Pathways to Protecting from Autoimmunity the Resurrected/Regenerated Beta Cells

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Diabetes Center at UCSF
Is islet replacement a viable option?

**Total:** \( n = 26,660 \)
- **Non USA:** \( n = 6,548 \)
- **USA:** \( n = 20,014 \)
1989-Current (n=462)
ERA 1-3 : OKT3 Induction
ERA 4 : Thymoglobulin Induction

<table>
<thead>
<tr>
<th>Maintenance</th>
<th>Incidence of pancreas rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERA 1: OKT3/CSA/AZA/PRED</td>
<td>80%</td>
</tr>
<tr>
<td>ERA 2: OKT3/CSA/MMF/PRED</td>
<td>50%</td>
</tr>
<tr>
<td>ERA 3: OKT3/TACROLIMUS/MMF/PRED</td>
<td>15-20%</td>
</tr>
<tr>
<td>ERA 4: STEROID AVOIDANCE</td>
<td>10-15%</td>
</tr>
<tr>
<td>Thymoglobulin Induction</td>
<td></td>
</tr>
<tr>
<td>low dose tacrolimus/</td>
<td></td>
</tr>
<tr>
<td>sirolimus/MMF</td>
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</tbody>
</table>
Why isn’t pancreas only more common?

- Difficult operation
- Immunosuppression is still intense
- Difficulty in recognizing rejection
Is islets transplantation feasible?

Safer, Simpler Procedure than Pancreas Transplant

Better quality of life?

Lower requirement of immunosuppression?
Graft Survival (insulin independence)
Islet Transplant Registry


* Only well documented cases
Five-Year Follow-Up After Clinical Islet Transplantation

Edmond A. Ryan,1 Breay W. Paty,1 Peter A. Senior,1 David Bigam,2 Eman Alfadhli,1 Norman M. Kneteman,2 Jonathan R.T. Lakey,2 and A.M. James Shapiro2
Publication of the initial results of the Edmonton protocol in 2000 (1) raised hopes that many of the technical and immunologic hurdles of islet transplantation had finally been solved and that a new era for the treatment and cure of type 1 diabetes had arrived. Unfortunately, while short term results utilizing this specific protocol were repeated by other groups around the globe, long-term follow-up revealed that islet transplantation with this particular protocol is far less successful than originally hoped (2,3). Thus, although 5 years after transplantation 85% of recipients had measurable plasma C-peptide, well-controlled HbA1c levels, significant diminution in amount of daily insulin required, and virtually no clinical hypoglycemia (3), only 10% of patients experienced freedom from exogenous insulin use. While this still may represent partial success in alleviating the debilitating symptoms that brought them to islet transplant in the first place, such a claim needs to ultimately be established in a controlled trial, like other medical advances. Moreover, toxicities from the calcineurin inhibitors combined with sirolimus used for immunosuppression produced worrisome trends in renal function (4). Given continued insulin dependence, the shortage of donor organs, the complications of immunosuppression, and the great expense of this procedure, sober reassessment of the clinical applicability of this protocol and particular experiment is needed.
Why a decay in islet function?

- Rejection?
- Autoimmunity?
- Drug toxicity?
- No precursor cells?

Extensive studies by Shapiro, Nepom, Alejandro and others suggest that autoimmune destruction is a major cause of graft loss.
CTLA4Ig is tolerogenic in mice – blocks alloimmune and autoimmunity in some settings

Lenschow et al. Science, 1992
**Immunosuppressive Protocols**

**Efalizumab**

- **SIROLIMUS** (Target trough 8-12 ng/L) (substitute mycophenolate if not tolerated)
- **EFALIZUMAB** 1 mg/kg/wk
- **ATG**
- **0.5 mg/kg/wk** Drug withdrawn in all pts on May, 2009

**Belatacept**

- **SIROLIMUS** (Target trough 8-12 ng/L) (substitute mycophenolate if not tolerated)
- **Belatacept (10 mg/kg/mo)**
- **ATG**
- **5 mg/kg/mo**
- **5 mg/kg/2mos**

*Note: ATG dosage is indicated as a range, and the columns represent the timeline of the protocols.*
Graft Function

Time from initial transplant (days)

Discontinued Efalizumab

Insulin Dependent
Insulin Independent
Intermittent Use

BELA-1
BELA-3
BELA-5
EFA-1
EFA-3
EFA-5

Tx # 2 (day 445)
Tx # 2 (day 442)
Tx # 2 (day 400)
HbA1c and c-peptide levels after Islet Transplantation

Belatacept

Efalizumab

HbA1c (%)

Time from Transplant (d)

C - Peptide (ng/ml)

Time (minutes)
5-year Graft Survival Rates in Islet and Pancreas Recipients

Islet

Kaplan-Meier Estimates (n=20)

% Insulin Independence

Years Post Transplant

50%

Pancreas

% 80
70
60
50
40
30
20
10
0


PAK PTA SPK Px SPK Kd

50%!
Islet transplantation works but is not a CURE

1. There will not be enough cadaveric islets to go around
2. Current immunosuppression regimens require life-long treatment that can result in severe complications including increased infections, cancer and organ toxicity

- High quality islets can be produced from cadaveric donors
- Immunosuppressant cocktail prevents rejection
- >200 patients (>300 procedures) ~90% 1 year
Renewable Islet Cell Source: Pancreatic Progenitors (PE)

ViaCyte has optimized the protocol for making PE from human embryonic stem cells (hESC)

In vitro protocol...

