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“Descendants” of Adult Human Beta Cells Survive and Expand *In Vitro*

JDRF-funded researchers from Israel and France used a novel, cell-lineage approach to follow the fate of cultured adult human beta cells *in vitro*. What they found is that, in contrast to the behaviour of mouse beta cells grown in the laboratory, adult human beta cells not only “dedifferentiate” *in vitro* but can be significantly expanded in culture—a key property if these cells are to be developed for use as an islet replacement treatment. Dedifferentiated beta cells are beta cells that have reverted to a less specialized form. They may be important therapeutically, the authors explain, because they might retain enough of their original genetic structure to allow researchers to turn them, via “re-differentiation,” back into functional, insulin-producing beta cells.

The research, published in a recent online issue of the journal *Diabetes*, was performed in the laboratory of Shimon Efrat at the Sackler School of Medicine in Tel Aviv, Israel, by graduate students Holger Russ and Yael Bar. Philippe Ravassard, of the National Center for Scientific Research in Paris, France, also contributed to this work. In addition to providing grant support for the research, JDRF sponsors the European Consortium for Islet Transplantation’s Islets for Research

distribution program, one of the suppliers of the human islets used in these experiments.

“We believe that the dedifferentiated cells may be good candidates, perhaps better than other cells derived from various stem cells,” Dr. Efrat said, “for the generation of functional beta cells by re-differentiation.” He added that for now, however, “this is a hypothesis that needs to be tested, by screening various compounds and protocols for their capacity to induce such re-differentiation, and/or by transplanting these cells into animal models of diabetes.”

Previous studies have shown that adult beta cells, although highly specialized, or differentiated, maintain a capacity to replicate in the body by self-duplication. Scientists have thus looked to expand these cells in the laboratory as a means to solve the severe shortage of donor pancreases available for islet-cell transplantation. However, success in expanding adult human islets *in vitro* into insulin-producing beta cells has been limited. Attempts to grow these cells in the lab often result in only a small degree of cellular expansion (only a few doublings of the complete population of cells); and in the loss of insulin expression, a so-called “loss of the beta-cell phenotype.”

Another obstacle to developing an effective protocol for expanding these cells *in vitro* is the absence of a stable, betacell-specific biomarker for monitoring cellular changes—in a flask of cultured beta cells that no longer produce insulin, how does one know if the reason is dedifferentiation, which is preferred, or beta-cell death followed by the expansion of unwanted islet cells of a non-beta-cell origin?

Seeking to address these challenges, Dr. Efrat and colleagues applied a cell-lineage tracing approach to

track the fate of cultured beta cells—the molecular technique was previously used only in animals. After generating single cells from isolated human islets, they infected the cells with two genetically engineered viruses. The composition of these viruses enabled the researchers to sort and trace the beta cells harbouring them. Key to this process was labeling the beta cells with green fluorescent protein (GFP), a traceable marker. Because GFP's expression was tied to the production of a second viral protein under the control of the insulin promoter, called Cre recombinase, it was “assured that GFP will be turned on only in beta cells,” Dr. Efrat explained. “From that point on,” he added, “even if beta cells lost insulin expression, as well as their other markers, they maintained stable expression of GFP. This allowed tracing their fate and isolating them by cell sorting. Non-beta cells were not labeled, since they could never express the Cre recombinase. This method labels over half of the beta cells, so it is quite efficient.”

The researchers showed that beta cells can be efficiently and specifically labeled *in vitro*. More importantly, they provided direct evidence for the survival and dedifferentiation of cultured adult human beta cells, and further demonstrated that the dedifferentiated cells proliferate extensively in the laboratory. For instance, GFP-positive, insulin-negative cells derived from beta cells were shown to double up to 16 times, the doubling occurring about every seven days. “We are looking into ways to increase the expansion of these cells, although even at the present rate it is quite impressive—in theory cells from one donor can suffice for two to the power of 16 recipients if the cells regain their full function,” Dr. Efrat said.

Interestingly, the proliferation of isolated, labeled beta cells seemed to depend on factors secreted by the other cells that were present in the original mixed islet culture—that is, dedifferentiated beta cells will expand *in vitro* in the absence of other pancreas cell types but only if provided with growth medium conditioned by these other cells.

Similar analyses of mouse islet cells revealed a much lower proliferation of labeled cells under similar culture conditions.

The researchers will next focus on ways to re-differentiate these cells. “We are examining the changes in expression of known genes and pathways [involved in insulin regulation], to gain insights into potential candidates for manipulation. In addition, we are planning an unbiased screen of chemical libraries for compounds with a potential redifferentiation capacity,” Dr. Efrat noted.

