Pituitary Cotransplantation Significantly Improves the Performance, Insulin Content, and Vascularization of Renal Subcapsular Islet Grafts

Alberto M. Davalli, Luisa Scaglia, Marco Brevi, Francesca Sanvito, Massimo Freschi, Ugo Cavallaro, Albert F. Parlow, and Antonio E. Pontiroli

Because the pituitary contains hormones with β-cell trophic activity, we evaluated whether cotransplantation of pituitary tissue with pancreatic islets might be beneficial for islet graft function and survival. Streptozotocin diabetic nude mice were transplanted under the kidney capsule with 150 handpicked islets alone or mixed with two diced pituitaries and were then followed for 4 weeks. Mice transplanted with mixed islet/pituitary grafts had higher levels of circulating prolactin (PRL) than mice transplanted with islets only, while serum cortisol, growth hormone, and follicle-stimulating hormone were similar in the two groups. After transplantation, recipients of mixed islet/pituitary grafts showed a more pronounced decrease in glycemic levels and higher systemic insulin levels than mice transplanted only with islets. Mixed islet/pituitary grafts were macroscopically characterized by an excellent vascularization and were biochemically characterized by higher insulin and PRL content than pure islet grafts. Histologically, posttransplantation remodeling originated a hybrid organ in which healthy, well-vascularized islets were adjacent to pituitary cell clusters. Transplantations performed to address the specific effect of the anterior versus the intermediate pituitary lobes indicated the former as responsible for the improved function of cotransplanted islets. Mixed islet/pituitary grafts composed of anterior lobes were also the best vascularized and were histologically characterized by the presence of many folliculo-stellate cells. In conclusion, we obtained evidence that pituitary cotransplantation significantly improves the function, insulin content, and vascularization of suboptimal islet grafts. Evidence suggesting that ectopically produced PRL and/or locally released angiogenic peptides might play a causal role is provided. ***Diabetes*** **48:59–65, 1999**

From the Unit of Metabolic Diseases (A.M.D., L.S., M.B., A.E.P.), the Unit of Biotechnology (U.C.), and the Department of Pathology (F.S., M.F.), Istituto Scientifico San Raffaele, Milan, Italy; and the National Hormone and Pituitary Program (A.F.P.), Harbor-UCLA Medical Center, Torrance, California.

Address correspondence and reprint requests to Dr. Alberto M. Davalli, Unit of Metabolic Diseases, Istituto Scientifico San Raffaele, Via Olgettina 60, 20132-I Milan, Italy. E-mail: alberto.davalli@hsr.it.

Received for publication 17 November 1997 and accepted in revised form 4 September 1998.

b-FGF, basic fibroblast growth factor; FS, folliculo-stellate; FSH, follicle-stimulating hormone; GH, growth hormone; islet/pit.AL, islet/pituitary anterior lobe; islet/pit.IL, islet/pituitary intermediate lobe; IPGTT, intraperitoneal glucose tolerance test; NGF, nerve growth factor; PBS, phosphate-buffered saline; PRL, prolactin; RIA, radioimmunoassay; STZ, streptozotocin; VEGF, vascular endothelial growth factor.

Islet transplantation has the potential to normalize glucose levels in people with type 1 diabetes (1–3); however, important obstacles must still be overcome to improve this procedure in the clinical setting (4). Even when initially successful, human islet grafts survive and function significantly less than whole-pancreas transplants (5), and the reasons responsible for this remain essentially unknown. However, it is almost unanimously accepted that the β-cell mass initially transplanted within an average islet preparation might be inadequate to sustain the long-term metabolic demand and would therefore undergo functional exhaustion over time (6). In addition, posttransplantation hypoxia causes an immediate loss of islet tissue (7) that further reduces the number of β-cells, which may successfully engraft. It appears therefore extremely important to develop strategies aimed to promote survival and function of transplanted β-cells; this is particularly needed for human islet allotransplantation where the mass of implanted islets is often borderline.

In this study, we examined whether cotransplantation of pituitary tissue, together with pancreatic islets, is beneficial for the islet graft. The rationale of this approach resides in the evidence that the β-cell mass of stabilized islet grafts can increase in response to adequate stimulation (8) and that prolactin (PRL) and growth hormone (GH) are potent stimuli for β-cell replication. PRL and placental lactogens are considered responsible for the increased β-cell mass observed during pregnancy (9–11). The presence of PRL receptors in pancreatic β-cells and the increase of their expression during pregnancy have been recently demonstrated (12) and confirm the physiological role of PRL in the adaptation of the endocrine pancreas to this situation of increased metabolic demand. Moreover, a positive regulation of the β-cell mass has been documented also in animal models of chronically elevated PRL and GH levels (13), where the hypertrophy of the endocrine pancreas is a typical feature. Finally, PRL and GH potently stimulate β-cell replication also in vitro (10,14).

β-Cells are extremely sensitive to hypoxia (15), thus explaining why pancreatic islets are so richly vascularized (16,17) and why posttransplantation ischemia is so detrimental for transplanted islets (7). Besides the six classic trophic hormones, the pituitary also expresses an extraordinary number of growth factors and cytokines, the local production of which mediate the function and cellular organization of the anterior pituitary (18). In particular, the folliculo-stellate (FS) cells of the pituitary release large amounts of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF),

which were, in fact, originally purified from their conditioned media (19–21). Normally, VEGF is expressed in pancreatic islets (22), and a significant increase in its mRNA is observed under hypoxic conditions (23,24). Hypoxia-induced increase in VEGF expression is supposed to trigger angiogenesis and revascularization of transplanted islets by endothelial cells of host origin (25). Unfortunately, while hypoxia may be instrumental for stimulating revascularization, it also triggers apoptosis (26,27). Increasing intragraft expression of VEGF and b-FGF, by cotransplanting pituitary tissue together with the islets, might ameliorate whole-graft vascularization and exert a positive effect on transplanted β-cells.

