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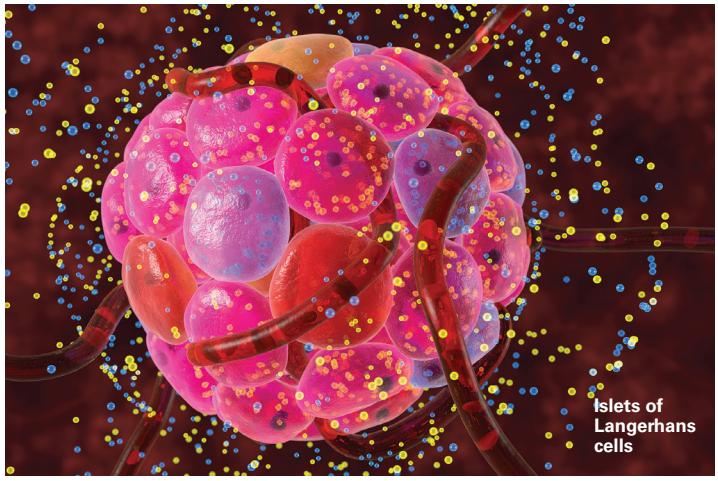
The AJT Report

News and issues that affect organ and tissue transplantation

Stem Cell Derived Beta Cells: The State of the Science

Conference brings together experts to discuss leading developments in the field



DAVID MACK/SCIENCE SOURCE

On September 19, 2016, scientists and physicians from four continents and 12 countries traveled to Boston to discuss stem cell derived beta cells at the International Pancreas and Islet Transplant Association (IPITA)-Juvenile Diabetes Research Foundation/Helmsley-Harvard Stem Cell Institute Key Opinion Leaders Meeting on Stem Cell Derived Beta Cells. "Recently, there have been landmark achievements that have really reinvigorated the field," said Jon S. Odorico, MD, IPITA president, in his welcoming remarks. The goals of the conference were to present this cutting-edge science of pluripotent stem cell derived beta cells and develop an understanding of supporting technologies. Such technologies include encapsulation, function and immune response assays.

The State of the Art of Beta Cell Differentiation

Douglas Melton, PhD, co-scientific director of the Harvard Stem Cell Institute in Boston, presented in the first session of the morning and announced, "All of us are working on this problem of trying to change stem cells into functional beta cells for diabetics." To accomplish this, researchers must address issues of efficiency, longevity, immunogenicity and posttransplantation protection. In his presentation, he described the recent functional improvements in human pluripotent stem cell derived islet-like clusters, then focused much of his talk on the fact that, during the process of beta cell differentiation, only a portion of stem cells develop into the beta cells classically identified for transplantation.

The beta cell induction protocol generates beta, alpha, gamma, delta and epsilon cells, as well as other cells that are difficult to classify. In most cases, generated cells are identified based upon cell surface markers, and it is not clear whether they are truly functional. Dr. Melton's research team has focused attention on the non-beta cells, and he explained his team's approach to understanding these cells in his presentation. Dr. Melton also expressed the growing realization that the physiologic cluster that constitutes an islet includes additional, not yet fully characterized, cells. He put forth a challenge: "We should really be trying to make an islet and not just beta cells." However, he continued, which cell types constitute an islet? Dr. Melton proposed that the two challenges are intertwined and the answers may be complicated by the fact that all cells can exist in a number of physiological states.

Assessing Function: Is It a Mature Beta Cell?

As researchers create pancreatic islets or stem cell derived surrogates, other scientists work in parallel to determine how best to reliably identify a differentiated and functioning beta cell. José Oberholzer, MD, director of the Chicago Diabetes Project at the University of Illinois at Chicago (UIC), explained that regulatory agencies have no precedent for approving an uncharacterized therapeutic. Therefore, any stem cell advancement into clinical trials depends upon the ability to reliably generate and identify cells targeted for transplantation. Dr. Oberholzer thus described the efforts of the Juvenile Diabetes Research Foundation International–UIC Microfluidic-Based Beta-Cell Functional Analysis Facility to develop defined criteria for comparative studies in patients.

Their facility has a biochip-based micro- and nanofluidic, multiparametric perfusion assay that can analyze beta cell physiology and phenotype islet surrogates from various sources.

KEY POINTS

- **Recent advances indicate near-term clinical testing of stem cell derived products to treat type I diabetes.**
- **Challenges still exist before successful clinical application.**

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Dr. Oberholzer acknowledged that the pumpless biochips are "still a little bit artisanal." However, he said, the technology is flexible and can answer questions previously difficult to address. For example, historically, the challenges of creating oxygen gradients have made it difficult to study the effects of hypoxia re-oxygenation. The biochip approach makes it possible to study islet function in hypoxic conditions in microencapsulated islets.

