

This Newsletter has been brought to you by the generous support of Bayer.



In this issue:

- Towards evaluating pancreatic beta cells in vivo
- Using dendritic cells to control autoimmunity
- Scientific presentations focus on adults with Type 1 diabetes

Imaging Technologies for Evaluating Insulin-Producing Beta Cells “In Vivo” – Why, How, When?

As part of the strategy to find a cure for Type 1 diabetes and its complications, JDRF is increasingly reaching out to the greater scientific community with specific research challenges, asking for applications or expressions of interest that detail how a particular research problem might be solved, and then funding those projects that show the most promise.

A major interest for JDRF that it has recently asked scientists to explore is the development of imaging technologies that noninvasively and safely provide information about the health of the insulin-producing cells in a patient’s pancreas. Focusing on the key information of beta cell mass (how many insulin-producing cells there are) and function (how effectively they sense glucose and produce insulin in response), these imaging technologies would identify such things as the number of beta cells or islets that a person harbours; or assess a specific cellular function, such as insulin production or gene activity.

According to JDRF, developing these technologies will be an invaluable resource in reaching three objectives that are key steps along the path to a cure:

- Following the natural history and pathogenesis of diabetes in order to develop cure therapeutics;
- Monitoring response to cure therapeutics in human clinical trials; and
- The early detection of diabetes.

Because pancreatic islets, and the insulin-producing beta cells within them, are deeply embedded and scattered throughout the pancreas in relatively low numbers—islets comprise only about 1 per cent to 2 per cent of the total cell mass in the pancreas—direct in vivo monitoring of these cell populations is difficult. (In vivo means in the body, compared with in a test tube in the laboratory, or in vitro.) Thus the development of imaging technologies for evaluating human pancreatic beta cells, or “functional beta cell imaging,” focuses not on the number or integrity of the beta cells themselves but rather on the beta cell biomarkers that serve as surrogates of their mass and function.

Biomarkers are objective biological characteristics, such as protein levels in the blood, that can be used to measure safety, make a diagnosis, indicate stage of disease, and predict or monitor response to treatment. Biomarkers of beta cell mass and function might include cell surface markers that identify newly formed or regenerated beta cells, molecular markers that characterize the stem cells/progenitor cells of the pancreas, and beta-cell-specific proteins in the plasma or other body fluids that correlate with the different stages of diabetes. The identification and validation of biomarkers is ongoing and must of course precede the development of any beta-cell-specific imaging technology. JDRF has noted that there is a critical need for biomarkers in all areas of Type 1 diabetes, because current measures for

assessing pancreatic health are of limited use in predicting the disease, and are not universally accepted by regulatory agencies. Robust biomarkers—those whose measurement can be made from a readily obtained sample or made directly in patients—would reduce the physical, financial, and logistical costs of clinical trials and are thus most sought after. The timely development of any imaging technology will require collaboration between scientists with imaging expertise and those with diabetes/beta-cell expertise. Adrienne Wong, Ph.D., scientific program manager for JDRF's Replacement program, explains: "Techniques and technologies derived for imaging other solid organs, cancer, Alzheimer's and Parkinson's disease, and for tracking stem cells, vascular changes, and inflammation could be a shortcut to a relevant tool for Type 1 diabetes imaging. FDA-approved imaging agents currently in use for other purposes may be especially useful in the vascular area."

State of the field

JDRF is looking to commit up to \$5 million per year towards projects pursuing imaging technologies. A larger amount, up to \$10 million per year, will fund the broader initiative of discovering and validating biomarkers of Type 1 diabetes, including those for beta cell mass and function. (A number of promising grant proposals are currently being screened for their potential to identify new biomarker targets and to further refine existing approaches.)

To begin focusing attention on this issue, JDRF co-sponsored and co-organized a workshop with the National Institutes of Health in 2006 (Imaging the Pancreatic Beta Cell in Health and Disease), bringing together researchers with expertise in both beta cell biology and imaging modalities like positron emission tomography (PET), magnetic resonance imaging (MRI), and optical imaging. Dr. Wong noted that progress is being made and that several promising imaging concepts will soon be tested in large animal models.

