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## In this issue:

- Progress on the stem cell front
- A gene in mice may hold clues to treatments for people
- In the near future, a clinical trial of regulatory T cells

## Producing Beta Cell Precursors from Stem Cells

Using a novel screening method, JDRFI-funded researcher Shuibing Chen and colleagues from the Harvard Stem Cell Institute identified a small molecule that can help drive human embryonic stem cells along the path to becoming insulin-producing beta cells.

When embryonic stem cells were combined with the molecule and a key growth factor, nearly half of them developed into an essential, early precursor cell from which all of the specialized cells of the pancreas are derived, including beta cells. Other research has sought to form insulin precursor cells from embryonic stem cells were far less effective.

Embryonic stem cells have the ability to develop into any type of cell or tissue as they mature, and hold the promise of being turned into insulin-secreting cells that could be transplanted into people with type 1 diabetes.

By uncovering a better way to generate pancreatic progenitor cells from human embryonic stem cells, this research could lead to less costly and faster ways of creating the large, unlimited supply of cells that would be needed for testing and treatment.

The research, published in the journal *Nature Chemical Biology*, was funded through a postdoctoral fellowship award to Dr. Chen and took place in the laboratory of Dr. Douglas Melton, one of the world's preeminent stem cell scientists.

### How and Why

Adapting a method that is widely used to discover new drugs, Dr. Chen and colleagues screened a chemical library of 5,000 different compounds for their ability to increase the production of a key pancreatic precursor cell from embryonic stem cells. Dr. Chen's approach was atypical in that her search focused on small molecules rather than only on traditional growth factors and related compounds. Small-molecule inducers, as they are

called, are typically less expensive to produce and, according to most experts, more easily controlled and possibly more effective in driving the differentiation of stem cells.

Dr. Chen's long-term objective is to identify the right combination of compounds that would allow her to mimic the natural signals occurring during the development of the pancreas. With this information, she hopes to create a clinically useful protocol for differentiating stem cells into functional, transplantable beta cells.

### Main Findings

A compound called ILV stood out in its ability to move embryonic stem cells further toward beta cells. ILV directed the differentiation of a starting stem cell population to the next-step pancreatic progenitor cell stage. This effect became more pronounced (greater numbers of progenitor cells were formed) when more of the compound was used and when it was combined with a specific growth factor.

The researchers also showed that by adding additional agents, the stem cells were able to develop even further into various mature cells of the pancreas, including those that produce insulin.

Animal tests strongly supported ILV's potential. When ILV-treated stem cells were transplanted into mice, some of the cells matured and began to produce both insulin and c-peptide, suggesting the ILV- and growth-factor treated stem cells are "capable of progressing through the pancreatic differentiation program *in vivo* [once transplanted into a body]."

### On Deck

Next steps for the researchers will be to identify, at each step of the differentiation pathway, other small molecules that might promote the development of embryonic stem cell to beta cell. Studies are needed to determine the best cell stage at which to transplant—that is, what will have the best possible outcome for people with diabetes.

### Key Point:

Researchers have developed a more efficient way to produce stem cells that are further along on the path to becoming insulin-producing beta cells. The finding is important progress toward producing the large number of beta cells needed for testing and treatment purposes in people with type 1 diabetes.

## A Gene that Contributes to the Development of Autoimmunity

JDRFI-funded researchers from the University of North Carolina at Chapel Hill have identified a gene in diabetic mice that helps to explain the autoimmune changes that lead to the development of type 1 diabetes.

The gene, called MerTK, appears to block the immune system's early attempts to control autoimmunity—uncontrolled, it eventually causes the onset of diabetes. The gene was specifically shown to prevent the removal of T cells that are primed to attack the insulin-producing beta cells of the pancreas. If MerTK is found to play a similar role in people, it could become a new drug target for treating or even preventing type 1 diabetes.

The study, published in the journal *Proceedings of the National Academy of Sciences*, was led by Roland Tisch, Ph.D., a 2008 JDRFI project grant recipient.

### MerTK and Autoimmunity

Dr. Tisch and his colleagues had previously shown MerTK regulates the behaviour of important immune cells called antigen presenting cells. These cells are constantly interacting with various molecules found within the body and serve a “show-and-tell” function. When they encounter a foreign protein, for example, they display information about that protein to the immune system's T cells, which then act to remove the potential threat. Antigen-presenting cells also process natural proteins like insulin, but the response here is very different. During early development in healthy individuals, T cells that react to insulin are targeted for deletion so they do not initiate autoimmunity; in persons with type 1 diabetes, however, these T cells are not all eliminated, and they eventually make their way to the pancreas where they begin to do harm.

The researchers asked the following question: If MerTK controls antigen presenting cells, might it also be contributing to the development of type 1 diabetes through a similar mechanism? To assess MerTK's role, Dr. Tisch and his colleagues created mice that no longer expressed the gene—scientists routinely “knock out” a gene to see what happens in its absence. The mice, though now without MerTK, still carried predetermined risk factors for type 1 diabetes and should have developed the disease.

Blocking the gene's actions protected the mice from getting diabetes—a confirmation that having the gene causes the disease. Importantly, the suspected mechanism was confirmed, as the researchers showed protection was due to an enhanced elimination of T cells that react to beta cell proteins.

