Islet Graft Assessment in the Edmonton Protocol
Implications for Predicting Long-Term Clinical Outcome
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The success of the Edmonton Protocol for islet transplantation has provided new hope in the treatment of type 1 diabetes. This study reports on the assessment of 83 human islet grafts transplanted using the Edmonton Protocol since 1999. Cellular composition, as assessed by immunohistochemistry, showed a lower islet purity (~40%) than has been reported in previous studies using dithizone staining to quantitate islet equivalents. Furthermore, grafts were found to contain substantial populations of exocrine and ductal tissue. Total cellular insulin transplanted was 8,097.6 ± 3,164.4 μg/patient, and was significantly lower in bottom gradient layer grafts than top gradient layer or whole/combined grafts (P < 0.0005). A static incubation test for islet function gave a stimulation index of 3–4, although this measure did not correlate with posttransplant metabolic outcome. Furthermore, we confirmed a previously reported trend in which donor age affects islet yield and purity. It is important to note that a significant positive correlation was observed between the number of islet progenitor (ductal-epithelial) cells transplanted and long-term metabolic success as assessed by intravenous glucose tolerance test at ~2 years posttransplant. In summary, careful assessment of islet graft composition is needed in a clinical transplantation program to accurately estimate islet purity and assess the contribution of other cell types present, such as islet progenitor cells. Diabetes 53:3107–3114, 2004

The Edmonton Protocol (1) and follow-up studies (2,3) have shown that islet transplantation is now a therapeutic alternative for some patients with type 1 diabetes. Despite improvements in the isolation of human islets (1,4,5), donor recoveries and characterization have continued to vary. The assessment of islet equivalents and the proportion of dithizone-positive aggregates have been the standard measures used to estimate yield and purity (6,7), respectively. However, these techniques are not necessarily quantitative largely due to observer subjectivity, and more accurate methods to assess human islet grafts are needed. Pipeleers and colleagues (8,9) have shown that islet graft composition with respect to endocrine as well as nonendocrine cell types can influence long-term metabolic function of rat islet grafts, and have also characterized human islet allografts to select the best preparations with respect to endocrine content and donor-recipient compatibility (10).

Graft function is another important factor when characterizing human clinical preparations. Methods to test human islet function include transplantation in immunodeficient mice (4), assessment of insulin biosynthesis (11), in vitro perfusion (12), and static incubation (13,14). The ability to correct hyperglycemia in an animal model is the most accurate assessment; however, this requires a large number of islets and several days to produce an outcome. Of the two in vitro techniques, static incubation is the fastest and easiest way to obtain an index of insulin secretory activity.

The assessment of clinical human islet grafts in a comprehensive manner would allow the refinement of isolation protocols and the selection of optimal cell preparations for transplant based on comparisons with patient outcome measures. We report here the characterization of 83 human islet grafts transplanted into 35 recipients since the inception of the Edmonton Protocol. Preparations were analyzed for endocrine and nonendocrine cellular composition, total cellular insulin content, total cell number, and β-cell function during static incubation. In addition, graft characteristics were compared with donor age and established postransplant patient outcome measures (2,3).

RESEARCH DESIGN AND METHODS
Islet isolation and transplantation. Pancreata were removed from cadaveric donors with prior informed written consent and stored in chilled University of Wisconsin solution (range 1–15.5 h) before islet isolation. Alternatively, several organs (n = 14) were preserved using a two-layer method (University of Wisconsin Solution/perfluorocarbon) (15) for 2–3 h before isolation. Islet isolation was performed as previously described for human islets (1,5). After gradient purification, tissue was collected from the topmost islet-rich gradient layers and transplanted as one preparation. Alternatively, in the less successful purifications, islets were collected separately from both the topmost layer (designated as "top") as well as the more dense middle layers (designated as "bottom"); these preparations were transplanted sequentially during the same procedure. Immediately after islets were isolated, duplicate samples were taken for immunohistochemistry and dithizone/
ductal cells, 1/1,000 rabbit anti-human somatostatin (Dako) to identify proportions of all four endocrine cell types (determined. Total endocrine cell purity was calculated by summing the obtained by immunostaining, the absolute number of each cell type was cellular mass per graft, total DNA content was divided by 6.6 pg DNA per