A novel imaging approach in mice

While functional imaging of endogenous (non-transplanted) beta cells in humans is the priority, studies of transplanted islets in animals are also critical for early tests of safety and can sometimes lead to novel applications in people. For example, JDRF-funded researchers in Europe and the U.S. recently developed a

Key Point:

Non-invasive imaging technologies that can track the health of insulin-producing beta cells over time are being sought by JDRF investigators. Such technologies will be invaluable in the study and management of Type 1 diabetes.

new method for non-invasive in vivo imaging of pancreatic islets using the front chamber of the eye as a natural body window. Islet cells introduced into the mouse eye just behind the iris (the coloured part of the eye) were shown to engraft on the iris, become vascularized, retain their composition, respond to stimulation, and reverse diabetes. Imaging was accomplished using a specialized technique called laser-scanning microscopy, which allowed repeated in vivo imaging of islet vascularization, beta cell function, and beta cell death at the cellular level. This new experimental model might be used, the researchers suggested, to study beta cell function and survival under both normal and diabetic conditions, as well as in broader applications such as studies of pancreatic development and drug screening.

The research, which took place at the JDRF Center for Islet Transplantation at the University of Miami in Florida, headed by Camillo Ricordi, is described in a recent issue of the journal *Nature Medicine*. The project was led by Stephan Speier in the laboratory of Per-Olof Berggren.

Harnessing Dendritic Cells to Make Destructive T Cells More Tolerant

Type 1 diabetes is an autoimmune disease that develops when specific immune cells—called T cells—mistakenly mount an immune response against insulin-producing beta cells in the pancreas, leading to their destruction. Scientists attribute the autoimmunity to defects in "self-tolerance." Of the various T cell types, one appears to be strongly associated with the development of diabetes: cytotoxic T cells known as CD8+ T cells. Studies in mice show, for example, that CD8+ T cells are required for onset of disease; and in people with Type 1 diabetes, these same T cells can be detected in the blood. Given the central role of CD8+ T cells, researchers have been looking into ways to make them more tolerant of the beta

cell and its protein components, since both appear to be a trigger of autoimmunity in persons at risk for Type 1 diabetes. One such approach is to harness the natural role of the dendritic cell, an immune cell whose specialized function is to process and display antigen on its cell surface for viewing by T cells and other action-taking immune cells.

What researchers know from experiments in healthy mice is that dendritic cells present beta cell antigens to T cells in a “tolerogenic” manner, causing potentially destructive CD8+ T cells to initially proliferate but then leading to their deletion or unresponsiveness.

What JDRF-funded researchers have now discovered is that a similar scenario might be established in Type 1 diabetes in people. A team of scientists from three laboratories—Albert Einstein College of Medicine and The Rockefeller University in New York City; and the Jackson Laboratory in Bar Harbor, Maine—was able to deliver a small beta cell protein straight to the dendritic cells of mice that develop diabetes; after recognizing and responding to this antigen, diabetes-causing T cells that had been introduced into the mice began to divide, but they were later deleted by the immune system—indicating a positive tolerogenic response.

Perhaps most noteworthy, the researchers said, is that “CD8+ T cell tolerance could be achieved even in the face of ongoing autoimmunity and in mice with multiple reported tolerance defects and dendritic cell abnormalities.”

While this research is at the discovery stage only, with much still to be done to demonstrate its effectiveness in the clinical setting, it represents an important milestone within the broader goal of finding a cure for Type 1 diabetes. Dr. Teresa P. DiLorenzo, the study’s principal investigator, explains: “The advantage of antigen-specific approaches such as ours is that they do not carry with them the unacceptable and dangerous side effects of systemic immunosuppression, which include increased risk of infectious diseases and cancers.”

Candidates for the treatment might one day include at-risk individuals, the newly diagnosed, as well as those receiving islet transplants.

