



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



simplewins™



Bayer HealthCare
Diabetes Care

In this issue:

- Targeting the B cell prevents diabetes in mice
- Successful encapsulation of beta cell precursors
- Why beta cell regeneration slows with age
- With the switch of a gene, non-insulin producing cells make insulin

Different Immune Cells Could Provide Target for Diabetes Therapy

New research findings support pursuing therapies that target a different kind of immune system cell as a way to treat type 1 diabetes.

A team of Australian researchers led by JDRFI-funded scientist Shane Grey was able to completely prevent type 1 diabetes in mice with a therapy that targets immune B cells, rather than T cells.

Most therapies aimed at reversing the immune response that causes type 1 diabetes target T cells—the immune cells ultimately responsible for the destruction of the insulin-producing beta cells. But previous research has pointed to a key role for another type of immune cell: B cells. B cells are among the first immune cells to enter the pancreas, and they have a crucial role in priming T cells to launch their autoimmune attack. Among the strongest evidence for a central role for B cells is that diabetes does not develop in mice that are deficient in B cells.

In the Australian research, prediabetic mice that received a B cell therapy were completely protected from type 1 diabetes throughout the study's 50 weeks. By contrast, mice not given the therapy showed rising blood sugar levels and eventually developed diabetes.

The researchers found the B cell therapy, which can broadly be categorized as an antibody therapy, prevents diabetes by reducing the total number of B cells in the body. This effect blunts the degree of B cell/T cell interaction, minimizing a trigger of the disease. It also increases the number of regulatory T cells, enabling the immune system to "reign in" potential autoimmune activity from destructive T cells.

The finding advances our understanding of how diabetes develops and progresses—and suggests that depleting the B cells may be a powerful tool for preventing and treating type 1 diabetes in people.

No other study, thus far, has reported such "profound and complete protection from hyperglycemia," according to the journal *Diabetes*.

The path to discovery

Inspired by previous studies showing that mice with no B cells did not develop diabetes, the scientists set out to understand the role these cells play in causing the disease, said Dr. Grey, who is affiliated with the Garvan Institute of Medical Research in Darlinghurst, Australia. To study B cells during the natural progression of diabetes, they relied on a "messenger molecule" called BAFF. Without this molecule, B cells cannot develop, and they found by using a BAFF blocker, they could turn B cell production on and off, and monitor the effect on diabetes.

Hoping to disrupt the pathway that leads to diabetes, the researchers administered the BAFF blocker twice a week, when the mice were nine to 15 weeks of age. The mice ultimately received 12 injections over a six-week period.

The scientists were surprised by the results: None of the mice that received the blocker—called BCMA-Fc—developed diabetes.

Without B cells, regulatory T cells gain control

The treatment protected the mice long-term, even though they still had T cells capable of starting an immune response and attacking insulin-producing cells in the pancreas. Suspecting regulatory mechanisms might be at work, the researchers analyzed the frequency and number of regulatory T cells, which are critically important in the control of immune reactions. They found the treated mice built up increasing numbers of these cells as they aged. The researchers also proved the regulatory T cells maintained normal blood sugar levels, by demonstrating their removal brought on type 1 diabetes.

Findings

The findings confirm B cells play a definite role in turning on the T cells that kill insulin-producing cells. "In fact, we show that for some stages of diabetes, the B cell is the most important activator of T cells and that by preventing this B-T interaction, one can halt the disease process," Dr. Grey said. The study also explains why turning off B cells prevents diabetes. "We show that when B cells are gone, immune regulatory cells can prevent the autoimmune attack from re-occurring," he said.

Ultimately, the research demonstrates strategies to reduce B cells could be powerful in treating type 1 diabetes. Dr. Grey and

his team are now exploring testing the therapy in people.

Clinical trials involving similar therapies

Additional clinical trials are testing other therapies that may lower the number of immune B cells in type 1 diabetes patients.

An ongoing Phase II/III clinical trial supported by JDRFI will determine whether an anti-CD20 antibody called rituximab can preserve beta cells in people who have been newly diagnosed with the disease. Rituximab is already approved by the FDA to treat a type of lymphoma, and scientists believe it may also work to reduce B cells in type 1 diabetes patients.