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitive endoderm</td>
<td>Primitive gut tube</td>
<td>Posterior foregut</td>
<td>Pancreatic endoderm and endocrine precursors</td>
</tr>
<tr>
<td>ActA + WNT</td>
<td>KGF</td>
<td>RA + Cyc + Nog</td>
<td>No factors</td>
</tr>
<tr>
<td>RPMI</td>
<td>RPMI</td>
<td>RPMI</td>
<td>DMEM</td>
</tr>
<tr>
<td>no FBS</td>
<td>0.2% FBS</td>
<td>2% FBS</td>
<td>1% B27</td>
</tr>
</tbody>
</table>

1 day | 2 days | 3 days | 3 days


...recapitulates natural embryonic development of the human pancreas
Function of hESC-Pancreatic Epithelium After Implantation into Immunodeficient Mice

hESC-Islet Graft
Implant days: 377

Human Islet Graft
Implant days: 360

Serum C-peptide (pM)

Controls
d246
d365
d30
d44
d71
d94
d30
d44
d71
d94
d30
d44
d71
d94

Fasting
30 min. glucose
60 min. glucose

0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000

Adult islets
hES cell-derived pancreatic endoderm

Glucagon
Somatostatin
Insulin
hES-derived PE cells are immunogenic

Day 14

C57BL/6

SCID

PDX Ins

GCG/SST/Ins

www.diabetes.ucsf.edu
hES PE transplant using co-stimulation blockade

- Anti-mThymocyte (500 µg/dose)
  - or
  - Anti-CD154 (500 µg/dose)
  - plus
  - CTLA4Ig (500 µg/dose)

hES Cell Derived PE

Stage: 1 2 3 4

Day of Transplant

Histology

Glucose Stimulation

www.diabetes.ucsf.edu
Combination Immuno-therapy promotes short-term graft survival: Day 3 through Day 14.

- **Post-Transplant:**
  - Day 3
  - Day 7
  - Day 14

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 14</th>
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</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocompetent</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C57BL/6</td>
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<tr>
<td>hCTLA+anti-CD40L</td>
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<tr>
<td>Immunosuppressed</td>
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<tr>
<td>NOD.SCID</td>
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<tr>
<td>Immunodeficient</td>
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H&E 20x

www.diabetes.ucsf.edu
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<th>Preserved Human Nuclear staining, PDX, and Insulin through Day 14</th>
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<tr>
<td>Post-Transplant:</td>
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<td>Day 3</td>
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<td><img src="image6.png" alt="Image" /></td>
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<tr>
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<td><img src="image7.png" alt="Image" /></td>
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<td><img src="image8.png" alt="Image" /></td>
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<tr>
<td><img src="image9.png" alt="Image" /></td>
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**HuN**
**PDX**
**INS**
**20X**
Days Post Transplant - Tx CTLA4Ig+anti-CD40L

Day 84

Day 109

Day 141
C-peptide production after alternative forms of immune suppression

3 month IVGTT

Human c-peptide (pM)

1000
100
10
1

B6  NOD  RAPA  CD154+ CTLA4Ig  ATG+ CTLA4Ig  SCID
Therapies that work in immunocompetent animal are not working in autoimmune mouse models.
Membrane System is Critical to the Encaptra™ Device

The membrane is designed to:
- Exclude host immune cells
- Retain graft cells
- Facilitate vascularization immediately adjacent to graft tissue

Two components:
- Outer membrane: Biocompatibility & vascularization
- Inner membrane: Cell impermeable yet open to transport of nutrients, proteins, etc.
Where do we go from here?
Can we use Tregs as Therapeutics?

**In vivo** antigen-specific expansion of T\textsubscript{reg} cell population

**Ex vivo** antigen-specific expansion of T\textsubscript{reg} cell population
Adoptive Treg immunotherapy works in NOD mice

Tregs block pathogenic T cell differentiation

Ag-specific Tregs are most effective

Tregs effectively suppress diabetes

Ag-specific Tregs reverse diabetes
Isolated human CD4+CD25+CD127lo T cells can be expanded efficiently from peripheral blood.

CD4+ T cells

CD127

FOXP3

Absolute cell number


5 billion Tregs
Expansion of donor-specific Tregs using CD40-activated allogeneic B cells
SUMMARY

- Islet cell replacement is a viable option
- Targeted immunosuppression (co-stimulation blockade and ATG induction) can lead to 5 year survival equal to pancreas transplantation
- Human PE cells derived from hES can develop into glucose responsive islets in mice
- Current immunotherapies do not block PE graft rejection in diabetic mice
- New immune depleting protocols coupled with Treg therapy can lead to prolonged islet survival in diabetic mice
- Current efforts are underway to move Treg therapy into the clinic
Collaborators

Diabetes Center
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ViaCyte

NIAID

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