RESEARCH DESIGN AND METHODS

Animals. Outbred male CD1 mice (8–10 weeks old) were used as tissue donors for allotransplantation of streptozotocin (STZ)-diabetic nude mice. Male nude mice (Nu/Nu, 6–8 weeks old) were rendered diabetic by injecting a single dose of STZ (180 mg/kg body wt i.p.). Diabetes was confirmed by the presence of hyperglycemia (>19.4 mmol/l), weight loss, and polyuria. Random (nonfasted) glycemic levels were measured (between 9:00 and 10:00 A.M.) on whole blood obtained from the snipped tail with a portable glucose meter (One touch II; Johnson & Johnson, Milpitas, CA). The animals were kept under conventional conditions in climatized rooms with free access to water and food.

Islet isolation and islet graft composition. Islets were isolated and purified by using the technique already described in detail (28). Aliquots of 150 islets (100–150 μm in diameter) were handpicked and transplanted alone or mixed together with pituitary tissue.

Pituitary graft composition. Mice used for the islets were also used as pituitary donors. Immediately after the removal of the pancreas, the pituitary was exposed and removed. Under the stereomicroscope (Leica MZ6 [Leica, Mikroskpie, Weilburg, Germany], the pituitary was cleaned of membranes, and the posterior lobe (neurohypophysis) was peeled off and discarded. The anterior and intermediate lobes (from two pituitaries) were pooled, diced into small fragments (~0.1–0.2 mm in diameter), and transplanted alone or with the islets. In additional experiments, anterior lobes were surgically separated from the intermediate lobes, and homologous lobes (from two pituitaries) were transplanted together with the islets.

Groups of graft recipients. The following five groups of recipients were used: group 1 received a pure pituitary graft (n = 4); group 2 received a pure islet graft (n = 17); group 3 received a mixed islet/pituitary graft (n = 17); group 4 received a mixed islet/pituitary anterior lobe (islet/pit.AL) graft (n = 5); and group 5 received a mixed islet/pituitary intermediate lobe (islet/pit.IL) graft (n = 5).

Transplantation and follow-up. Grafts were performed under the kidney capsule by using a 1-ml Hamilton syringe (Hamilton, Reno, NE) as previously described in detail (7). Only animals in which the loss of tissue during the procedure was considered <5% entered into the study. After transplantation, mice were followed for 28–30 days, with body weight and nonfasted blood glucose monitored at day 14 and 28. The day before they were killed, after an 8-h fast, mice were bled for measurements of glucose, insulin, PRL, GH, FSH, and cortisol serum levels. Insulin was measured by radioimmunoassay (RIA) using a commercial kit and a rat insulin standard (Incstar, Stillwater, MN). PRL, GH, and FSH were also measured by RIAs (anti-mouse PRL [final dilution 1:400,000]; anti-rat GH [final dilution 1:3,000,000]; anti-human FSH [final dilution 1:200,000]). Serum cortisol was measured by using a competitive enzyme immunoassay (Aia-pack cort; Tosoh, Kyobashi, Japan).

Intraperitoneal glucose tolerance test (IPGTT). An IPGTT was performed 3 weeks after transplantation after an 8-h fast. Mice were injected intraperitoneally with a 10% glucose solution at a dosage of 2 g/kg body wt, with glucose measurements at 0, 30, 60, 90, and 120 min.

Characterization of harvested grafts

Graft vascularization in vivo. At the time mice were killed, a laparotomy was performed in the anesthetized recipient, and the kidney bearing the graft was exposed and photographed at standard magnification (20) with a camera connected to a stereomicroscope (Leica MZ6). Color prints (20 × 30 cm) were used to quantitate the total (surface) graft and vessel areas. To do so, whole graft and vessels perimeters were accurately traced on an acetate sheet placed above the print, corresponding areas were then measured by placing the acetate over a 1-mm^2 paper sheet. Every single square millimeter entirely within the perimeter of the graft or vessels was counted, and the areas were then expressed in arbitrary units. The relative (percentual) graft vascularization was then calculated by dividing the area occupied by the vessels for the total graft area.

Graft insulin, PRL, and GH content. Harvested grafts were extracted as previously described (7) and insulin, PRL, and GH contents were assayed by RIA.

Graft morphology and cellular composition. Morphological and immunohistochemical analyses were performed on formalin-fixed, paraffin-embedded specimens. After deparaffinization and rehydration, 5-μm sections were stained with hematoxylin-eosin and immunostained for insulin (guinea pig anti-porcine 1:1,500; Eurodiagnostica, Malmo, Sweden), glucagon (rabbit anti-porcine 1:8; Dako, Carpintera, CA), prolactin (rabbit anti-rat prolactin 1:200; Eurodiagnostica), S-100 (rabbit anti-mouse, 1:6,000; Dako), and CD31 (mouse anti-human, 1:10; Dako). Slides were blocked for 30 min in 5% normal goat serum and 0.1% Triton X-100 in phosphate-buffered saline (PBS) and exposed overnight at 4°C to primary antibodies. Sections were then washed and incubated with the corresponding secondary antibodies for 1 h at room temperature. The specific staining was detected using the ABC immunoperoxidase system (Vectastatin, Vector, Burlingame, CA) and 3-amino-9-ethyl carbazole or diaminobenzidine as chromogens.