Another Phase II clinical trial is evaluating the use of rituximab to increase the success of islet transplantation in type 1 diabetes patients by helping islets survive. The trial aims to determine whether this therapy is safe and effective. ■

Key Point:

Type 1 diabetes can be prevented in mice using a therapy that lowers a type of immune cell called B cells, which are important in starting the immune destruction of the insulin-producing cells in the pancreas. The findings confirm the important role of B cells in triggering the disease and also point to a potential new treatment for people.

Beta Cells Survive and Flourish in an Encapsulation Device

JDRFI-funded researchers have made important discoveries in encapsulation that could improve the success of islet transplantation.

In a study in mice, scientists showed for the first time that transplanted cells that become insulin-producing cells can survive being encapsulated in a durable “device” protecting the cells against an immune attack. Equally important, those cells then developed into insulin-producing cells that could control rising blood sugar levels. By contrast, adult insulin-producing cells that were encapsulated in the same way exhibited poor survival.

The results suggest encapsulating cells before they are differentiated and become beta cells—using stem cells, for example—may be a more successful approach to replacing insulin-producing cells in people with type 1 diabetes and a new way to take advantage of emerging cell-based therapies.

“Our data suggest that long-term protection of human beta cells in type 1 diabetic patients without immunosuppression is a realistic goal,” said Pamela Itkin-Ansari and colleagues from the University of California, San Diego and the Burnham Institute for Medical Research in La Jolla, California. Their findings were reported in the journal *Transplantation*.

Transplanting insulin-producing cells is an important treatment option for adults with type 1 diabetes who have hypoglycemia unawareness, a life-threatening condition in which people can no longer sense dangerously low blood sugar levels. But the procedure has two main limitations. First, people who get

transplants need to take immunosuppressive drugs for the rest of their lives to prevent the rejection of the donated islets, even though these drugs can cause health problems and damage the transplanted cells. Second, there are too few donors to make transplantation a widely available option.

A solution to both problems would be to transplant cells from another source and to encapsulate these cells in a material that protects them from the immune response. Shielding the transplanted cells would mean there is no need for immunosuppressive drugs—and by using stem cells as the transplant source, people with type 1 diabetes would have access to a potentially unlimited supply of transplantable cells. To date, however, most islet encapsulation studies have used delicate devices made of semi-solid materials that may deteriorate over time. Such encapsulation is not ideal for stem cells in people because there is a concern that stem cell preparations may harbour cells with the potential to cause cancer.

With their principal goal to identify a way to safely deliver stem cell-derived therapies, Dr. Itkin-Ansari and her team set out to test a durable “macroencapsulation” device made by TheraCyte, Inc. The device, already shown to be biologically inert in people, has an inner layer with pores small enough to allow insulin, but not the enclosed cells, to flow out.

Main findings

To see whether cells within the encapsulation device would survive and function well, the researchers first tested it in mice unable to mount a strong immune response. This allowed them to eliminate the variable of immune rejection. They transplanted the mice with either encapsulated adult human islets or cells that would become insulin-producing cells. Other mice were transplanted with unencapsulated islets.

The encapsulated, insulin-producing cell precursors thrived within the device. Ten weeks after transplantation, they were producing insulin, glucagon, and other hormones that indicated normal function. In fact, the fraction of encapsulated cells producing insulin had nearly tripled since the transplantation.

The percentage of replicating cells was strikingly high—almost four times as high as in the mice that did not receive encapsulated cells. This suggests the environment within the device not only allowed the cells to replicate, but may also have promoted the process.

In most of the mice, the encapsulated cells were able to produce insulin in response to glucose by five months after transplantation. This was considered slow compared with the mice that received unencapsulated cells. However, when the mice were given a drug that selectively killed only mouse insulin-producing cells, the encapsulated, transplanted human precursor cells produced enough insulin to control the inevitable rise in blood glucose. Untransplanted mice became diabetic within 48 hours.

Significant cell death, then recovery

The researchers also did mouse-to-mouse transplants to assess how well the encapsulated tissue “took” and to see whether the new encapsulation device would provoke the immune system

into an attack. They monitored the encapsulated precursor cells over a 50-day period.

What they observed was a dynamic process of cell death followed by regrowth and ultimately, robust long-term survival of the transplant.

Perhaps most noteworthy was the encapsulation device did not stimulate a detectable immune response.

