New islets—no immunosuppressives

By Tim Fulmer, Senior Writer

Islet cell transplantation could offer an alternative to the lifelong supplementation with insulin that is standard of care for type 1 diabetics. However, transplantation typically requires some form of immunosuppression that can put patients at risk for opportunistic infections.¹ A trio of papers now offers strategies that could help restore islet cell function without the need for immunosuppression.²⁻⁴

In separate articles in the *Proceedings of the National Academy of Sciences*, a **Northwestern University Feinberg School of Medicine** group reported on the chemical induction of host immune tolerance to donor islets in mice and a **Harvard Medical School** team demonstrated selective depletion of autoimmune T cells in fresh blood samples from type 1 diabetics. The latter strategy is already being tested in humans.

The third study, published in the *Journal of Clinical Investigation*, suggests that CD8 T cells that specifically target preproinsulin (PPI) are at least partly responsible for islet destruction in type 1 diabetes and thus reinforces the case for selective depletion of type 1 diabetes-specific autoimmune cells as a therapeutic strategy.

In all cases, a key challenge will be to show whether removing autoreactive T cells from the equation leads to long-term insulin independence and glycemic control.

Getting tolerant

In order to induce tolerance, the Northwestern group started out by isolating and treating donor splenocytes with the cross-linking agent ethylene carbodiimide (ECDI). The splenocytes, which are antigen-presenting cells similar to dendritic cells, were intravenously injected into the host mouse both prior to and after islet cell transplantation.

In a mouse model of nonautoimmune, streptozotocin-induced diabetes, the strategy prolonged the survival of donor transplants compared with that of transplants in mice that received untreated donor splenocytes or splenocytes from a mouse other than the donor. The islets also produced therapeutically detectable amounts of insulin.

Finally, long-term tolerized mice were able to accept a second donor islet graft without further treatment.

The authors proposed that ECDI-treated donor splenocytes could induce host tolerance through multiple pathways, including limiting the antigen-presenting cell-mediated stimulation of donor-specific T cells and upregulating tolerizing T regulatory cells (Tregs).

Stephen Miller, a principal investigator and corresponding author on the *PNAS* paper, told *SciBX* that next steps include looking at the

tolerance-induction strategy in murine models of autoimmune diabetes. In those models, the goals are to prevent both the rejection of an islet graft from an allogeneic donor and the development of long-term autoimmunity against islet autoantigens.

“We’re now studying the strategy in the non-obese diabetic or NOD mouse, an established preclinical model of autoimmune diabetes,” he said. “In this model, we’re faced with the challenge of not only inducing tolerance to the allogeneic islet cell transplant, as we did in the *PNAS* paper, but also overcoming residual host autoimmunity,” said Miller.

In that model, he said, the group has set about modifying tolerization “to include ECDI-treated splenocytes that display autoantigens as well as alloantigens. Thus, we predict we’ll be able to prevent both graft rejection and recurrence of autoimmunity in type 1 diabetes.”

Miller, professor of microbiology and immunology at the Feinberg School of Medicine, said he also plans to study the tolerization strategy in a mouse model of allogeneic graft transplantation that can support the growth of human peripheral T cells.

Xunrong Luo, assistant professor of medicine and surgery at the Feinberg School of Medicine and a principal investigator on the paper, told *SciBX* that “pancreatic islet cell transplantation avoids the need for insulin injections or an insulin pump, which are often unable to physiologically maintain appropriate serum glucose levels and can put patients at risk for hypoglycemia.”

Historically, said Luo, islet cell transplants required the use of immunosuppressives such as steroids, which can injure or kill islets after transplantation.

Luo noted that the so-called Edmonton protocol has eliminated the use of steroids in islet transplantation,⁵ but she said the long-term outcome of islet grafts is still not optimal due to other factors, including β -cell toxicity from other immunosuppressives used in the protocol.

