Enumerating β-Cells in Whole Human Islets: Sex Differences and Associations With Clinical Outcomes After Islet Transplantation

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Islet transplantation is an experimental therapy for type 1 diabetes. Though it is increasingly successful, limitations include unpredictable declines in islet graft function (1). There is still inadequate knowledge of specific human islet characteristics that predispose to successful and durable islet graft function and what types of donors are more likely to have islets with these beneficial traits. The relationship between the composition of dissociated human islets (as opposed to whole islets) and transplant outcomes has been studied (2), with a positive association between recipients' acute insulin response to glucose (AIR_g) posttransplant and number of pancreatic ductal cells in the preparation (a positive association with number of β-cells approached significance). However, as dissociation inherently damages islets, β-cells enumerated after dissociation may no longer reflect the number of β-cells within whole islets ultimately transplanted. Using an epidemiological approach, we therefore investigated *1*) the independent association of the β-cell composition of transplanted whole human islets with recipient outcomes and *2*) the donor characteristics associated with β-cell composition.

Pancreata were biopsied prior to islet isolation (3), then immunostained for insulin, glucagon, and somatostatin (Fig. 1). An average of 867 immunoreactive cells were enumerated for each donor, and percent staining positive for insulin (β-cells) was calculated. Recipients were part of phase 1/2 ($n = 1$) or phase 3 ($n = 13$) clinical trials. Donor and recipient characteristics (e.g., age, sex, BMI) and serial assessments of β-cell function (i.e., fasting and stimulated C-peptide, insulin, and glucose and HbA_{1c}) up to 15 months posttransplant were collected (≤295 total longitudinal time points).

Forty-seven biopsies were enumerated (mean donor age 47.9 years and BMI 31.3; 48.9% female). Using multivariable regression, donor characteristics significantly associated with greater β-cell percent included former/current alcohol use ($P = 0.008$), a lower white blood cell count prior to procurement ($P = 0.04$), and female sex ($P = 0.01$), in which the model estimated females had on average 6.0% more β-cells relative to males. Donor age, BMI, cause of death, and smoking were not associated with β-cell percent nor were they confounders.

Islets from 19 of these pancreata (57.9% female) were transplanted into 14 recipients (78.6% female). The mean β-cell percent of biopsies from donors whose islets were ($n = 19$) or were not ($n = 28$) transplanted did not differ (68.3% vs. 69.4%, $P = 0.66$). Using multivariable regression (repeated-measures with clustering), a greater percentage of β-cells in transplanted islets was associated with higher fasting C-peptide and lower stimulated glucose, even after adjusting for the number of islets transplanted and other confounders (e.g., recipient age, sex, weight, prior transplants). β-Cell percent was not associated with HbA_{1c} or AIR_g.

As alcohol use was obtained from next of kin and may be incomplete, future research should evaluate associations with islet composition more thoroughly. While it is known that pregnancy upregulates β-cell mass, this study revealed potential sex differences in whole human islets where (nonpregnant) females may have a higher percent of β-cells relative to males. In addition, a higher β-cell percent in transplanted islets produced significant improvements in recipient outcomes. One explanation for this potential sex difference is that estradiol protects islets from apoptosis and enhances glucose-induced insulin secretion (4). These findings may be important as currently only 35% of donors used for islet transplantation are female

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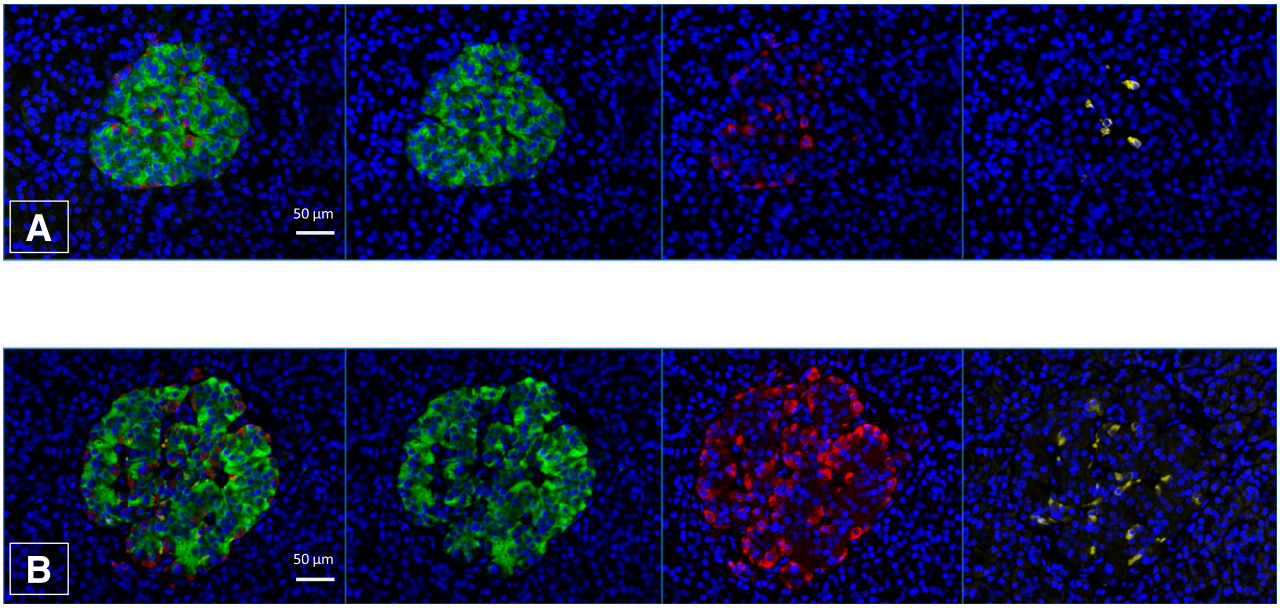

Figure 1—Representative images of islets stained with immunofluorescence in human donor pancreas sections with similar total cell counts with high (*A*) and low (*B*) β-cell percent. Blue, nuclei; green, insulin (β-cells); red, glucagon (α-cells); yellow, somatostatin (δ-cells).

(5). However, larger clinical studies are needed to confirm whether female islets provide a therapeutic advantage.

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References

1. Balamurugan AN, Naziruddin B, Lockridge A, et al. Islet product characteristics and factors related to successful human islet transplantation from the Collaborative Islet Transplant Registry (CITR) 1999-2010. Am J Transplant 2014; 14:2595–2606
2. Street CN, Lakey JR, Shapiro AM, et al. Islet graft assessment in the Edmonton protocol: implications for predicting long-term clinical outcome. Diabetes 2004;53:3107–3114
3. Gangemi A, Salehi P, Hatipoglu B, et al. Islet transplantation for brittle type 1 diabetes: the UIC protocol. Am J Transplant 2008;8:1250–1261
4. Tiano JP, Mauvais-Jarvis F. Importance of oestrogen receptors to preserve functional beta-cell mass in diabetes. Nat Rev Endocrinol 2012;8:342–351
5. Collaborative Islet Transplant Registry Coordinating Center. Collaborative Islet Transplant Registry Eighth Annual Report, 2012. Available from http://www.citregistry.org/reports/reports.htm. Accessed 16 March 2015