In Diabetic Mice, Antibody Therapy Activates Disease-Controlling, Regulatory T Cells

A team of researchers from the University of Florida in Gainesville has shown that a type of antibody therapy first investigated in the late 1970s can alter disease progression in diabetic mice when administered at or just before disease onset. The antibody therapy—called ATG, or anti-thymocyte globulin—appears to function via two separate mechanisms, according to the study authors:

- First, by transiently reducing the number of T cells, and thus initiating a form of immunosuppression; the premise here is that eliminating T cells, some of which contribute to the destruction of insulin-producing beta cells, would slow pancreatic beta-cell loss.
- Second, and most intriguing, by increasing the frequency and activity of regulatory T cells, a type of T cell whose role is to dampen, or suppress, the immune response. This “induction of immunoregulation” by ATG was shown to result from the restoration of self-tolerance—that is, the induced regulatory T cells “taught” the potentially destructive cells of the immune system to recognize but not respond to its own cells and molecules.

“To our knowledge,” the authors write, “our results provide the first indications that a short course of ATG

given alone can restore self-tolerance...a facet that has been previously ascribed to anti-CD3.” In light of its immunoregulatory potential, they support further studies of ATG, saying that the therapy “might be applicable not only to Type 1 diabetes but also to other diseases associated with dysregulated immune responses.”

The research, supported in part by grants from JDRF, was led by Mark Atkinson and is published in a recent issue of the journal *Diabetes*.

A closer look at ATG

Antibodies, which are proteins with the ability to bind specific cells and molecules of the immune system, represent an important therapeutic approach in Type 1 diabetes. Monoclonal antibodies like anti-CD3, for example, have been shown to reverse or arrest the progression of diabetes in both mice and humans with the disease. (JDRF industry partners MacroGenics and Tolerx, in conjunction with Eli Lilly and GlaxoSmithKline, respectively, are currently developing and commercializing anti-CD3 antibodies, which have proven effective in clinical trials at slowing disease progression in the newly diagnosed.)

But while monoclonal antibodies may have taken center stage, researchers have also been using other antibody-based treatments to address Type 1 diabetes—including anti-lymphocyte serum, of which ATG is one form. In studies conducted more than 30 years ago, anti-lymphocyte serum reversed Type 1 diabetes in rats, and more recently, did so in diabetic mice. An approved form of anti-lymphocyte serum that is closely related to the ATG used in Dr. Atkinson’s studies of mice, and widely known as Thymoglobulin, has long been known to deplete T cells *in vivo* and is used in a variety of therapeutic settings in humans to control a person’s immune response. However, much of the previous scientific research on anti-lymphocyte serum did not account for the role of regulatory T cells, which have recently been identified as important

cellular players whose immunosuppressive potential might be harnessed to treat autoimmune diseases.

Given the therapeutic potential of ATG, but a limited and incomplete understanding of its actions, particularly related to regulatory T cells, Dr. Atkinson and colleagues performed a number of *in vivo* and *in vitro* mouse experiments aimed at defining the physiologic, immunologic, and metabolic activities of this agent. Their key interests were to determine how ATG modulates the autoimmune response against beta cells and whether it delays or reverses Type 1 diabetes at the various stages of disease.

What exactly is anti-lymphocyte serum?

A comparison helps: If monoclonal antibodies represent the specialist approach—one antibody, one target—then antilymphocyte serum represents the broader, generalist approach—many antibodies, many targets. In the case of ATG used in Dr. Atkinson’s studies, the therapeutic is made up of a diverse repertoire of antibodies that are generated by injecting rabbits with immature T cells (thymocytes) extracted from the thymus glands of mice—the rabbits respond to the mouse antigens by forming anti-mouse thymocyte globulin (or ATG, with globulin being another name for antibodies). Blood serum samples from the rabbits thus contain a range of anti-lymphocyte antibodies that will bind to T cells and other immune cells of the exact specificity when injected into the mice in the experiments. The researchers conducted their tests in nonobese diabetic mice, which are characterized by the eventual spontaneous development of Type 1 diabetes.

Key findings

Most striking, ATG was shown to delay the development of Type 1 diabetes in a time-dependent manner. While the mice injected with ATG at four and eight weeks of age did not show a significant delay in disease onset compared with control mice, mice injected at 12 weeks of age remained disease-free for

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significantly longer times. At 30 weeks of age, 89 per cent of the mice treated with ATG at 12 weeks had blood glucose levels within the normal range, whereas only 22 per cent of the control mice remained “normoglycemic.” (Mice 12 weeks of age are considered to be in the late pre-diabetic phase.)

