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The isolated islet of Langerhans as a micro-organ and its transplantation to cure diabetes mellitus: Celebrating the legacy of Paul Lacy.

WUMS Historica Medica Lecture 43
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Stan Misler
Paul E. Lacy, M.D., Ph.D  
(1924-2005)  
Late Kroc Professor Emeritus,  
Department of Pathology,  
Washington University Medical School  

Two careers in one: “I started my career wanting to learn about the structural basis and cell mechanics of insulin secretion and ended it with a mission to try to cure diabetes with islet transplantation. Gosh, what a surprising turn a life in science can take!”
"Grandpa Islet"

Encourager, enabler, galvanized of others
Proselytizer for islet transplantation: inspiring speaker and amazing gatherer of industrial grant support

Asked “why not” when others more timid asked “wherefore and how”
Personal legacy of openness and generosity of spirit as a mentor may be as important as his legacy to science
“It’s time not to talk about what we’ve done because we’re only as good as the ideas we inspire”

What hath Paul Lacy wrought?

What followed him?
1. The islet as micro-organ
“As a pathologist, I believe what I see.”

A. Pioneering islet microscopy:

1. Characterization of $\alpha$, $\beta$, $\delta$ cell structure:
   $\beta$–cell identified by granule depletion on glucose and sulfonylurea stimulation and $\beta$–cell kill off by streptozotocin

2. Conceptualization of granule “emiocytosis” (exocytosis) for hormone exit

3. Recognition importance of granule maturation; roles of Ca in exocytosis & cytoskeleton in granule movement; preferential approach of granules to membrane after glucose stimulation; Dark field microscopy
B. Lacy’s Gift: isolated islet of Langerhans

Hormone secretion and glucose oxidation by human islets

12-fold peak increase

4-fold sustained decrease

Single islet cells & Cytosolic components of islet cells esp. hormone containing granules

Glucose-induced cell depolarization

biphasic insulin secretion

+cytoskeletal disrupting agents
“Draw me a simple picture of what is happening between uptake of glucose and insulin secretion….and while you’re at it where are the sore spots in type 2 Diabetes (mellitus)”
C. Consensus model of operation of insulin-secreting β cell

1. Glucose uptake by plasma membrane glucose transporters ->
2. Oxidative metabolism of glucose, including by mitochondria, resulting in increased cytosolic ATP and decreased ADP ->
3. Closure of ATP sensitive K channels, K\text{ATP} against a background of open non-selective cation channels C(NS) ->
4. Membrane depolarization ->
5. Opening of voltage dependent ion channels including Ca channels ->
6. Ca entry with binding to protein complex anchoring insulin granule to plasma membrane ->
7. Fusion of insulin granule membrane with plasma membrane and exocytotic release of insulin granule contents. Also some release of GABA from smaller vesicles
1. A potassium channel (K\textsubscript{ATP}) closed by bath applied glucose or sulfonylurea in cell attached patch of intact cell is also closed by bath applied ATP and reopened by bath applied ADP in the inside-out excised membrane patch.
2. Stimulus – depolarization coupling in beta cells:

- glucose metabolism -> closure of K\text{ATP} channels against background of non-selective cation channels, C(NS) -> cell depolarization -> electrical activity due to sequential opening of Na, Ca and K channels
3. Unraveling exocytosis in single b cells
Exocytosis = incorporation of granule membrane into plasma membrane resulting in increased membrane capacitance (prop. surface area; measured by patch clamp mode) as well as simultaneous release of granule’s chemical contents sensed electrochemically (after preloading with serotonin) or seen as emptying of fluorescent marker (total internal reflectance fluorescence microscopy)
Quantal release in β-cells as measured by capacitance increase ($\Delta C_m$) and amperometry ($I_{amp}$)

Single action potentials $\rightarrow$ single granule exocytosis

Prolonged depolarization $\rightarrow$ release of 10s of quanta
4. Cellular basis of gut secreted incretin GLP-1 and vagus nerve secreted Ach in enhancing glucose-induced insulin secretion: enhanced closure of KATP and recruitment of insulin granules for exocytosis
2. Lacy model of biphasic insulin secretion in modern terms
6. Islet paracrine or “social” interactions

β-cells at center of islet perfused first and secrete best when electrically coupled

GABA released by β-cell contributes to depolarization-based inactivation of α-cell

Somatostatin from δ cells reduces secretion from β and α cells: G-protein coupled receptor and stimulation of phosphodiesterase
T2DM, afflicting overweight and older persons with hyperglycemia, has been widely attributed to the “tune-out” of peripheral fat, muscle and liver cells to the effect of insulin thereby promoting hyperglycemia that was toxic to most cells. However newer evidence suggests that β-cell defects are important initiators of the phenotype.

(i) Mutations that provide a gain of function of $K_{ATP}$ (i.e., channels inherently less sensitive to ATP-induced closure) produce T2DM. Actual damage to β-cells can be prevented by normalizing insulin secretion with treatment with a sulfonylurea.