Targeted delivery and strategy

To deliver the beta cell antigen to the dendritic cell, Dr. DiLorenzo, Dr. Ralph M. Steinman, and colleagues constructed a hybrid antibody, linking the antigen to an antibody that specifically binds to a receptor on the dendritic cell. Once this antibody delivered its “goods,” the dendritic cell could then process the beta cell antigen, presenting it to the pathogenic CD8+ T cell line. (Dendritic cells that express the complimentary receptor are found at high levels in areas of the body where T cells gather—a feature that brought the beta cell antigen and T cell into common territory.)

Dr. DiLorenzo said the terms “immunization” and “vaccination” are sometimes used to describe immunotherapeutic interventions like theirs, but this is misleading: “These terms are associated with childhood shots, in which a dose of antigen leads to an immune response that will allow the individual to destroy the corresponding pathogen if and when it is encountered later in life.”

In contrast, the idea behind their antigen-based therapy is to prevent a destructive immune response. “Under normal circumstances, dendritic cells present self-proteins—including beta cell antigens—to T cells in a manner that leads to deletion or inactivity of the T cells, thus protecting the body from autoimmune disease. We believe that our immunotherapeutic intervention helps this process by increasing the amount of beta cell antigen that is available to these tolerizing dendritic cells,” Dr. DiLorenzo explained.

Key findings

Using a labeling technique to trace the effect of the antigen-based treatment three days after it was administered, the researchers noted extensive T cell proliferation in various immune-cell gathering sites, including the peripheral lymph nodes, the pancreatic lymph nodes, and the spleen. However, when the T cell response was evaluated approximately two weeks later, they found that the CD8+ T cells had been largely eliminated.

To test if T cell tolerance was in fact achieved, Dr. DiLorenzo and colleagues rechallenged a group of treated mice with more of the same antigen and a strong agent to

boost its effect. The rechallenge did not generate a pronounced T cell response, thereby confirming the generation of sustained tolerance to the beta cell antigen.

According to the authors, their finding that dendritic cells can mediate T cell tolerance in mice “could not have been predicted from the existing literature.” They speculate that tolerance predominated over ongoing autoimmunity because the antigen treatment was able to target dendritic cells located in the peripheral lymph tissues. The researchers plan to test the antigen treatment on T cells in the body, as opposed to transferred CD8+T cells, after developing the appropriate reagents. They will also need to conduct prevention and intervention studies in mice before exploring these possibilities in humans.

It is likely that future antigen-based treatments will be broader in scope, in that most researchers now believe that multiple beta cell antigens are targeted for autoimmune attack, and thus a focus on T cells reactive to a single antigen may have somewhat limited clinical benefit. “Our vision is that with continued antigen identification work, a manageable set of key antigens will emerge, with insulin likely to be among these,” Dr. DiLorenzo said. “Then, a cocktail of reagents targeting multiple T cell reactivities will ultimately be the therapy of choice.”

The study is reported in a recent issue of the journal *Proceedings of the National Academy of Sciences*. Contributing to the work were researchers from the Albert Einstein College of Medicine (Teresa DiLorenzo, Arunika Mukhopadhyaya, Tadashi Hanafusa); The Rockefeller University (Ralph Steinman, Kristin Tarbell, Yoshiko Iwai); and the Jackson Laboratory (Yi-Guang Chen, David Serreze).

2008 Global Diabetes Research Forum: Science, Hope for Adults with Type 1 Diabetes

Research findings and innovative approaches offer the promise of new therapies and the potential for cures for adults living with Type 1 diabetes, according to researchers at JDRF’s 2008 Global Diabetes Research Forum, which took place this past June in Washington D.C.; the event, held each year at JDRF’s Annual Conference, highlighted promising scientific developments and important directions in Type 1 research.

Key Point:

Dendritic cells that have been targeted to express beta cell antigens offer a way to eliminate the T cells that cause diabetes. The study provides solid support for the development of antigen-specific therapies for treating and preventing Type 1 diabetes in humans.