1/1,000 rabbit anti-human pancreatic polypeptide (Dako) to identify Peyer's exocrine cells, 1/1,000 guinea pig anti-porcine insulin (Dako, Carpinteria, CA) diaminobenzidine), as previously described (16). Primary antibody concent-

ABC/DAB method (Avidin-Biotin complex visualized with the chromagen ethanol.

water-based Bouin fixative for 12 min before being stored at 4°C in 70%

bond slides (Marienfeld, Lauda-Koenigshofen, Germany) and fixed in a described (16); cell suspensions were then placed on positively charged histo-

enzymatically dissociated into single cell preparations, as previously de-

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immunostaining of single cell islet samples was performed using the Statview (SAS Institute, Cary, NC). Correlation analysis between graft com-

Statistical analysis. Data are expressed as means ± SD. The statistical significance of differences was calculated by a one-way ANOVA and the post hoc Scheffe's test or unpaired Student's t test. The analysis of correlations between graft function/composition and donor/isolation characteristics was by Pearson’s correlation coefficient and Fisher’s r to z test performed on Statview (SAS Institute, Cary, NC). Correlation analysis between graft com-

RESULTS

Phenotypic composition of clinical human islet grafts. Table 1 illustrates the cellular composition and absolute cell number of 83 clinical human islet grafts. The majority of these preparations (n = 69) was evaluated and transplanted as whole preparations as multiple islet-rich gradient tissue layers were combined during the isolation procedure. Alternatively, during some isolations (n = 14), the top and bottom islet layers were collected from the Cobe processor and subsequently assessed and transplanted sequentially. In these cases, values are reported for the layers individually and as a weighted average for both combined, taking into account the total cell mass of each fraction.
Table 1 also shows that the proportion and mass of β-cells in each graft was variable, with 23.4 ± 11.9% insulin-positive cells and a mass of 147.4 ± 160.7 × 10⁶ cells. The proportion of insulin-positive cells was slightly, but not significantly, lower than that found in top layer alone preparations (28.6 ± 15.3%), although the smaller overall cell mass of the top layer preparations resulted in a smaller absolute number of β-cells (94.2 ± 94.7 × 10⁶). Separated bottom layer preparations, in contrast, showed a significantly lower proportion (7.1 ± 4.9%; P < 0.0001) and absolute number (21.7 ± 15.5 × 10⁶; P < 0.05) of β-cells as compared with both top and whole preparations. When values were combined for top and bottom layers, the β-cell proportion and absolute number were not significantly different from that observed for whole preparations. Similar results were seen when comparing the remaining three islet endocrine cell types.

Grafts were found to contain a significant and variable amount of both exocrine (amylase-positive) and ductal (cytokeratin-19-positive) cells (Table 1). Bottom layer preparations were generally less pure than top layer and whole preparations, as evidenced by a significantly higher proportion of ductal (36.7 ± 15.5%; P < 0.05 vs. whole preparations) and exocrine (44.9 ± 9.4%; P < 0.005 vs. top layer preparations) cells. These differences, however, were not significant when considering the absolute numbers of ductal and exocrine cells present in the grafts due to differences in overall graft mass.

Immunostaining was also performed to examine the presence of other cell types in clinical islet grafts, such as immune cells and vascular endothelial cells. It was found that <1% of the cells expressed CD45 (n = 10) and smooth muscle cell α-actin (n = 10). For this reason, analysis of these markers was discontinued in the overall graft analysis protocol.

The total mass of each cell type implanted per patient was calculated by adding the individual values for all grafts transplanted. Patients received an average of 320.0 ± 206.5, 132.8 ± 96.5, 63.7 ± 46.0, and 76.2 ± 76.1 × 10⁶ β-, α-, δ-, and PP-expressing cells, respectively. In comparison, a higher and more variable number of ductal and exocrine cells were received by these patients (ductal: 454.9 ± 306.7 × 10⁶; exocrine: 754.0 ± 609.3 × 10⁶).

Comparison of dithizone-based purity and endocrine purity as assessed by immunostaining. Islet purity, as assessed subjectively at the time of isolation based on dithizone staining (4,6) in 82 clinical preparations, was compared with the results using immunostaining to quantitate total endocrine cellular composition (as described in research design and methods). The dithizone-based purity assessment suggested that these preparations were composed of 66.9 ± 15.8% islets, a number significantly higher than indicated by the endocrine immunostaining results for whole (41.1 ± 16.3%; P < 0.0001), top layer (46.7 ± 20.1%; P < 0.0005), and bottom layer (18.1 ± 11.0%; P < 0.0001) preparations. In addition, a significant difference was seen in total endocrine purity using immunostaining in bottom layer grafts versus both whole (P < 0.0001) and top layer (P < 0.0001) grafts.