Pituitary PRL and GH content. At the time of death, the naïve pituitaries from recipients of pure islet and mixed islet/pituitary grafts were retrieved, weighed, and extracted in 1 ml of $NaHCO_3$ 0.01 mol/l for measurements of PRL and GH content by RIAs. The results were compared with those obtained in normal (nontransplanted) Nu/Nu mice.

Pancreas histology. At the time of death, the pancreases of mice transplanted with pure islet- and mixed islet/pituitary grafts were harvested and immunostained for insulin to detect residual β-cells.

Statistical analysis. Data are expressed as means ± SE. Comparisons between two groups were performed by using the paired or unpaired Student's t test, two-tailed, as appropriate. Multiple comparisons between groups were done by using the one-way analysis of variance. P values <0.05 were considered significant.

RESULTS

Posttransplantation metabolic follow-up. The recipient's parameters measured 4 weeks after transplantation are shown in Table 1. Gain in the recipient's body weight was

TABLE 1
Recipient parameters measured 4 weeks after transplantation

Graft composition	Gain in body weight (g)	Fasting blood glucose (mmol/l)	Fasting plasma insulin (pg/ml)	Fasting serum cortisol (ng/ml)	Fasting serum PRL (ng/ml)	Fasting serum GH (ng/ml)	Fasting serum FSH (ng/ml)
Pure pituitary	–8.6 ± 1.2*	18.9 ± 0.5‡	0.0 ± 0.0‖	ND	ND	ND	ND
Pure islet	4.9 ± 0.8	8.1 ± 0.4	379 ± 42	6.8 ± 1.8	14.5 ± 7.2	17.2 ± 2.4	25.5 ± 4.3
Islet/pituitary	6.6 ± 0.7	4.8 ± 0.3§	680 ± 50¶	6.7 ± 2.5	102 ± 14#	20.7 ± 2.2	27.7 ± 3.1
Islet/pit.AL	4.6 ± 1.2	4.5 ± 0.4§	804 ± 38¶	ND	ND	ND	ND
Islet/pit.IL	3.6 ± 1.0†	5.6 ± 0.3§	452 ± 41	ND	ND	ND	ND

Metabolic and hormonal parameters in the different groups of graft recipients were measured the day before death. Body weight and fasting blood glucose levels were measured in all transplanted animals; insulin levels were measured in all recipients' groups (n = 5 in each group, excluding mice transplanted with pure pituitary grafts, n = 4). Serum cortisol, PRL, GH, and FSH levels were measured only in mice transplanted with pure islet and mixed islet/pituitary grafts (n = 6 in each group for cortisol; n = 4 in each group for PRL, GH, and FSH). *P < 0.0001 vs. all groups; †P < 0.05 vs. islet/pituitary; ‡P < 0.001 vs. all groups; §P < 0.01 vs. pure islet; ‖P < 0.001 vs. all groups; ¶P < 0.01 vs. pure islet; #P < 0.001 vs. pure islet. ND, not determined.

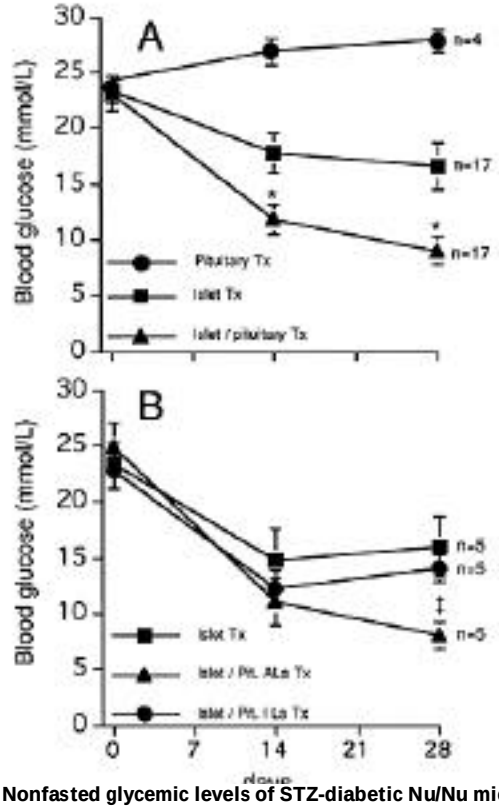

FIG. 1. *A*: Nonfasted glycemic levels of STZ-diabetic Nu/Nu mice transplanted with pure pituitary (pituitary Tx), pure islet (islet Tx), and mixed islet/pituitary (ALs plus ILs) grafts (islet/pituitary Tx). After transplantation, blood glucose decrements were only marginal in mice transplanted with pure islet grafts. In contrast, when pituitary tissue was cotransplanted together with the same number of islets, a significant reduction of glycemic levels was observed; *P < 0.01 vs. islet Tx. *B*: A series of pure islet grafts is compared with mixed grafts in which the pituitary part was alternatively made of anterior (islet/pit.AL Tx) or intermediate lobes (islet/pit.IL Tx). At day 28, glycemia did not differ among pure islet and islet/pit.IL grafts, while it was significantly lower in mice transplanted with mixed islet/pit.ALs grafts; ‡P < 0.05.

