGF-I delivered from a microporous scaffold will enhance islet function post-transplant in a murine model of diabetes.
2. To determine if the effect of IGF-I on function post-transplantation is secondary to decreased islet cell apoptosis and/or increased islet cell proliferation.

These outcomes can be assessed by determining the number of mice that become euglycemic and the time required for the restoration of euglycemia as well as by performing immunohistochemistry assays.

Background and Significance

Though cell replacement therapy offers the potential to treat type 1 diabetes, the best method by which to transplant islet cells is still under investigation. Intrahepatic infusion of pancreatic islets is the standard approach to clinical islet transplantation, but it has a variety of limitations due to the microenvironment of the site, leading to the need to transplant a large number of islets. , in collaboration with from the Department of Chemical and Biological Engineering and Dr. from the Department of Surgery, has recently demonstrated that islets can be successfully transplanted on a microporous polymer scaffold in the intraperitoneal space. This method is advantageous for a variety of reasons. First, it removes the islets from the microenvironment of the liver. Second, the scaffold has many unique properties that may allow for better islet survival. Its porous nature allows for vascularization of the islets, as well as nutrient transport via diffusion through the scaffold. Finally, the scaffold is capable of delivering bioactive molecules to the islets, which provides a method of creating a better microenvironment. Thus, this approach to pancreatic islet transplantation has the potential to increase overall transplant efficacy.

Two of the current impediments to successful islet transplantation are the death of a substantial number of islet cells in the early transplant period and the limited supply of islets. An enhancement of the microenvironment into which islets are transplanted will allow for the transplantation of fewer islets into any given individual. In performing the proposed experiments, we hope to gain a better understanding of how the microenvironment of the islets may affect islet cell survival and proliferation post-transplantation. These studies will thus continue to enrich research on the treatment of type 1 diabetes.

IGF-I is a peptide that is produced mainly by the liver but is also expressed locally by all tissues in the body, including the pancreas. It is important in the regulation of cell growth and proliferation during all stages of development and adult life, interacting with both the insulin receptor and its own distinct tyrosine kinase receptor. In animal studies, IGF-I has been shown to act synergistically with glucose to enhance beta cell proliferation. Recent research suggests that this peptide plays a role in the development and maintenance of beta cell mass. Based upon these properties of IGF-I, we believe that it will enhance islet cell function and survival after transplantation.

This will be a multidisciplinary project, requiring the cooperation of the laboratory of with the laboratories of both ... and ... The lab has expertise in the area of constructing synthetic biodegradable polymer scaffolds, while 's laboratory is expert in mouse models of islet transplantation. laboratory is responsible for designing the studies, following the mice post-transplantation, and defining the effects of the scaffold microenvironment on islet cell function, survival, and proliferation. This is a well-established, ongoing collaboration supported by a Juvenile Diabetes Research Foundation (JDRF) program project grant and an NIH-funded Bioengineering Research Partnership.

Preliminary Data

IGF-I has been shown in previous studies to increase pancreatic beta cell proliferation by acting through both the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K) pathways, although the details of its mechanism of action have not been fully elucidated. We have shown that IGF-I stimulates islet cell progression through the G1 phase of the cell cycle and that this is accompanied by an increase in expression of cyclin D1, which is important for cell cycle progression. A kinase known as p70 S6 kinase is downstream of Akt in the PI3K pathway and is activated by the mammalian target of rapamycin (mTOR). mTOR (and p70 S6 kinase) are inhibited by rapamycin. p70 S6 kinase is known to be important for protein synthesis, but its role in cell proliferation is less clear. Our studies employed rat insulinoma INS-1 cells as a model for pancreatic beta cells to examine the molecular mechanisms of the effect of IGF-I on cell cycle progression. My summer research demonstrated that treatment with IGF-I increased the phosphorylation of p70 S6K. Treatment with both IGF-I and rapamycin inhibited IGF-I-stimulated phosphorylation. As noted, cyclin D1 expression was increased in cells treated with IGF-I. This increase in cyclin D1 expression was inhibited, at least in part, by rapamycin. Flow cytometry was used to examine the effect of rapamycin on IGF-I-stimulated cell cycle progression. IGF-I treatment increased the percentage of cells in S-phase. This increase was inhibited in cells treated with rapamycin. From these results of my summer research, we were able to conclude that the effect of IGF-I on progression of islet cells through the cell cycle is mediated, in part, by activation of mTOR. It is important to note that rapamycin is used clinically as an immunosuppressive agent following islet transplantation. Islets residing in the liver would be exposed to high concentrations of rapamycin following oral administration. This is further evidence for the need to find a site for islet transplantation outside the liver.