Mass and insulin content of human islet grafts. Clinical islet grafts were also assessed for cellular insulin and DNA content and number of islet equivalents (Table 2). Bottom layers contained a significantly lower amount of cellular insulin (P < 0.0005) than all other preparations, despite the fact that the total DNA content of top and bottom layers was similar. Thus, there was a significantly lower insulin-to-DNA ratio in the bottom layer as opposed to the top layer preparations (P < 0.01). Furthermore, the number of islet equivalents was significantly lower in bottom layer preparations (63.6 ± 43.5 × 10⁶; P < 0.0001) versus whole (344.2 ± 103.3 × 10⁶) and top layer (383.5 ± 158.6 × 10⁶) preparations, whereas top and bottom grafts combined (447.1 ± 169.3 × 10⁶) contained a significantly higher number (P < 0.005) of islet equivalents than the whole preparations. The average total cellular insulin, DNA, and islet equivalents summed over all grafts received by a patient was 8,097.6 ± 3,164.4 μg, 12,064.1 ± 6,438.9 μg (1,827.9 ± 975.6 × 10⁶ cells), and 815.9 ± 195.2 × 10³ islet equivalents, respectively.

In vitro insulin secretory activity. Insulin secretion from islet preparations was assessed during static incubation (14,16). The insulin secretory activity was not significantly different when comparing whole, top layer, and bottom layer preparations (Table 3). Thus, the calculated stimulation indexes were also similar. The only significant difference observed was that of insulin release per 10 islet equivalents at high glucose in bottom layer grafts (P < 0.005 vs. whole and top layer); however, because of a higher basal release at 2.8 mmol/l glucose, this did not make the stimulation index significantly different.

Effect of donor age on islet graft composition/function. Isolations from donors age <25 years yielded fewer islets than those from donors age 25–50 or >50 years. (Table 4), although this difference did not reach statistical
Effect of graft composition on long-term clinical outcome. We compared several established patient outcome measures, including insulin independence and insulin response to arginine or glucose challenge, to islet graft composition and glucose-stimulated insulin secretion in an attempt to define a predictor(s) of clinical islet transplant success.

We assessed whether the total number of β-cells transplanted could provide a prediction of short-term metabolic outcome. Figure 1 shows a comparison of β-cell number transplanted and both the acute insulin response to arginine (AIRarg) and AUC for insulin (AUCins) for the same patient group as was assessed in a previous report with respect to the number of islet equivalents transplanted (3). Although β-cell number versus AIRarg showed a positive correlation approaching statistical significance ($r = 0.609; P = 0.08$) (Fig. 1A), neither metabolic measure was strongly associated with absolute β-cell number transplanted.

We also attempted to define a correlation between graft cellular composition and long-term transplant success. Two groups of patients transplanted >19 months earlier were selected based on whether they remained insulin independent ($n = 6$) or required subsequent insulin injections posttransplant ($n = 5$). These groups were chosen by an endocrinologist blinded to any graft assessment data; the average time posttransplant did not differ between these groups (success group 19.8 months and failure group 19.1 months). One difference observed between these groups was the absolute number of ductal-epithelial cells (cytokeratin-19 positive) transplanted. Patients in the success group were implanted with a total of $500.5 \times 10^6$ (median $435.3 \times 10^6$) ductal cells, whereas those in the failure group received $277.3 \pm 224.3 \times 10^6$ (median $232.3 \times 10^6$) cells ($P = 0.093$). When assessed as success and failure groups, no difference was seen between the mean number of β-cells transplanted between the groups ($276.0 \pm 58.2 \times 10^6$ in success group, $421.2 \pm 400.2 \times 10^6$ in failure group; $P = 0.40$).