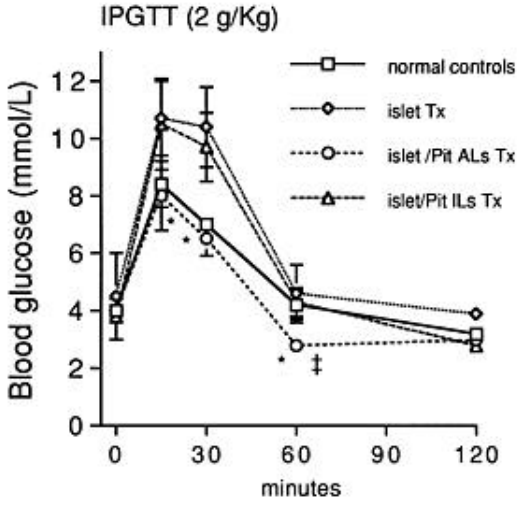

FIG. 2. IPGTTs performed in mice transplanted with pure islet grafts (islet Tx), in mice transplanted with mixed islet/pituitary grafts in which the pituitary part was alternatively made of anterior (islet/pit.ALs Tx) or intermediate lobes (islet/pit.IL Tx), and in normal controls. Mice transplanted with islet/pit.AL grafts had glycemic excursions very similar to those of normal controls and significantly lower than mice transplanted with pure islet or with mixed islet/pit.IL grafts; *P < 0.01 vs. islet Tx and islet/pit.Tx, ‡P < 0.02 vs. normal controls (n = 5 in each group).

similar among the different groups, with the exclusion of recipients of pure pituitary grafts (which lost ~9 g) and recipients of mixed islet/pit.IL grafts, which gained less than mice transplanted with mixed islet/pituitary grafts. Fasting glycemic levels were lower in mice that received any kind of mixed graft than in those receiving only islets or only pituitaries. Mice transplanted with mixed islet/pituitary and islet/pit.AL grafts, had higher insulin levels than recipients of pure islet grafts. Circulating PRL levels were higher in mice transplanted with mixed islet/pituitary grafts than in mice transplanted only with islets, while GH, FSH, and cortisol levels were similar in the two groups. Two and four weeks after transplantation, nonfasted blood glucose levels of mice transplanted with mixed islet/pituitary grafts were significantly lower than those of mice transplanted with only islets or only pituitaries (Fig. 1*A*). Transplantations performed to explore the specific impact of the anterior versus the posterior pituitary lobe, showed significantly lower glycemic levels in mice transplanted with mixed islet/pit.AL grafts than in recipients of mixed islet/pit.IL grafts and of pure islet grafts (Fig. 1*B*). Mice transplanted with mixed islet/pit.AL grafts showed also lower glycemic increments in response to an IPGTT, as compared with mice transplanted with pure islet- or mixed islet/pit.IL grafts (Fig. 2).

Graft vascularization. In Fig. 3*A* a typical islet graft 28 days after transplantation is shown, while in Fig. 3*B* and *C* two representative mixed islet/pituitary grafts performed into a normal and a diabetic recipient are shown, respectively. At a difference with pure islet grafts, an extremely rich vascularization was observed in mixed grafts that showed many large vessels seldom observed in pure islet grafts (Fig. 1*A*). When the area occupied by the vessels was calculated as the percentage of the total (surface) graft area, it was ~3% in pure islet grafts, and 10, 15, and 8% in mixed islet/pituitary, islet/pit.AL, and islet/pit.IL grafts, respectively (Table 2). Mixed islet/pit.AL grafts were significantly better vascularized than mixed islet/pit.IL grafts. In pure pituitary grafts, the percentual graft vascularization was ~9%. Mixed islet/pituitary grafts appeared to be very well vascularized also at the microscopic level (Fig. 4); seriate sections stained for CD31 (a marker of endothelial cells) and insulin, showed well-vascularized β-cells scattered within pituitary tissue (Fig. 5).

Graft morphology and immunohistochemistry. Post-transplantation remodeling originated a hybrid organ in which pituitary cell clusters were adjacent to pancreatic islets. Immunohistochemistry for insulin and glucagon showed well-granulated β- and α-cells (Fig. 6*A*,*B*). Staining for prolactin showed the presence of numerous positive cells scattered within the pituitary cell clusters (not shown). Mixed islet/pit.IL grafts were characterized by the presence of saccular formations, near healthy islets, which resemble the hypophyseal cleft and many cyst-like structures often containing cellular debris (Fig. 6*C*). Mixed islet/pit.AL grafts were characterized by the presence of many cells that stained positively for S-100 (Fig. 7*A*). Although in lower

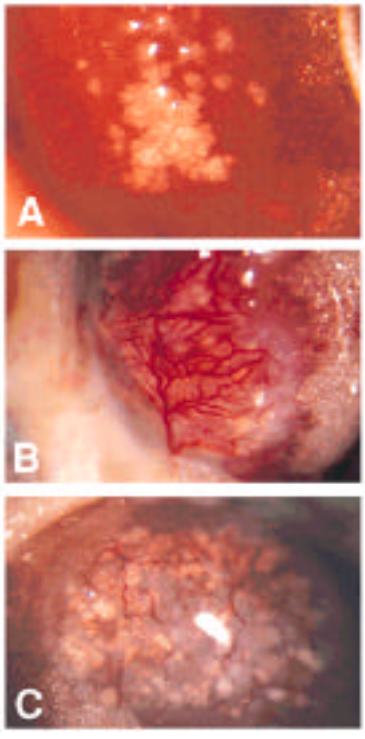

FIG. 3. In vivo graft vascularization of a representative pure islet graft (*A*) and of mixed islet/pituitary grafts performed into a normal graft (*B*) and diabetic recipient (*C*) 4 weeks after transplantation. The excellent vascularization of mixed grafts is evident, characterized by the presence of many large vessels usually absent in pure islet grafts (original magnification ×20).

number, similar cells were also detected in mixed islet/pit.IL grafts (Fig. 7*B*).