Research Design and Methods

Fabrication of the poly(lactide-co-glycolide) (PLG) microporous scaffolds will be performed in the lab using a three-step gas foaming process. This process will include the incorporation of IGF-I into PLG microspheres via a double emulsion technique. The transplant studies will be performed in C57BL/6 mice using a syngeneic model of streptozotocin-induced diabetes. Studies will assess the impact of delivery of IGF-I from the scaffold on islet cell apoptosis and proliferation. Islets will be

isolated from C57BL/6 mice and seeded onto the scaffolds at a density of 200 islets/scaffold. For these studies, three groups of mice will be examined. Mice will be transplanted with either: (i) a control scaffold without IGF-I placed into intraperitoneal fat; (ii) a scaffold containing IGF-I placed into intraperitoneal fat; or (iii) 200 islets under the kidney capsule (the standard model of islet transplantation in mice – although this site does not work well in primates, including humans).

The purpose of Aim 1 is to determine whether IGF-I will enhance islet function post-transplant in a murine model of diabetes. To complete this Aim, we will determine: (i) the number of mice in each group in whom euglycemia (glucose < 200 mg/dl) is achieved and (ii) the time at which euglycemia is reached in those mice that acquire a normal glucose level. Serum glucose levels will be measured every two days post-transplant. The first day on which a sustained glucose level less than 200 mg/dl is achieved will be designated as the time to resolution of euglycemia. These studies will require a minimal mass of islets. Based upon previous studies in laboratory, we will transplant 125 islets/mouse and will study the three groups described above. We hypothesize that the group transplanted with scaffolds containing IGF-I will show an increased number of mice in whom euglycemia is achieved, as well as a decrease in time to euglycemia. Data will be analyzed by comparing the natural logarithm of the number of days required to reach euglycemia among groups using analysis of variance (ANOVA). Previous power analyses showed that 12 mice/group are necessary for attaining 90% power using a two-sided t-test to demonstrate a 50% decrease in the days to reach euglycemia.

The goal of Aim 2 is to ascertain whether IGF-I decreases islet cell apoptosis and/or increases islet cell proliferation. In these experiments, the three groups of mice described above will be studied. The scaffolds or kidney will be harvested at 5, 15, or 30 days post-transplantation. Four mice per group will be studied. Because complete engraftment of islets, as reflected by revascularization, occurs over two weeks, harvesting the islets at those time points will allow us to observe islet engraftment early, mid-way, and late in the process. We hypothesize that the group transplanted with scaffolds containing IGF-I will display the lowest percentage of apoptotic islet cells. The amount of apoptosis will be determined by terminal dUTP deoxynucleotidyl-transferase nick end-labeling (TUNEL) staining using the ApopTag in situ apoptosis detection kit. This assay identifies TUNEL-positive, and thus apoptotic, nuclei using a horseradish peroxidase recognition method. For the quantification of apoptosis, a blinded observer will examine 100 islet cells in five non-overlapping sections from each scaffold (or islets under the kidney capsule) and subsequently determine the percentage of apoptotic islet cells.