Treatment with ATG was also shown to reverse Type 1 diabetes at disease onset—defined as the overt onset of hyperglycemia. Of the seven mice injected with ATG at this time, four showed a significant disease reversal, their blood glucose levels dropping rapidly from an average of about 400 mg/dL to about 150 mg/dL one week later, with a sustained benefit up to the end of the six-week monitoring period. In contrast, none of the control mice, who were not given ATG, showed any sign of disease reversal.

Further investigations pointed to the regulatory T cells as mediating these benefits. When given to mice at 12 weeks of age, ATG reversed insulinitis, an inflammation of the pancreatic islets caused by an infiltration of lymphocytes, which can destroy the insulin-producing beta cells. Not only were significantly lower levels of T cell infiltration observed in these mice compared with the control mice, but a less severe form of insulinitis developed over time. This suggests, according to the authors, that “above and beyond the initial [T cell] depletion afforded by ATG, the agent may induce protective mechanisms attenuating migration of cells to pancreatic islets.” Supporting this further were the findings (in ATG mice) of improved response to glucose and a rapidly increased frequency of antigen-presenting cells in the spleen and pancreatic lymph nodes.

The researchers were also able to show that ATG therapy directly and dramatically increased the frequency and functional activity of the regulatory T cells—the latter via the suppression of T effector cells, which are the cells that target and destroy beta cells in Type 1 diabetes.

The one caveat, however, is that because ATG delayed disease only at specific stages, there is a time-dependent window of opportunity for delaying or reversing Type 1 diabetes with the use of this drug, based on its capacity to enhance the activity of regulatory T cells.

From bench to bedside

Dr. Atkinson and other researchers at the University of Florida, including Dr. Michael Haller, continue to study how ATG induces immunoregulation in mice. They are also moving this safe and promising Type 1 treatment, which Dr. Atkinson said, “seems to work both alone and in combination with other drugs,” into clinical trials.

Their findings support a recent human trial of ATG that was independently initiated by Steve Gitelman, M.D., at the University of California, San Francisco, in affiliation with the Immune Tolerance Network, which is supported by JDRF. The trial aims to determine whether ATG treatment can halt the progression of newly diagnosed Type 1 diabetes when given within six weeks of disease diagnosis. Dr. Gitelman noted that, “We are very hopeful that we can extend the findings of Dr. Atkinson and colleagues from mouse to man, and alter the natural course of Type 1 diabetes by turning off the destructive autoimmune response with only a brief treatment with ATG. We need to determine if this approach will be even more successful than the initial clinical trials with the anti-CD3 monoclonal antibody.” Further information about this trial can be found at www.clinicaltrials.gov by entering the search terms “Gitelman and START.”

Describing research not yet published, Dr. Atkinson and Dr. Haller also recently observed that the combination of ATG and the cancer drug Granulocyte Colony-Stimulating Factor (G-CSF), when given to mice with recent-onset disease, will essentially reverse hyperglycemia in nearly all treated animals. “In almost every mouse receiving those two drugs,” Dr. Atkinson

said, “their diabetes reverses, and it’s permanent.”

Combination therapies do, however, face practical challenges in terms of their implementation, and as a first step in the process, Dr. Haller started a small trial of G-CSF in recent-onset Type 1 patients (an effort also supported by JDRF; for more information, visit www.clinicaltrials.gov and enter the search terms “Haller and G-CSF”). In June, the two research groups (University of Florida and the University of California, San Francisco) will meet to discuss the plans for a human trial using the two drugs in combination.

Human Stem Cells, When Transplanted Into Mice, Are Glucose-Responsive, Insulin-Secreting

Scientists from the San Diego, California–based company Novocell successfully developed a primitive cell line from human embryonic stem cells that, when implanted into mice, was able to reverse chemically induced diabetes. JDRF partly funded the development of the stem cells lines used in this research, which was published in the journal *Nature Biotechnology*.

The primitive cells, chosen for implantation because their biologic profile closely matched that of an important precursor pancreatic tissue called pancreatic endoderm, started producing human insulin in the mice after one to three months. The implanted cells were also shown to produce blood levels of C-peptide comparable to those seen in mice transplanted with about 3,000 isolated human islets; and to protect the mice from hyperglycemia.

The study demonstrates that, “under the right conditions inside the body, human embryonic stem cells can be differentiated down the path to eventually becoming insulin-secreting beta cells,” said Julia Greenstein, Ph.D., director of the Beta Cell Replacement Program at JDRF. “This advance is important,” she added, not only “because of its potential to possibly accelerate progress in

understanding the development of insulin-producing cells, but also for the field of beta cell replacement—one of the five ‘cure therapeutic’ research areas JDRF has identified as offering the most promise in leading to a cure for Type 1 diabetes.”

Dr. Greenstein also noted that the research is in its early stages, and future research will require that the endodermal cells be purified, because a small number of the mice developed tumors. ■