(ii) Day’s long exposure to high concentrations high free fatty acids such as palmitate results in disordering of tight coupling of Ca channels to insulin granules in IRP thus reducing depolarization – induced exocytosis (inc $C_m$) at unchanged calcium current
2. The isolated islet for transplantation
Ricordi: Gartner innovation curve (or hype cycle) defining steps in evolution of clinical islet transplantation

- **Peak of Inflated Expectations**: Edmonton protocol
- **Plateau of Productivity**: Long-term graft survival and function
- **Slope of Enlightenment**: Immunosuppression refinements
- **Trough of Disillusionment**: Low % long-term insulin independence

**Technology Trigger**: Semi-automated islet isolation
A. Lacy’s vision of “curative” islet transplantation in unstable Type I diabetes

• “Islet greediness”: “Harvest pancreas’ worth of islets from a single human donor, purify them, and culture them to reduce their antigenicity

• “Transplant islets into a safe location” in the recipient where they would take root and provide insulin independence, or at least eliminate the daily highs and lows of blood sugars

• Strategies might be needed to prevent immune attacks to avoid long-term use of immuno-suppressants
B. Best guess as to Lacy’s basic assumptions underlying islet transplantation

(i) Reduced model of islet function

- **β-cell-centricity**: Need for moment-to-moment function of beta cells to secrete insulin to control blood glucose. Hyperglycemic function of α-cells secondary concern and should be maintained as in perifusions.

Post Lacy: Restoration of C peptide secretion -> decreased neuropathy and increased myocardial and renal blood flow.

- Innervation and incretins not essential: glucose-induced biphasic secretion seen in isolated, cultured islets. However, incretins (e.g., synthetic analogs of GLP-1) might be used to enhance islet function in marginal cases.

- Islet cells poorly antigenic and if well treated may not need long term immunosuppression: limited antigenicity arises from passenger immune cells and endothelium.

- Islet cells intrinsically plastic: hypertrophy and replacement of worn-out cells occurs in intact islets.
(ii) Ease of islet transplantation as compared with whole pancreas transplantation

- Many choices of target sites: subcapsular in kidney or spleen; intraperitoneal; but especially intrahepatic via injection/embolization into portal vein to provide first pass source of insulin for hepatocytes
- Whole pancreas Tx complicated by high M/M
- No need for exocrine drainage.
C. Proof of principle
(Ballinger, Scharp and Lacy, 1973)

Syngeneic islet transplantation: 400-600 islets into portal veins of streptozotocin-treated, single dose immunosuppressed rats ->
2-12 weeks: abolition of polydypsia, polyuria and post-prandial hyperglycemia; establishment of near normal resting glucose and insulin levels; and reversal of histological lesions of mesenteric autonomic nerve supply
5 months: well granulated intrahepatic beta and alpha cells forming direct contacts with hepatocytes
D. “Trigger technology” = high yield isolation of islets from pancreas of large mammals

Collagenase digestion of pancreas helped by marbles pounding whole pancreas
1. Procedure: Isolation of islets from pancreata and infusion into liver via percutaneous transhepatic catheterization of portal vein under fluoroscopic or ultrasound guidance

E. Clinical islet transplantation at the “peak of inflated expectations” beginning in 1990
Portogram of injection

MRI showing engraftment (steatosis = local fatty liver)

Courtesy: D. Brennan, WUMC
Effective clinical trials of islet transplantation have been limited by the inability to transplant enough viable human islets into patients with type I (insulin-dependent) diabetes mellitus to eliminate their exogenous insulin requirement. We report the first type I diabetic patient with an established kidney transplant on basal cyclosporin immunosuppression who was able to eliminate the insulin requirement after human islet transplantation into the portal vein. We successfully isolated ~800,000 islets that were 95% pure from 1.4 cadaver pancreases containing 121 U of insulin. Islets were proven viable by in vitro insulin response to glucose challenge. After 7 days of 24°C culture, the islets were transplanted into the portal vein under local anesthesia. Seven days of Minnesota antilymphoblast globulin (20 mg/kg) administration followed the islet transplantation, with maintenance of the cyclosporin. Blood glucose was kept under strict control via intravenous insulin for 10 days posttransplantation, when all insulin therapy was stopped. Off insulin, the average 24-h blood glucose level remained <150 mg/dl, with the fasting glucose level at 115 ± 6 mg/dl and the 2-h postprandial level at 141 ± 8 mg/dl for 22 days posttransplantation (the time of this study). The C-peptide values post-Sustacal testing, although initially rising slower, exceeded the normal range, with peak values of 1.0–1.6 pmol/ml. This preliminary result represents the first essential step required to determine the feasibility of islet transplantation by future clinical trials. *Diabetes* 39:515–18, 1990
2. First 15 years of trials, the best results -> “trough of disillusionment” 2005

- Edmonton + Miami + Minnesota
- Type 1 DM X 25 years; hypoglycemic episodes but no renal dysfunction
- Semi-selected pancreati (ABO but not HLA compatible donors without history of DM); cold ischemia < 12h
- Initial transplant 400,000 islet equivalents (IE) (5 cc), 0-2 boosters -> average total 800,000 IE; later 10,000 -14,000 IE /kg body weight
- Induction with antiCD25 antibody (later strong lymphodepleting induction) and steroid free immunosuppression with low dose calcineurin inhibitor and high dose mTOR inhibitor; more recently conversion to mycophenolate acid to avoid nephrotoxicity
- 82% insulin dependent at 1 yr; 20% at 5 years (82% graft survival by C-pee)
- Better glycemic control (HbA1c 7 vs. 9) with fewer episodes of hypoglycemia even if not insulin-dependent after transplant of “marginal” tissue
- Chronic complications: mouth ulcers, anemia, diarrhea, ovarian cysts, acne, increased need for antihypertensives, statins
- Unsolved: peripheral/autonomic neuropathy; hypoglycemic counter-regulation/awareness
F. Overcoming the myopia in early transplant vision or “the slope of enlightenment”

Initially little intimate knowledge or standardization of the islet as a micro-organ.