In addition to this analysis, when examining a larger group of patients individually ($n = 19$), a statistically significant positive correlation was observed between the

### TABLE 3
Insulin secretory activity of clinical islet preparations as assessed by static incubation

<table>
<thead>
<tr>
<th></th>
<th>2.8 mmol/l glucose</th>
<th>20 mmol/l glucose</th>
<th>Stimulation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular insulin content (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole preparations</td>
<td>66</td>
<td>4.3 ± 5.1</td>
<td>11.2 ± 7.7</td>
</tr>
<tr>
<td>Top preparations</td>
<td>14</td>
<td>4.5 ± 3.2</td>
<td>12.2 ± 6.9</td>
</tr>
<tr>
<td>Bottom preparations</td>
<td>14</td>
<td>3.1 ± 3.1</td>
<td>10.5 ± 6.7</td>
</tr>
<tr>
<td>Insulin release per 10 islet equivalents (ng)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole preparations</td>
<td>59</td>
<td>2.5 ± 2.5</td>
<td>5.8 ± 4.2</td>
</tr>
<tr>
<td>Top preparations</td>
<td>14</td>
<td>2.6 ± 2.1</td>
<td>7.4 ± 5.1</td>
</tr>
<tr>
<td>Bottom preparations</td>
<td>14</td>
<td>3.2 ± 2.9</td>
<td>10.9 ± 7.1*</td>
</tr>
<tr>
<td>Insulin release per β-cell (pg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole preparations</td>
<td>31</td>
<td>1.5 ± 1.3</td>
<td>3.6 ± 2.6</td>
</tr>
<tr>
<td>Top preparations</td>
<td>12</td>
<td>1.2 ± 0.9</td>
<td>3.2 ± 2.5</td>
</tr>
<tr>
<td>Bottom preparations</td>
<td>12</td>
<td>0.8 ± 0.3</td>
<td>3.0 ± 1.5</td>
</tr>
</tbody>
</table>

Data are means ± SD. Values for insulin release are expressed as the percent of total cellular insulin content released, nanograms per islet equivalent, or picograms per individual β-cell. The stimulation index is expressed as the ratio of insulin release at 20 mmol/l glucose to insulin release at 2.8 mmol/l glucose. *$P < 0.005$ vs. release per 10 islet equivalents in whole preparations. Three preparations did not contain a sufficient tissue volume after 18–24 h culture to accurately measure function and thus were not included in this assessment.

significance. However, fewer islet preparations from young donors (<25 years) were considered suitable for transplantation (20%) than those from donors age 25–50 (38%) or >50 (39%) years. No significant differences were found in endocrine, ductal, or exocrine mass or cellular proportion regardless of the donor’s age-group (Table 4). Similarly, although the islet stimulation index was lower in preparations from older donors and the insulin content per β-cell was lower in younger donors, neither of these differences reached statistical significance due to the large degree of variation within these age-groups.

### TABLE 4
Effect of donor age on human islet graft composition and function

<table>
<thead>
<tr>
<th></th>
<th>Age &lt;25 years</th>
<th>Age 25–50 years</th>
<th>Age &gt;50 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>31</td>
<td>23</td>
</tr>
<tr>
<td>Islet equivalents ($\times 10^6$)</td>
<td>290.0 ± 58.9</td>
<td>339.6 ± 104.9</td>
<td>362.6 ± 99.5</td>
</tr>
<tr>
<td>Endocrine cell mass ($\times 10^6$)</td>
<td>251.3 ± 197.4</td>
<td>267.2 ± 219.5</td>
<td>289.7 ± 368.8</td>
</tr>
<tr>
<td>Endocrine cell proportion (%)</td>
<td>40.3 ± 15.8</td>
<td>42.8 ± 15.4</td>
<td>40.3 ± 17.1</td>
</tr>
<tr>
<td>Ductal cell mass ($\times 10^6$)</td>
<td>185.8 ± 188.1</td>
<td>193.8 ± 255.0</td>
<td>184.7 ± 181.6</td>
</tr>
<tr>
<td>Ductal cell proportion (%)</td>
<td>21.6 ± 7.6</td>
<td>23.3 ± 13.2</td>
<td>25.2 ± 12.2</td>
</tr>
<tr>
<td>Exocrine cell mass ($\times 10^6$)</td>
<td>452.4 ± 578.9</td>
<td>379.5 ± 550.0</td>
<td>245.4 ± 357.3</td>
</tr>
<tr>
<td>Exocrine cell proportion (%)</td>
<td>38.7 ± 18.3</td>
<td>35.3 ± 17.5</td>
<td>31.7 ± 13.2</td>
</tr>
<tr>
<td>Stimulation index</td>
<td>4.4 ± 6.7</td>
<td>4.8 ± 4.1</td>
<td>2.8 ± 1.4</td>
</tr>
<tr>
<td>Insulin content per β-cell (pg)</td>
<td>23.9 ± 15.9</td>
<td>34.8 ± 25.3</td>
<td>35.1 ± 25.4</td>
</tr>
</tbody>
</table>