A similar, extremely low number of insulin-positive cells were detected in the pancreases retrieved from mice transplanted with pure islet grafts (n = 5) and with mixed islet/pituitary grafts (n = 5, not shown).

Graft insulin, PRL, and GH content. Insulin and PRL content of mixed islet/pituitary grafts were significantly higher than those of pure islet grafts (Table 2). GH content tended to be higher in mixed islet/pituitary than in pure islet grafts (6.7 ± 2.7 vs. 1.1 ± 0.5 total μg) even though the statistical significance was not obtained.

Pituitary PRL and GH content. PRL content of naive pituitaries retrieved from mice transplanted with pure islet and mixed islet/pituitary grafts and normal control Nu/Nu mice was 482 ± 56 (n = 8), 450 ± 51 (n = 8), and 611 ± 190 (n = 4) ng/mg of pituitary weight, respectively, NS. Also naïve pituitary GH content did not change in the three groups being 13.4 ± 2.3, 13.9 ± 2, and 15.7 ± 4.6 μg/mg of pituitary weight, respectively, NS.

DISCUSSION

We obtained the evidence that cotransplantation of pituitary tissue with pancreatic islets is beneficial for islet graft function and insulin content. Implantation of ectopic pituitaries did not induce an excess of GH, FSH, and cortisol in the recipients, while PRL levels were significantly increased (Table 2). This agrees with previous reports showing that the pituitary-grafted rat is a model of relatively pure hyperprolactinemia (29). Pituitary cells physiologically under tonic hypothalamic stimulation, when ectopically transplanted (i.e., kidney capsule) and suddenly deprived of trophic factors, undergo atrophy (30), probably through an apoptotic program (31). GH content of mixed islet/pituitary grafts tended to be higher than that of pure islet grafts; however, since serum GH levels were similar in these two groups of recipients, we speculate that ectopic somatotroph cells were actually undergoing atrophy and that if grafts were retrieved a few days later, their GH content would have been more significantly reduced. Conversely, lactotrophs, which are physiologically under central dopaminergic inhibition, survive after renal subcapsular transplantation (30). Accordingly to that, we observed many PRL-positive cells (not shown) and an elevated PRL content in mixed islet/pituitary grafts (Table 2).

Insulin content of mixed islet/pituitary grafts was about three times higher than that of pure islet grafts (Table 2). Since we previously have shown that insulin content of stabilized islet grafts accurately reflects their β-cell mass (7), it is entirely possible that ectopically derived PRL might have upregulated the mass of transplanted β-cells. By the evidence, this hypothesis further suggests that cotransplantation of intermediate pituitary lobes, which lacks lactotrophs, does not induce any beneficial effect on islet graft function. That

TABLE 2
Graft vascularization and hormone content

Graft composition	Total graft area (G) (AU)	Total vessel area (V) (AU)	Ratio V/G	Graft insulin content (total ng)	Graft PRL content (total ng)	Graft GH content (total μg)
Pure pituitary	8,100 ± 594	741 ± 32	9.1	0.0 ± 0.0	ND	ND
Pure islet	4,200 ± 603	125 ± 24	2.9	500 ± 20	108 ± 35	1.1 ± 0.5
Islet/pituitary	10,480 ± 1,902*	1,109 ± 84‡	10.5	1,400 ± 100¶	1,300 ± 265	6.7 ± 2.7#
Islet/pit.AL	8,250 ± 845†	1,289 ± 182‡	15.6	ND	ND	ND
Islet/pit.IL	6,380 ± 710	541 ± 89§‖	8.4	ND	ND	ND

Characteristics of the different types of grafts 4 weeks after transplantation. Whole graft (G) and total vessel (V) (surface) areas were measured on photographs taken in anesthetized mice at the time of death (n = 5 in each group, excluding pure pituitary grafts, n = 4). Graft insulin (n = 6 in pure islet and in mixed islet/pituitary grafts, n = 3 in pure pituitary grafts), PRL and GH (n = 5 in pure islet and in mixed islet/pituitary grafts) contents were extracted from the retrieved grafts and measured by RIA. *P < 0.02 vs. pure islet; †P < 0.01 vs. pure islet; ‡P < 0.001 vs. pure islet; §P < 0.01 vs. pure islet; ‖P < 0.02 vs. islet/pituitary and islet/pit.AL, #NS (P = 0.0505). AU, arbitrary units.

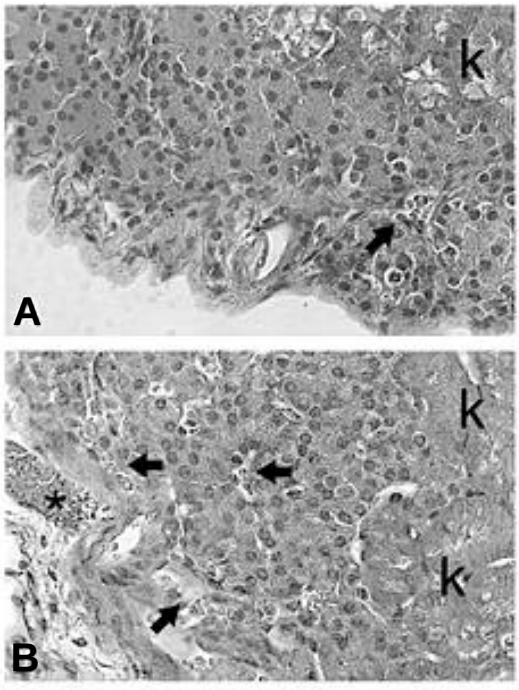

FIG. 4. Hematoxylin-eosin stain of a representative pure islet graft (*A*) and of a mixed islet/pituitary graft (*B*). In both of the photomicrographs from left to right are kidney capsule, islet tissue, and kidney (K). Islet tissue of mixed islet/pituitary grafts (*B*) was characterized by the presence of many capillaries containing red blood cells (←) and merging into large venules (*) (original magnification ×400).