Miller told *SciBX* that an invention disclosure has been filed for the tolerance-induction strategy and that he and Luo would be interested in licensing the technology.

“For probably any islet cell transplantation protocol, key clinical goals will be long-term independence from insulin therapy and improved control over serum glucose,” said Elliot Chaikof, chief of the Division of Vascular Surgery at **Emory University School of Medicine**. “Nonimmunosuppressive strategies for protecting donor islets from host immune response will likely be fundamental to achieving those goals. The *PNAS* paper nicely illustrates the potential strategy of using chemically modified donor splenocytes to induce tolerance to donor islets. The result is a form of toleragenic vaccine.”

Going forward, Chaikof said he wants to see work in other animal models “to better understand the significance of donor splenocyte number and the potential of cellular heterogeneity within that population to influence tolerance induction. Presumably, the fewer splenocytes necessary the better, in order to avoid potential cell-harvesting issues in the donor.”

Chaikof and colleagues are exploring the use of conformal barrier technology in islet cell transplantation.⁶ “In our preclinical work, we’ve sought to develop ultra-thin coatings that allow donor islets to sense glu-

cose and secrete insulin while protecting them from local inflammatory and immunological responses,” he said.

Getting antagonistic

In the second *PNAS* paper, Denise Faustman and colleagues at **Massachusetts General Hospital** and Harvard Medical School used human blood samples to test whether tumor necrosis factor (TNF) and TNF receptor agonists can selectively trigger apoptosis in autoreactive T cells and thus treat autoimmune disorders without immunosuppressants.

Faustman is director of the immunobiology laboratory at the hospital and associate professor of medicine at Harvard Medical School. Her previous work in a NOD mouse model of autoimmune diabetes showed that certain autoimmune T cells were more susceptible to the proapoptotic effects of TNF than normal T cells.^{7,8} Thus, she said, her group “became interested in exploring TNF receptor agonism as a general strategy to treat autoimmune disease beyond just diabetes.”

In the current paper, Faustman and colleagues found that both TNF and a TNF receptor 2 (TNFR2) antibody agonist selectively triggered cell death in a subpopulation of autoreactive CD8 T cells isolated from the serum of type 1 diabetics. TNF and the TNFR2 agonist killed similar subpopulations of CD8 T cells in sera from patients with other autoimmune diseases, including lupus, psoriasis and multiple sclerosis (MS).

“At first glance, the strategy of agonizing TNFR might appear surprising or counterintuitive in this context, since anti-TNF antibody therapies are marketed to treat autoimmune diseases like rheumatoid arthritis and Crohn’s disease,” noted Faustman. “However, a close look at clinical reports in these areas reveals that roughly half of RA patients and only a third of Crohn’s patients respond to long-term anti-TNF therapy.”

Furthermore, Faustman noted that anti-TNF therapies have been shown to worsen disease in some patients with MS. “Thus, based on our own data and past findings, we think boosting TNF stimulation may be warranted in some autoimmune situations,” she said.

Faustman has already begun testing the strategy in type 1 diabetics. Patients are receiving low-dose bacille Calmette-Guérin (BCG) on top of their insulin replacement therapy and the study is monitoring patient T cell responses.

“Based on our published preclinical data, we anticipate that after depletion of autoreactive T cells with BCG, insulin-producing islets will regenerate without the need for islet transplants and immunosuppressives,” she said.

BCG is a live attenuated mycobacterial vaccine that stimulates TNF production; it is marketed to treat tuberculosis.

In the longer term, Faustman hopes to secure funding to test TNFR2 agonists in other autoimmune diseases including lupus and Sjögren’s syndrome.

According to Faustman, the two *PNAS* papers “illustrate different strategies for achieving the same goal—killing off autoimmune T cells without the use of immunosuppressives. Miller’s group does this by inducing T cell tolerance to the islets, whereas we selectively

deplete T cells targeting the islets. In both cases, the lesson is that simply transplanting a large number of islets is not sufficient to treat autoimmune diabetes. The autoreactive T cells that lie at the root of autoimmunity must also be addressed.”