Data are means ± SD and represent percent, absolute cell numbers, or ratios for the given number of human islet grafts derived from donors aged <25, 25–50, and >50 years.
number of ductal cells transplanted and AIRg at ∼2 years posttransplant ($r = 0.490$, $P < 0.05$) (Fig. 2D). In this same group of patients, no significant correlation was observed between the number of ductal cells transplanted and AIRg at only 3 months posttransplant ($r = 0.421$, $P = 0.118$) (Fig. 2C) or between the number of β-cells (Fig. 2A and B) or exocrine cells (Fig. 2E and F) transplanted and AIRg at 3 months and 2 years posttransplant. Furthermore, no significant correlation was observed between the total islet equivalents or total pack cell volume transplanted and AIRg at 2 years posttransplant (data not shown). A significant positive correlation was seen between the number of ductal cells transplanted and the change in AIRg from 1 month to 1 year posttransplant ($r = 0.513$, $P < 0.05$) (Fig. 3).

The posttransplant metabolic outcome was also compared between patients receiving immunosuppression under the Edmonton Protocol and those receiving a modified infliximab protocol. No significant difference in AIRg at 3 months or 2 years posttransplant was seen between these patient groups.

**DISCUSSION**

Although methods exist to subjectively assess islet mass (i.e., islet equivalents) and graft purity, few researchers are rigorously assessing the cellular composition and function of these grafts. We have reported here data from 83 human islet preparations transplanted into 35 patients beginning with the inception of the Edmonton Protocol. These data were compared with donor age and long-term posttransplant metabolic function in recipients to define graft-dependent predictor(s) of subsequent isolation and transplant success.

In our analysis, dissociated cell preparations were used as opposed to staining of whole fixed cellular aggregates (17,18) to more accurately assess the proportion of each cell type. Although, based on dithizone staining, human islet preparations are routinely estimated to be >50% in purity (19), and often approach 80–90% (4) purity, our results demonstrated this to be an overestimation. In fact, the difference in overall endocrine purity of our preparations as indicated by immunostaining versus that indicated by dithizone-based estimations was highly significant. Our results indicated 50% endocrine purity to be an exceptional isolation outcome, even for separated top layer–only preparations. Furthermore, our findings indicated an approximate endocrine composition of 60% β-cells, 23% α-cells, 10% δ-cells, and 10% PP-cells. Although PP cells were more abundant than α- or δ-cells in bottom layer preparations, overall they were less common (this result was considered to be an anomaly of the specific bottom layer preparations used in this study).

Analysis of cellular composition showed that islet grafts were composed of mostly nonendocrine cells. Using cytokeratin-19 as a ductal cell marker (21,22), it was observed that a significant proportion of ductal cells (20–30%) were present. Furthermore, a large proportion (20–50%) of these preparations consisted of amylase-positive exocrine cells. As expected, the less pure bottom layer preparations contained a significantly higher proportion of both ductal and exocrine cells. Correspondingly, these preparations also had a significantly lower proportion of endocrine cells than the purer top layer preparations. These findings underscore the need to determine the positive and negative effects of having nonislet tissue present in clinical islet grafts, including the possible presence of islet progenitor cells that could contribute to new β-cell mass through differentiation posttransplant.

The measurement of total cellular insulin content and total cell number in islet grafts can also give an estimate of purity. Previous studies have suggested the total cellular insulin content of the adult pancreas to be ∼140 μg/g tissue (23). Assuming an average pancreas weight of 90 g, this translates to ∼12,600 μg total insulin. We obtained an average of 3,000–4,000 μg insulin in each islet preparation. Therefore, we either recovered only 25–30% of the β-cell mass or β-cell degranulation occurred during the isolation process. Our patients received a total of ∼8,000 μg of insulin, suggesting that even after undergoing 2–3 transplant procedures, islet recipients receive only ∼65% of the cellular insulin content present in a normal pancreas. Furthermore, each patient received an average of $320 \times 10^6$ β-cells over multiple transplants, with an average of 815,000 islet equivalents, thereby suggesting an average β-cell number of 400 per islet equivalent.