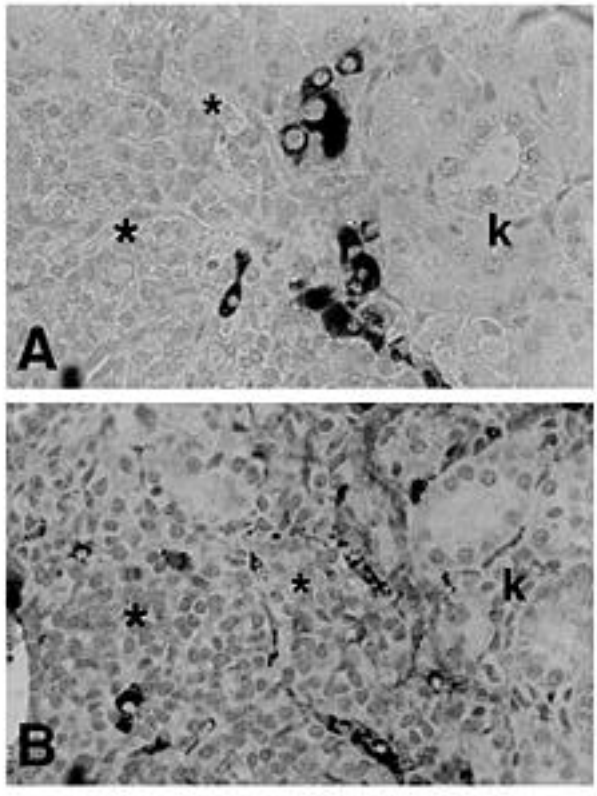

FIG. 5. Seriate sections of a mixed islet/pituitary graft stained for insulin (*A*) and for CD31 (*B*). Insulin-positive cells, between pituitary (*) and kidney tissue (K), are in close vicinity with endothelial cells stained positively for CD31 (original magnification ×400).

hyperprolactinemia caused by ectopic pituitary transplantation was moderate is noteworthy. In fact, although sevenfold higher than in mice transplanted only with islets, circulating PRL levels did not induce downregulation of endogenous pituitary lactotrophs.

Prolactin is not the only secretory product of mammotroph cells, and in fact, nerve growth factor (NGF) is cosecreted with PRL by anterior pituitary cells (32). NGF was not measured in this study, but its circulating and/or intragraft levels might also be increased in mice transplanted with mixed islet/pituitary grafts. Because NGF has been implicated in islet cell development and differentiation, insulin expression, and islet innervation (33–36), the possible contribution of NGF to the beneficial effect on islet graft function cannot be ruled out.

The improved metabolic control of mice transplanted with mixed islet/pituitary grafts might appear paradoxical when considering the potential diabetogenic effect of PRL (37). Nevertheless, the actual diabetogenicity of elevated PRL and GH levels had already been questioned by the classic studies of transplantation of pituitary tumors in rats that failed to show the induction of diabetes even in presence of extremely high hormone levels (38–40). Only in the presence of a severely reduced β-cell mass (80% pancreatectomy), transplantation of GH- and PRL-releasing tumors was followed by overt diabetes (41). Therefore, in our transplanted mice, the diabetogenic effect of pituitary hormones, essentially caused by the induction of insulin resistance, must be compensated for by a concomitant increase in graft β-cell mass and/or function. In addition, it has been recently shown that ectopic pituitary transplantation may actually potentiate insulin effects, at least in males (42–44).

Posttransplantation remodeling of mixed islet-pituitary grafts originated a hybrid organ in which clusters of pituitary cells were adjacent to intact healthy islets (Fig. 6). Insulin stain showed well-granulated β-cells accounting for ~80–85% of total islet cell population. Glucagon-positive cells were ~10–15% of the total, similar to what was observed in pure islet grafts (not shown). A striking characteristic of mixed islet/pituitary grafts was their excellent macroscopic vascularization (Fig. 3), which was significantly higher than what was observed in pure islet grafts (Table 2). In addition, mixed islet/pit.AL grafts were better vascularized than mixed islet/pit.IL grafts (Table 2) and were histologically characterized by the presence of many FS cells, positively stained for the protein S-100 (45), and particularly abundant in the anterior lobe (Fig. 7). Because FS cells release both b-FGF and VEGF, which are the most potent angiogenic peptides so far characterized (19–21), they might be responsible for the excellent vascularization of these grafts. Increased (and perhaps accelerated) revascularization might reduce posttransplantation hypoxia and consequent β-cell loss and can facilitate insulin release by engrafted cells. In fact, VEGF has been implicated in the development and maintenance of capillary fenestrations typical of endocrine glands (46).