The three-layer microfluidic device requires only small amounts of reagents and analytics. With this device, his group is able to perform real-time functional imaging and quantify calcium signaling and changes in mitochondrial potential. This single cell assay can thus evaluate transdifferentiated neo-beta cells to determine glucose sensitivity. The laboratory has also developed integrated high-throughput islet arrays that allow increased analytical power and a better understanding of the heterogeneity of stem cell derived islet surrogates, as well as better assessment of beta cell toxicity. Dr. Oberholzer said his team now has the ability to carry out dynamic, label-free, live-cell electron microscopy and can perform ultra-structural imaging of living cells and quantify insulin granule mass and size distribution.

We should really be trying to make an islet and not just beta cells.

—Douglas Melton, PhD

Monitoring and Modulating Immune Responses

Any attempt to transplant stem cells into patients with diabetes must also address the host immune response. Immunological response is of particular concern because type 1 diabetes is a T cell–mediated autoimmune disease, and 60% to 70% of patients test positive for HLA-A2. Patients have circulating autoreactive T cells that can be reliably measured. The T cells occur in human insulitic lesions *in situ* and can recapitulate insulitis in humanized mice.

Bart Roep, MD, PhD, chair of the department of diabetes immunology at City of Hope's Diabetes and Metabolism Research Institute in Duarte, Calif., described research being performed to characterize these autoreactive T cells. His group uses nanotechnology for high-throughput detection of low precursor frequency autoreactive CD8+ T cells in stored blood samples. The "Diab-Q-Kit" requires limited amounts of blood and allows for simultaneous detection of T cells reactive to multiple HLA-A2–restricted beta cell epitopes. These detailed analyses revealed that healthy individuals have naïve T cells specific for beta cell epitopes. Moreover, diabetes pathology is associated with a distinct immune signature, and patients who achieve remission following transplantation have a low level of autoimmune CD4+ and CD8+ cells prior to transplantation. In contrast, patients who relapse have autoreactive T cells.

Any effort to transplant beta cells must take into account these autoreactive cells. While human embryonic stem cells (hESC) appear to have immune privileged properties, these properties do not appear to persist in hESC derived cells. Immune intervention such as cell-impermeable macro-encapsulation may be required to successfully transplant hESC derived pancreatic progenitors.

Advances in Encapsulation Technologies for Stem Cell Derived Products

While successful islet transplantations are performed today, patients require not only a large supply of insulin-secreting cell products, but also chronic immunosuppression in order to prevent rejection and specific attack of beta cells. Patients, however, want a solution that does not require chronic immunosuppression. Researchers are meeting this need via the development of encapsulation technologies that can achieve islet immunoisolation and facilitate transplantation.

Initially, investigators attempted to encapsulate human islets in large capsules, but this approach did not result in physiological insulin secretion. Additionally, human islets in large capsules occupy a large volume of transplant material, making them more difficult to place in the body. Alice Tomei, PhD, assistant professor in the department of biomedical engineering at the University of Miami in Florida, and her team designed a microfluidic method to coat islets of different diameters. They achieved a constant thickness hydrogel coating that minimizes capsule thickness. The resulting minimal diffusion barrier allows oxygen and nutrients to reach the islets and facilitates physiological glucose-stimulated insulin response.

The conformal coatings also minimize thickness and allow for transplantation in vascularized sites, which decreases the risk of islet central hypoxia/necrosis. Consequently, transplantation of encapsulated islets in vascularized sites, such as the epididymal fat pad, translates into improved outcomes. "The first thing that you see is that you get much better engraftment," said Dr. Tomei. She proposed that conformal coatings may someday reduce or eliminate the need for chronic immunosuppression.

Road to the Clinic

Kevin D'Amour, PhD, president and CEO of ViaCyte, a San Diego–based research laboratory specializing in regenerative medicine, described the first-in-man combined biologic/device clinical product (VC-01), which currently is in the investigational new drug-enabling preclinical program. The stem cell based islet replacement therapy was designed to address unmet needs in islet replacement

Cyt49 cells were directed to differentiate into pancreatic endoderm cells, and the resulting PEC-01 cells were subjected to manufacturing and release testing. In particular, researchers examined OCT-4 expression during differentiation to evaluate the presence of residual hESC and consequent risk of teratoma. The researchers documented consistently rapid downregulation of OCT-4 as seen in data from more than 40 PEC-01 cell manufacturing runs. The PEC-01 cells were then encapsulated within a retrievable delivery device known as the Encaptra Drug Delivery System.

To date, the VC-01 appears to be safe and well-tolerated. Moreover, the Encaptra appears to be immune protective as designed.

FDA APPROVES FIRST "ARTIFICIAL PANCREAS"

THE U.S. FOOD AND DRUG ADMINISTRATION has approved Medtronic's Hybrid Closed Loop insulin delivery system (MiniMed 670G) for use in patients with diabetes who are 14 years of age or older. The MiniMed 670G system includes an advanced glucose sensor, which has enhanced accuracy and performance and lasts for seven days. The MiniMed 670G is the first device that automatically monitors blood glucose and delivers appropriate basal insulin doses.

Medtronic anticipates bringing the advanced insulin pump technology to market in the spring of 2017. Regulatory approval outside of the United States is expected in the summer of 2017. AJT