Autoreactive pilot

In addition to the *PNAS* articles, a *JCI* paper further highlights the importance of CD8 autoreactive T cells in autoimmune type 1 diabetes.

Mark Peakman and colleagues at the **King’s College London School of Medicine** identified two naturally processed epitopes of the human PPI molecule that were targeted by CD8 T cells isolated from the blood of type 1 diabetes patients. Moreover, clones of the PPI-targeted CD8 T cells killed human β -cells in an *in vitro* assay.

Peakman, who is a professor of clinical immunology at King’s College, told *SciBX* that the findings suggest two different strategies for potentially preventing or halting progression of type 1 diabetes.

“On the one hand, there may be therapeutic value in using exogenous insulin replacement therapy at very early stages of disease and with greater stringency to prevent or slow β -cell destruction. The rationale here is to reduce β -cell display of autoantigens like

PPI and thus protect the cells from attack by autoantigen-targeting T cells,” he said.

“A second potential therapeutic strategy supported by our findings is selectively depleting autoreactive CD8 T cells that target epitopes expressed by host β -cells,” said Peakman. Selectivity will be the key to the latter approach, he said.

“Given the important role that normal CD8 T cells play in host antiviral response, selectivity will be essential in order for this strategy to avoid potentially subjecting the patient to opportunistic infection,” Peakman said.

“Indeed, a general challenge facing the field is identifying critical epitopes and antigens that are targeted by host T cells in autoimmune diabetes. This work is ongoing in our lab and others, and ideally it will lead to the identification of T cell subpopulations that are critical to induction and progression of autoimmunity,” he added.

“Selectively knocking out these subpopulations would thus become the holy grail for a diabetes therapy with minimal immunosuppression,” said Peakman.

However, there is also evidence that the host autoimmune response is dynamic in type 1 diabetes, with evolving specificities for autoantigens over the course of the disease, a phenomenon known as antigen or epitope spreading. Thus it may not necessarily be the best strategy to deplete T cells that correspond to a single so-called critical autoantigen.

Indeed, according to Douglas Ringler, cofounder, president and CEO of **Tolerx Inc.**, new autoantigens could arise later in disease, triggering another autoimmune reaction that involves a subset of additional autoreactive T cells.

“Consequently, a key aspect of our anti-CD3 strategy in type 1

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Emory University
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diabetes has been to increase the number and function of host T regulatory cells, which then suppress the activity of potentially all autoreactive T cells,” he said. “By upregulating Tregs, we ensure that autoreactive T cells are generally kept in check and prevented from targeting autoantigens associated with islet cell destruction.”

The company’s otelexizumab (formerly TRX4) is an anti-CD3 antibody in a Phase III trial called DEFEND (Durable Response Therapy Evaluation for Early or New Onset Type 1 Diabetes), which is treating patients with new-onset type 1 diabetes who continue to receive insulin replacement therapy.

In a previous trial, the therapy helped preserve islet cell function and lowered the amount of insulin therapy for proper blood glucose control in patients recently diagnosed with type 1 disease.⁹

“The DEFEND trial is enrolling type 1 diabetics within three months of diagnosis. They still have as much as 30% of their islet cell mass present, and the goal is to protect and preserve the function of those islets over the long term and potentially reduce the levels of insulin replacement therapy required for glycemic control,” said Ringler. “However, we also think our therapy could potentially be combined with islet cell regeneration or transplantation therapies to address disease in brittle diabetics—those patients who no longer have any remaining functioning islet cells.”

In 2007, Tolerx granted **GlaxoSmithKline plc** rights to develop and commercialize otelexizumab. Ringler told *SciBX* that GlaxoSmithKline is now considering running trials to test the antibody in multiple other autoimmune indications.

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