The immune system was able to respond in this way, they determined, because key antigen presenting cells, called dendritic cells, became much more efficient at presenting antigens—including, presumably, insulin and other beta cell proteins—once the gene's effect was removed. These events

take place within a small organ called the thymus. Located behind the breast bone, the thymus is where T cells are “educated” by the dendritic cells and then selected for survival or elimination.

### A Potential Drug Target

Barry Jones, Ph.D., director of JDRFI's autoimmunity program, said diabetic mice clearly have a form of MerTK that prevents the deletion of self-reactive T cells from the thymus; and by implication, a failure of the immune system to eliminate aberrant thymic T cells plays a role in the development of type 1 diabetes.

“The importance of MerTK in human type 1 diabetes remains to be determined,” he emphasized, “but if a role is demonstrated, MerTK might be a potential target for therapeutic intervention in people at risk of developing type 1 diabetes.”

### Key Point:

Researchers have identified a gene involved in the development of autoimmunity in mice. If the gene is determined to have a similar role in people with the disease, it could become a new therapeutic target.

## A Potential T Cell Therapy Clears Laboratory Hurdles

JDRFI-funded researchers have discovered further evidence that regulatory T cells are a promising cell-based therapy for type 1 diabetes. Jeffrey Bluestone, Ph.D., and colleagues from the Diabetes Center at the University of California, San Francisco have shown that regulatory T cells can be isolated from people with type 1 diabetes, and then expanded in the laboratory to levels that could be therapeutically useful without a loss of function or stability.

A type of immune cell, regulatory T cells serve the important role of putting the brakes on immune responses. When other immune T cells begin to attack the insulin-producing beta cells in the pancreas, regulatory T cells orchestrate their removal and slow their destructive actions. Previous research shows regulatory T cells can prevent and even reverse type 1 diabetes in mice when administered in large quantities. The current study, published in the journal *Diabetes*, confirms the feasibility of that approach—isolating, expanding, and ultimately reintroducing the regulatory T cells—as a potential treatment for people.

The work was supported by a JDRFI Collaborative Center for Cell Therapy Grant to Dr. Bluestone, and a postdoctoral fellowship award to researcher Todd Brusko.

Barry Jones, Ph.D., director of JDRFI's autoimmunity program, said the study's solid pre-clinical results provide “clear mechanistic rationale” for a human clinical trial using regulatory T cells. A small phase I study, representing an initial assessment of the therapeutic benefits of such a therapy in people, is being planned.

The goal of the proposed therapy is to restore balance within the immune system by shifting immune system activity toward greater regulation and tolerance. Although the mechanism underlying this shift is not fully understood, there is strong and compelling evidence of the success of the approach in mice with diabetes. The transfer of regulatory T cells expanded in the laboratory somehow “can overcome intrinsic defects and restore tolerance in type 1 diabetes,” the scientists said, emphasizing early treatment would likely provide the best outcomes. “The therapeutic benefits of restoring tolerance early in type 1 diabetes would likely result in the preservation of endogenous beta-cell mass and subsequent reduction in complications resulting from hyperglycemia.”

### Method of Regulatory T cell Expansion

The researchers isolated regulatory T cells from the blood of nine adults who were recently diagnosed with type 1 diabetes, and from three healthy control subjects. The cells were then grown in the laboratory. The researchers evaluated the capacity of the cells to expand as well the behaviour of the expanded cells.

### Large Numbers of Cells are Grown

The researchers identified two separate populations of functional regulatory T cells that expanded to large numbers and could potentially be used for treatment. However, one group was deemed better for clinical use based on several findings:

- After two weeks in culture, these regulatory T cells increased by about 1,500-fold while still maintaining their ability to suppress other types of T cells;
- These cells were attained without using immunosuppressive drugs that help maintain the function of the cells, but inhibit proliferation and growth;
- The enriched T cells continued to express the proteins needed for proper development and function; and
- The expanded cells tended to produce more of a cytokine known to have regulatory properties and less of one known to activate pathogenic T cells.

### Next Steps and Considerations

Regulatory T cells are present in the blood in very low numbers, and so to use them in any type of treatment requires their isolation and expansion. But a major concern with expanding these cells from people with an autoimmune disease like diabetes is the very cells that initiate the autoimmune attack could also be expanded—an unwanted consequence called “outgrowth.” In the current study, the researchers minimized this risk by sorting the cells for markers found only on regulatory T cells.

One of the strengths of the study is the isolated and expanded cells represent a multitude of distinct regulatory T cell lines—each with a specific regulatory task. This aspect of the therapy, the researchers said, is a “necessary advancement” in the treatment of type 1 diabetes because it provides a broader, more encompassing assault against the T cells that are mediating the disease.

However, before regulatory T cells can be used as a cell therapy for type 1 diabetes, several questions must be addressed. These include how long the expanded cells will survive after they are transferred, where in the body they will reside, and whether they will retain their desired suppressive nature once transferred.

“This study outlines important tools and principles for translating [regulatory T cell] therapies into clinical treatments for patients with type 1 diabetes and other autoimmune disorders,” the researchers concluded. ■

### Key Point:

Regulatory T cells are emerging as an important cell therapy for the treatment of type 1 diabetes because they can suppress the actions of the immune cells that cause the disease.



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