The GDRF focused on adults with Type 1 this year because fully half of those diagnosed each year with Type 1 diabetes are adults. As research has improved diabetes care over the years, and people with the disease live longer and with fewer complications, adults with diabetes may have lived with their disease for more than 90 per cent of their lives. The forum also looked to focus attention on JDRF-funded science aimed at curing established Type 1 diabetes, as JDRF’s success in the recent past with cure therapeutic advances in the newly diagnosed setting—such as anti-CD3 compounds—may have overshadowed the fact that a significant portion of JDRF’s funding goes towards reversing the disease in people who have been living with diabetes for years, if not decades.

Producing Insulin—Even 50 Years After Diagnosis

Among the research presented were insights from the Medalist Study, an ongoing project at the Joslin Diabetes Center in Boston, Massachusetts, whose main objective is to answer the question, “Can complications of diabetes be avoided or stopped?” The study recruits patients with long-duration Type 1 diabetes, and has created a recognition program for people who have been insulin-dependent for 50 years or longer. George King, M.D., Senior Vice President and Director of Research at the Joslin Center, and the study’s lead investigator, said that research shows that individuals with established Type 1 diabetes (even those who have lived with it for 50 years or more) are still capable of producing insulin. The Joslin Study also found that even after 50 years, about 30 per cent of the patients studied did not experience any of the common complications such as eye, kidney, or nerve disease. These findings show that complications can indeed be prevented, and point to the way towards the possibility of improved clinical outcomes for all Type 1 diabetes patients.

Potential for Beta Cell Regeneration

Mark Atkinson, M.D., Director of The Diabetes Center of Excellence at the University of Florida, presented initial findings from nPOD—the Network for Pancreatic Organ Donors with Diabetes. Organized and funded by JDRF, the network was established last August to procure and characterize, in a collaborative manner, pancreatic and related tissues from organ donors with long-standing Type 1 diabetes as well as those who are islet-autoantibody positive. These tissues are being used to study how Type 1 diabetes develops, with the aim of finding a means to a cure.

Dr. Atkinson described findings from nPOD that have enabled researchers to assess the potential for islet cell regeneration. “Contrary to common dogma, what we’ve learned so far is that some pancreata from subjects with long-standing Type 1 diabetes have insulin-positive beta cells and some have many intact islets. This finding gives hope for islet cell regeneration or restoration,” Atkinson noted. He pointed out another key finding: that some islets have beta cells that don’t produce insulin. “If we know beta cells are there, then we can focus on finding ways to get them to produce insulin,” Dr. Atkinson explained.

Natural regenerative abilities in individuals without diabetes are also being explored. JDRF’s Chief Medical Officer, Paul Strumph, MD, presented findings that showed how beta cell mass expands in response to increased metabolic demands, such as growth during the first decade of life, obesity, and pregnancy. Possible

therapeutics may therefore be developed if scientists can mimic these or other biological mechanisms that increase the number of insulin-producing cells in the pancreas. “A little bit of insulin is not a cure, but it can be significant to reduce the complications of diabetes,” Dr. Strumph noted.

A New Era of Diabetes Research

All of the presenters agreed that researchers are on the cusp of a new era in diabetes research, one in which advanced technology and human clinical research should enhance the development of new therapeutics and an ultimate cure.

“Much of what we’ve known regarding the pathogenesis of Type 1 diabetes has dated back to studies performed with the human pancreas in the 1970s—before microwaves, the internet and cell phones, and before modern day medical research technology. Now we’re looking at this disease in whole new ways,” Dr. Atkinson explained.

Dr. Strumph added that there is more of an emphasis now on looking at the natural history of the disease to guide research opportunities in those with established Type 1 diabetes. JDRF devoted a significant portion of the \$160 million in research it committed last year to science involving patients with established Type 1 disease, with a particular emphasis in the areas of autoimmunity and regeneration; the organization plans to fund as much as \$195 million on diabetes research in the coming twelve months. ■



TOGETHER WE CAN
triumph over diabetes
simplewins™