"In transplanted mice," they explained, immune cells "were not recruited to the tissue surrounding the device. This was somewhat surprising, given that severe insulinitis (inflammation) was readily apparent in pancreases from the same mice. The data suggest the encapsulated beta cells are invisible to the immune system, and this bodes well for long-term clinical translation of the technology."

Next steps

Dr. Itkin-Ansari will use funding from the California Institute of Regenerative Medicine to follow up on this work. She will conduct similar tests of the encapsulation device using progenitor cells derived from embryonic stem cells, working in collaboration with the biotechnology company Novocell, which recently developed a method for producing pancreatic precursor cells from human stem cells. ■

Key Point:

The study provides proof-of-concept that insulin-producing cell progenitors can survive, proliferate, and mature in an encapsulation device to the point where they can correct diabetes. "This approach may be an important step in our ability to translate the transplantation of human embryonic stem cell-derived progenitors into clinical testing," said Julia Greenstein, Ph.D., Director of JDRF's Replacement research program.

Researchers Discover How the Regeneration of Insulin-Producing Cells Slows With Age

Two groups of JDRFI-funded researchers have identified processes that explain why insulin-producing cells lose their ability to regenerate with age. The findings shed light on the mechanisms that regulate normal expansion and decline of those cells—and could help lead to new therapies for type 1 diabetes.

Scientists believe insulin-producing beta cells can regenerate within the body either through the replication of existing adult beta cells or from stem cells in the pancreas. They see this ability as critically important for establishing and maintaining a normal, functioning pancreas. For example, the number of beta cells expands via regeneration during pregnancy to meet the increasing demands of the mother and growing fetus. Regeneration also accounts for the increase in beta cells that occurs with weight gain. However, the capacity of beta cells to regenerate and adapt diminishes as we age.

To better understand the molecular events involved, the two groups of researchers decided to focus their work on a specific

cluster of genes. They chose this route because the proteins produced by this cluster are found at higher levels in older beta cells and have a "braking," or inhibitory, effect on cell growth.

Their choice paid off. The research teams each identified a key protein that overcomes the gene cluster's inhibitory actions. They also uncovered the way each protein worked. Seung Kim, who led the research at Stanford University School of Medicine in California, pinpointed the role of a protein called Ezh2. Anil Bhushan, at the University of California, Los Angeles established the role of a protein known as Bmi-1. Both proteins turn genes on or off by altering gene packaging and accessibility within DNA.

Main findings

Dr. Kim's group observed that levels of Ezh2 decline with age in the insulin-producing cells of both people and mice. With this decline, there is an increase in cell cycle braking, and beta cells consequently have less capacity to proliferate. Ezh2 was found to directly regulate this process.

In one set of experiments, the scientists created mice with a limited ability to express the protein. The result was the mice were less able to regenerate beta cells, produced only low insulin levels, and eventually developed diabetes as they aged. And when the researchers knocked out the Ezh2 gene in the beta cells—so no Ezh2 protein was produced and nothing stood in the way of the inhibitory genes—the mice developed lethal diabetes. By contrast, mice maintained the Ezh2 gene in their beta cells did not develop diabetes. Together, these results confirm the loss of Ezh2 can cause diabetes, since beta cells are unable to regenerate without it.

Dr. Bhushan's group showed levels of Bmi-1 also decline with age. Working with mouse beta cells, they demonstrated the protein regulates the same cell braking. They determined as beta cells age, less of the protein is available, so the cell's braking genes are unchecked, and the cells' ability to replicate diminishes. Dr. Bhushan's group also showed Bmi-1 and Ezh2 work together in a cooperative fashion to regulate this process.

Implications for type 1 diabetes

The new findings have important implications with respect to therapeutic strategies for type 1 diabetes.

"The investigators independently identified pathways that regulate how beta cells regenerate, and that explain the loss of replicative capacity with age," said Patricia Kilian, Ph.D., Director of JDRFI's Regeneration program. "This is exciting, since it suggests that controlling these pathways might enable us to restore regenerative capacity to treat diabetes, even in older people. These results provide new tools and insights for finding a means to overcome the loss of beta cells."