Glucose-stimulated insulin secretion is an important
predictor of islet function. Calculating insulin secretory activity as the percent of the total cellular insulin content released, absolute insulin release per 10 islet equivalents and absolute release per individual β-cell gave similar stimulation indexes in the range of 3–4. This compares with a fivefold average increase reported by Ricordi et al. (4) using the perifusion method and is in agreement with an earlier study by Grant et al. (24), who also reported a fivefold stimulation index for human islets using a static incubation. Our observations, however, suggest that static incubation data are not necessarily a good predictor of clinical outcome, as no correlations were seen with post-transplant metabolic success. The fact that some successful patients receive islets with poor in vitro insulin release (i.e., stimulation index <1) suggests that functional recovery occurs posttransplant or that static incubation is not a representative test of islet function in vivo. Other tests of function should be evaluated in the future, including perifusion to show a more dynamic biphasic insulin release profile, assessment of insulin biosynthesis, and animal transplantation to test actual in vivo function of grafted islets.

It has been proposed that islets derived from younger donors exhibit metabolic advantages for clinical transplantation (23,25). A younger pancreas contains more insulin per gram of tissue as well as a lower concentration of exocrine digestive enzymes, thus limiting islet destruction by enzymes released from necrotic exocrine tissue during and after isolation (23). However, isolations from donors age <30 years are technically difficult and yield fewer islets than isolations from older donors (25,26). We have shown a similar trend to that previously reported (25,26),...
where isolations from donors age <25 years yielded fewer islet equivalents, although in contrast to the other studies, our results did not reach statistical significance. Islet β-cell mass was also examined with respect to donor age and a trend was observed for younger donors to exhibit a lower insulin content per β-cell, lower number of islet equivalents recovered per gram of pancreas, and a higher stimulation index than donors age >50 years. It should be noted that a smaller percentage of islet isolations from young donors were considered suitable for transplantation (20 vs. 38% of donors age 25–50 years and 39% of donors age >50 years). For this reason, the results of this study may be skewed toward indicating less of a difference among age groups, as only the best preparations from younger donor isolations were included in the clinical dataset.

Variability in graft cellular composition may be useful as a predictor of clinical transplant outcome. Our group has previously reported (2,3) that the AIRg and AIRarg indicate both graft mass and function posttransplant. Furthermore, Ryan et al. (3) have reported a correlation between the number of islet equivalents transplanted and the metabolic measures AIRg and AUCins. We examined whether the same metabolic measures correlated with the total number of β-cells transplanted in the same patients as the Ryan et al. study (3). A correlation was found between AIRg and β-cell number transplanted that approached statistical significance ($r = 0.609; P = 0.08$); however, there was no correlation between AUCins and number of β-cells transplanted. The reason for this discrepancy with the previous study is likely the inaccuracy of the method for counting islet equivalents as compared with immunostaining and quantification of dissociated cell samples.

Several patients under the Edmonton Protocol have remained insulin independent for >3 years (unpublished data); however, in some cases, graft failure not associated with immunorejection has necessitated a return to insulin therapy. Finegood et al. (27) used a mathematical model to predict an average β-cell life of 1–3 months and proposed a dynamic system of β-cell turnover in the adult pancreas. This suggests that islet cell turnover may also occur in clinical grafts to sustain long-term function after transplant. We examined whether the number of ductal-epithe-

### FIG. 3. Relation of change in AIRg from 1 month to 1 year posttransplant ($n = 17$) with total number of ductal cells transplanted in patients receiving islet grafts using the Edmonton Protocol.

**In conclusion, we have provided a summary of human islet grafts with respect to cellular composition, mass, and function. A discrepancy has been shown between purity estimated by dithizone staining and the more quantitative immunostaining of representative dissociated cell samples. In addition, we have shown that islet graft assessment may provide information about subsequent transplant outcome in that the presence of ductal cells may improve long-term metabolic outcome. It is important to note, however, that significant variability exists in islet isolation success, graft characteristics, and patient outcome. Thus, it remains difficult to accurately define characteristics of donors or graft composition that affect clinical outcome. However, with the improvement and standardization of islet isolation techniques, detailed information collected on human islet grafts in the future will allow the refinement of clinical islet transplantation procedures to build on the success of the Edmonton Protocol.**

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