We also will determine whether IGF-I increases islet cell proliferation. This will be accomplished using methods similar to those applied for the apoptosis studies. These experiments will employ the same three transplantation groups and times for harvesting the islets as those described above. We hypothesize that the group transplanted with scaffolds containing IGF-I will show the highest percentage of proliferating cells. To quantify cell proliferation, histochemical analyses will be performed using an antibody to proliferating cell nuclear antigen (PCNA). As PCNA is expressed during DNA replication and processing, its expression signifies the entry of a cell into the cell cycle. As before, a blinded observer will examine 100 islet cells in five separate regions, noting the number of PCNA-positive cells. In addition, the number of actual β-cells that are PCNA-positive can be determined by performing a dual insulin/PCNA stain. The percentage of PCNA-positive β-cells will be calculated.

III. The primary focus of the research effort in the laboratory is to develop new approaches to islet transplantation that will enhance islet survival, engraftment, and function in the post-transplant period. This is also the theme of proposal. Importantly, this research is being performed in the context of a multidisciplinary team that includes investigators from Endocrinology, Transplant Surgery, Chemical and Biological Engineering, Materials Science, and Chemistry. Thus, the research experience that will be afforded to will be enriched via participation in this collaborative, multidisciplinary environment. Importantly, the efforts of this research team are supported by a program project grant from the Juvenile Diabetes Research Foundation (JDRF) and an NIH-funded Bioengineering Research Partnership (BRP). To facilitate interactions among this research team, we have a weekly multidisciplinary research meeting focused on cell replacement therapy for diabetes. Participants include

members of the different laboratories involved in the JDRF program project and BRP. This multidisciplinary meeting typically features the presentation of research results and plans by a member of one of the laboratories. will not only attend this weekly meeting but my intent will be for her to present the findings of her research at the end of her research experience. Moreover, the members of the BRP have semi-annual retreats, during which each of the participating laboratories provides an update on ongoing activities. This multidisciplinary meeting will provide another opportunity for Ms. Fisher to observe the interworkings and collaborative efforts of a multidisciplinary team.

The laboratory occupies ~650 ft^2 of space among ~10,000 ft^2 of Endocrinology research space on Tarry 15 and 14. There are ~45 post-doctoral fellows, graduate and medical students, and technicians working in this research space with whom she will interact. In the laboratory, there are two Endocrinology fellows, two medical residents, and three experienced technicians, all of whom are working on approaches to cell replacement therapy for diabetes. As described below, will not only work closely with these individuals, but they will also play a key role in her training. I have mentored 13 post-doctoral fellows, many of whom are in academia or industry, and one pre-doctoral student who received her PhD. degree.

IV. As described above, will be integrated into a well-established research team with considerable expertise. All of these personnel will be available to provide help and training with the laboratory techniques needed to complete the described studies. Making the microporous scaffolds, preparing islets, and performing the islet transplants are too technically demanding to gain the needed expertise during a relatively short research experience. For this portion of her proposed studies, will work closely with the trained personnel from the different laboratories who perform these techniques, so as to have an appreciation for the methods. , a masters-trained technician in my laboratory who has experience with preparing islets and monitoring mice post-transplantation, will provide training and assistance with the post-transplant follow-up of the mice, an activity in which will play an active role. will also perform the analyses to examine apoptosis and cell proliferation described above. in the laboratory runs a histology core for the JDRF program project and has developed the needed assays. She will be available to assist and train in the needed techniques and methods of data analysis to complete the studies of apoptosis and cell proliferation.

The laboratory has a weekly meeting during which one of the members of the lab presents a critical review of an article or their research data. will participate in these meetings by presenting articles and her data. I will also plan to meet weekly with r to review progress, discuss experiments to be performed, and results generated to date. Also, my office is adjacent to the laboratory, and I will be available to interact wit on a daily basis as needed to complement our formal meetings. These varied training and mentoring opportunities will ensure that receives the needed training and mentoring in the Research Thesis Program.

References

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