(a) A priori, metabolic state of transplanted islets should determine their long-term survival and should be dependent on: (i) condition of source pancreas and islets and (ii) stability of islets as micro-organ out of their pancreatic milieu.

(b) However there was continued reliance on pre-transplant optical measures of immediate “viability”, “gentle standards” for quantitation of yield (what is an IE?) and for “in vitro” secretion after acute culture (2-3 fold increase).

Little or no extensive post-transplant assays of “in vivo” functional reserve (e.g., C peptide secretion)
G. Complex pre-transplant issues: Dependence on source and early treatment

(a) Pre-harvest of pancreas: donor age, adiposity, insulin reserve, & cytokine storm in brainstem death
Conundrum: islets of lean & <35y/o -> difficult to liberate but likely robust islets vs. islets of obese & > 50 -> large, encapsulated, easy to liberate islets with less function

(b) Post-harvest of pancreas and pre-isolation of islets: cold ischemia time usually > optimum 6-8 h (“left-over organs”); fluorocarbon O2 transport solution

(c) Continuing problems of isolation: enzyme potency and exposure time

(d) Post-isolation: shaggy vs. round; fresh vs. cultured

(e) Newer developments for rapid pre-transplant islet screening: Laser scanning cytometry; mitochondrial membrane potential assay; tests of increased O2 consumption with glucose stimulation
H. Complex post-transplant issues:
1. “Stranger in a strange hepatic land” -> acute and chronic loss of tissue and function

Islet transplants into liver survive less well than those infused into pancreas:
(i) decreased glucose oxidation and insulin secretion;
(ii) down-regulation of expression of genes of differentiation;
(iii) reduced probability of islet cell replacement by local stem cells and budding pancreatic ducts
2. Attacking islet ischemia and poor post-transplant proliferation

(a) 30% of islets have necrotic cores at time of infusion -> need for non-ischemic preparation

(b) Loss of 30-40% of viable islets by “hypoxia-reperfusion injury”
   -> ischemia Inhibitable by nicotinamide

(c) Rapid onset of apoptosis (including lymphocyte induced) + a, b -> loss of 80% of islets in 3 days.
   Inhibition by ex vivo transduction of islets with X-linked inhibitor of apoptosis?

(d) Poor acute and long-term functional revascularization and oxygenation
   Intra-islet pO2 = 5-10 torr vs. 40 torr in situ -> switch to anaerobic respiration with poor stimulus-evoked secretion. No current treatment

(e) Poor islet cell proliferation Improved by transduction of proliferation switch -> activation of Jak-Stat pathway by membrane permeant agonists?
I. Success with alternative transplantation modalities: lessons learned

- Freshly prepared, partially purified pancreatic tissue infused into portal vein results in long-term (up to 13 year) reduction in fasting glucose and HBA1c; correlated with mass of infused tissue.
  
  Implies that mass of co-transplanted acinar and ductal tissue not an impediment

- Solitary pancreatic transplantation or simultaneous with renal transplantation results in 70-80% survival of pancreatic function up to 3 years (Improvement due to reduced exocrine damage during harvest; bladder drainage of ductal secretion; tacrolimus-based immunosuppression).

  Implies that immunosuppression is not a swift and potent islet killer
J. The future
1. Finding a better microenvironment for islet transplantation: the bone marrow?
   “Who would have thunk it?”
2. Human islet transplantation likely to be superseded by a bioengineering approach…. but which?

- **Chemical engineering of encapsulation with alginate:** xenogenic porcine microencapsulation followed by (i) intraperitoneal injection or (ii) macro or hollow fiber encapsulation with subcutaneous patch implantation. This should avoid immunosuppression and in xenotransplant should protect against foreign oncogenes, viruses while still susceptible to macrophage attack.

- **Developmental engineering:** induction of embryonic stem cells (ES = inner cell mass of preimplantation blastocysts) or pleuripotential stem cells (iPS) for differentiation along β-cell lineage

- **Cell engineering (transdifferentiation):** directed transdifferentiation of hepatic and pancreatic exocrine ductal cells or acinar cell line reprogramming of these by adenovirus mediated transduction of PDX1 and NGN3, MAFA for ectopic expression

- **Whole organ bioengineering:** pancreatic extracellular matrix (ECM) with signaling molecules and growth factors to drive cellular repopulation, and enhance islet secretion and survival. Mimicable by biomolecular carriers?
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