Both research groups are actively following up on these findings with JDRFI funding. Researchers who collaborated with Dr. Kim include Rita Bottino from the University of Pittsburgh School of Medicine in Pennsylvania and Juan Contreras from the University of Alabama School of Medicine in Birmingham. Working with Dr. Bhushan at UCLA were Sangeeta Dhawan and Shuen-Ing Tschen. ■



Key Point:

Researchers have identified two proteins that promote the regeneration of insulin-producing cells but whose levels decline with age. The proteins, which act in similar ways, could lead researchers to new targets for stimulating beta cell regeneration as a therapy for type 1 diabetes.

Researchers Convert Cells in the Pancreas to Insulin-Producing Beta Cells

In findings that boost the prospects of regeneration as a treatment for type 1 diabetes, researchers have shown that cells found in the pancreas that normally do not make insulin can be changed into cells that do.

In a study in mice, they discovered by modifying the expression of a gene called Pax4, they could direct alpha cells, which do not produce insulin, into becoming insulin-producing beta cells.

The alpha cell's usual role is to make the hormone glucagon, which helps to restore low blood sugar to normal levels. To make the alpha cells begin producing insulin, the researchers targeted the Pax4 gene—doing so because the gene is known to regulate growth, development, and other key cellular functions. By forcing the expression of Pax4 in the alpha cell, the researchers were able to drive alpha cells to convert to beta cells.

The researchers also discovered the alpha cells that became new beta cells originated from progenitor cells in the pancreas and the drop in the number of alpha cells triggered additional progenitor cells to replace them.

Ultimately, the newly formed beta cells resulted in better glucose control and helped the mice survive.

"This study suggests that regenerating beta cells may be a viable pathway towards restoring beta cell function in type 1 diabetes," said Richard Insel, M.D., JDRF's Executive Vice President of Research. "It reinforces the concept that there are progenitor cells in the mouse pancreas that can generate new beta cells under special circumstances."

The research also points to some potential cell targets for regenerative therapies—both the progenitor cells and the alpha cells, Dr. Insel said. In addition, it identifies a critical protein and pathways that can be used to screen for drugs that target these cells.

The study, co-funded by JDRF, was published in the journal *Cell*. Lead researchers were Patrick Collombat of the Max-

Planck Institute for Biophysical Chemistry and Ahmed Mansouri of the University of Göttingen, both in Germany, working in collaboration with researchers at the JDRF Center for Beta Cell Therapy in Diabetes in Brussels.

New pathway for research

In type 1 diabetes, the immune system attacks the insulin-producing cells in the pancreas. One way to cure the disease is to restore insulin production either by regenerating new beta cells or by reprogramming other cells to work like beta cells.

JDRFI has been a leader in pursuing this avenue of research. In fact, regeneration was one of JDRFI's two largest areas for new research funding in the just-ended fiscal year.

"From minimal funding just a few years ago, beta cell regeneration and reprogramming have become one of the top new research areas for JDRFI," said Dr. Patricia Kilian, Director of JDRFI's Regeneration research program. To accelerate the progress of regeneration therapies, JDRFI is funding multiple research projects at several institutions, including the Broad Institute of MIT and Harvard, the Genomics Institute of the Novartis Research Foundation (GNF), and the Burnham Institute. These projects are using sophisticated screening techniques to identify small molecules that promote beta cell regeneration, including compounds that can influence what a cell becomes. In addition, JDRFI is supporting a wide range of projects with leading scientific investigators at top academic institutions, each targeting ways to replicate beta cells, regenerate them, or reprogram other cells to become beta cells.

Underscoring the importance of this rapidly evolving field, JDRFI recently announced it has entered into a collaborative research agreement with GNF to create a diabetes drug discovery and development platform. The four-year program, among the largest and most comprehensive collaborations in JDRFI's 40-year history, will establish a basic and translational research program in type 1 diabetes. The partnership will look to fill the current gap in translating basic research into new treatments for type 1 diabetes. The goal is to build a pipeline of diabetes therapies focused on beta cell regeneration, with a final goal of delivering a succession of new drugs to the clinic. ■

Key Point:

By targeting a specific gene in the alpha cells of the pancreas, researchers were able to convert these cells into insulin-producing beta cells. The finding provides important insight into a possible regenerative therapy for type 1 diabetes.



TOGETHER WE CAN
triumph over diabetes
simplewins™

 Bayer HealthCare
Diabetes Care