In conclusion, this study demonstrates that pituitary cotransplantation improves function and insulin content of cotransplanted islets and increases whole-graft vascularization. This strategy is capable of turning an inadequate islet

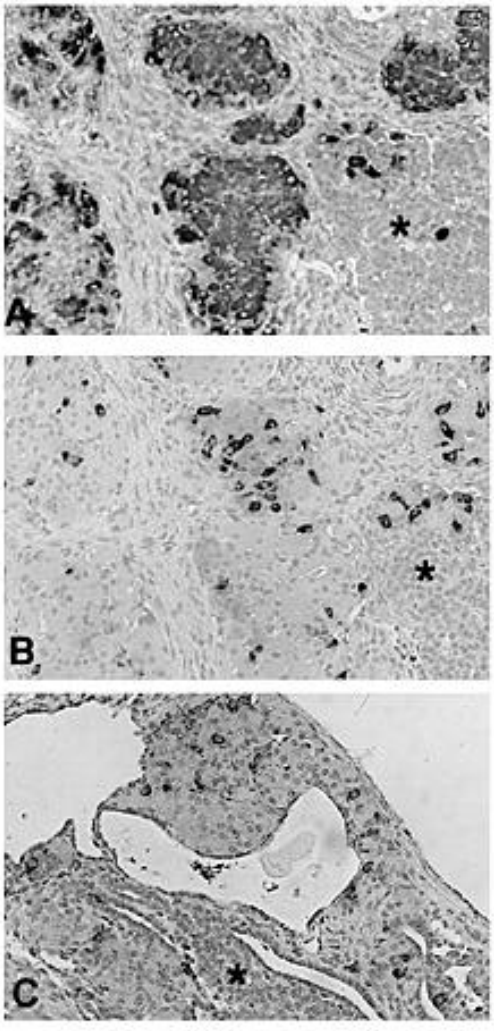

FIG. 6. *A* and *B*: Seriate sections of a mixed islet/pituitary graft stained for insulin and glucagon, respectively. Posttransplantation remodeling originated a hybrid organ in which islets, with well-granulated β- and α-cells, were adjacent to pituitary cell clusters (asterisk). *C*: Section of a mixed graft in which the pituitary part was made of intermediate lobes (mixed islet/pit.IL graft) stained for glucagon; these grafts were characterized by the presence of islets lined with saccular formations and cyst-like structures sometimes containing debris (original magnification ×200).

mass, otherwise destined to fail (47), into an efficiently functioning graft. We provide evidence suggesting that ectopically produced PRL and/or locally released angiogenic peptides might have played a causal role, even though other pituitary-derived factors might have contributed to this process. Further studies are now in progress to determine the individual effect of different pituitary hormones and growth factors on transplanted β-cells.

ACKNOWLEDGMENTS

This study was supported in part by Telethon-Italy (grant E.508, A.M.D.). A preliminary report of this study was presented at the 57th Annual Meeting of the American Diabetes Association, Boston, MA, 21–24 June, 1997.

We thank Letizia Sala for expert technical assistance and Dr. Marina Pontillo for serum cortisol measurements.

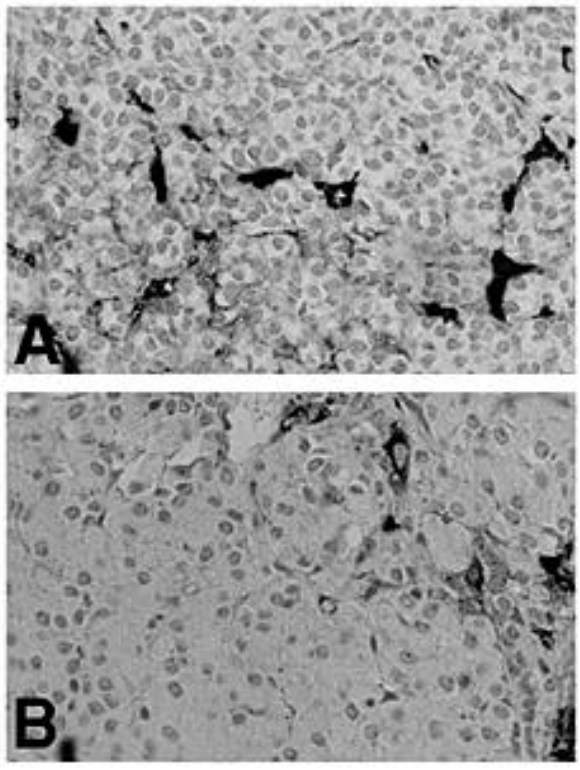

FIG. 7. Sections of a mixed islet/pit.AL (*A*) and of a mixed islet/pit.IL grafts (*B*) stained for the protein S-100 that in the pituitary is selectively expressed by the FS cells. AL cell clusters contained significantly more FS cells than IL clusters (original magnification ×400).

REFERENCES

1. Sharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Falqui L, Marchetti PM, Gingerich RL, Jaffe AS, Cryer PE, Anderson CB, Flye MW: Insulin independence after islet transplantation into type 1 diabetic patient. *Diabetes* 39:515–518, 1990
2. Warnock GL, Kneteman NM, Ryan E, Seelis REA, Rabinovich A, Rajotte RV: Normoglycemia after transplantation of freshly isolated and cryopreserved pancreatic islets in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 34:55–58, 1991
3. Socci C, Falqui L, Davalli AM, Ricordi C, Braghi S, Bertuzzi F, Maffi P, Secchi A, Gavazzi F, Freschi M, Magistretti P, Socci S, Vignali A, Di Carlo V, Pozza G: Fresh human islet transplantation to replace pancreatic endocrine function in type 1 diabetic patients. *Acta Diabetol* 28:151–157, 1991
4. Weir GC, Bonner-Weir S: Scientific and political impediments to successful islet transplantation. *Diabetes* 46:1247–1256, 1997
5. Hering BJ: Insulin independence following islet transplantation in man: a comparison of different recipient categories. *Int Islet Transpl Registry* 6:5–19, 1996
6. Alejandro R, Cutfield RG, Shienvold FL, Polonsky KS, Noel J, Olson L, Dillberger J, Miller J, Mintz DH: Natural history of intrahepatic canine islet cell autografts. *J Clin Invest* 78:1339–1348, 1986
7. Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir S, Weir GC: Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function. *Diabetes* 45:1161–1167, 1996
8. Montana E, Bonner-Weir S, Weir GC: Transplanted beta cell response to increased metabolic demand: changes in beta cell replication and mass. *J Clin Invest* 93:1577–1582, 1994
9. Parsons JA, Brelje TC, Sorenson RL: Adaptation of islets to pregnancy: increase in b-cell division and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology* 130:1459–1466, 1992
10. Brelje TC, Sharp DW, Lacy PE, Orgren L, Talamandes F, Robertson M, Friesen HG, Sorenson RL: Effect of homologous placental lactogens, prolactins, and growth hormones on islet β-cell division and insulin secretion in rat, mouse and human islets: implication for placental lactogen of islet function during pregnancy. *Endocrinology* 132:879–887, 1993
11. Brelje TC, Parsons JA, Sorenson RL: Regulation of islet β-cell proliferation by prolactin in rat islets. *Diabetes* 43:263–273, 1994
12. Sorenson RL, Stout LE: Prolactin receptors and JAK2 in islets of Langerhans:

an immunohistochemical analysis. *Endocrinology* 136:4092–4098, 1995
13. Parsons JA, Bartke A, Sorenson RL: Number and size of islets of Langerhans in pregnant, human growth hormone-expressing transgenic, and pituitary dwarf mice: effect of lactogenic hormones. *Endocrinology* 136:2013–2021, 1995
14. Nielsen JH, Linde S, Welinder BS, Billestrup N, Ole Madsen D: Growth hormone is a growth factor for the differentiated pancreatic β-cell. *Mol Endocrinol* 13:165–173, 1989
15. Dionne KE, Colton CK, Yarmush ML: Effect of hypoxia on insulin secretion by isolated rat and canine islets of Langerhans. *Diabetes* 42:12–18, 1993
16. Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 31:883–889, 1982
17. Brunicardi FC, Stagner J, Bonner-Weir S, Wayland H, Kleinman R, Livingston E, Guth P, Menger M, McCuskey R, Intaglietta M, Charles A, Ashley S, Cheung A, Ipp E, Gilman S, Howard T, Passaro E Jr: Microcirculation of the islets of Langerhans: Long Beach Veterans Administration Regional Medical Education Center Symposium. *Diabetes* 45:385–392, 1996
18. Ray D, Melmed S: Pituitary cytokine and growth factor expression and action. *Endocr Rev* 18:206–228, 1997
19. Ferrara N, Henzel WJ: Pituitary follicular cells secret a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* 161:851–858, 1989
20. Gospodarowicz D, Abraham JA, Schilling J: Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. *Proc Natl Acad Sci U S A* 86:7311–7315, 1989
21. Ferrara N, Schweiger L, Neufeld G, Mitchell R, Gospodarowicz D: Pituitary follicular cells produce basic fibroblast growth factor. *Proc Natl Acad Sci U S A* 84:5773–5777, 1987
22. Cristofori G, Naik P, Hanahan D: Vascular endothelial growth factor and its receptor flt-1 and flk-1, are expressed in normal pancreatic islets and throughout islet cell tumorigenesis. *Mol Endocrinol* 9:1760–1770, 1995
23. Shweiki D, Itin A, Soffer D, Keshef E: Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature* 359:843–845, 1992
24. Gorden DL, Mandriota SJ, Montesano R, Orci L, Pepper MS: Vascular endothelial growth factor is increased in devascularized rat islets of Langerhans in vitro. *Transplantation* 63:436–443, 1997
25. Vajkoczy P, Olofsson AM, Lehr H-A, Leiderer R, Hammersen F, Arfors KE, Menger MD: Histogenesis and ultrastructure of pancreatic islet graft microvasculature: evidence for graft revascularization by endothelial cells of host origin. *Am J Pathol* 146:1376–1405, 1995
26. Jacobson MD, Raff MC: Programmed cell death and Bcl-2 protection in very low oxygen. *Nature* 374:814–816, 1995
27. Himizu S, Eguchi Y, Kosaka H, Kamiike W, Matsuda H, Tsujimoto Y: Prevention of hypoxia-induced cell death by Blc-2 and Bcl-xl. *Nature* 374:811–813, 1995
28. Weir GC, Davalli AM, Hollister J, Bonner-Weir S: Islet isolation from rodent pancreas. *In Pancreatic Islet Transplantation*. Vol. 1. Lanza RP, Chick WL, Eds. New York, RG Landes, 1994, p. 53–59
29. Adler RA: The anterior pituitary-grafted rat: a valid model of chronic hyperprolactinemia. *Endocr Rev* 7:302–312, 1986
30. Auguado LI, Rodriguez A, Bilbao A, Rodriguez EM: Cytological changes in the pars distalis of the female rat hypophysis grafted under the kidney capsule. *Cell Tissue Res* 199:539–543, 1979
31. Tenniswood MP, Guenette RS, Lakins J, Mooibroek M, Wong P, Welsh P, Welsh J-E: Active cell death in hormone-dependent tissues. *Cancer Metastasis Rev* 11:197–220, 1992
32. Missale C, Boroni F, Sigala S, Buriani A, Fabris M, Leon A, Dal Toso R, Spano PF: Nerve growth factor in the anterior pituitary: localization in mammotroph cells and cosecretion with prolactin by a dopamine-regulated mechanism. *Proc Natl Acad Sci U S A* 93:4240–4245, 1996
33. Polak M, Scharfmann R, Seilheimer B, Eisenbarth G, Dressler D, Verma IM, Potter H: Nerve growth factor induces neuronal-like differentiation of an insulin-secreting pancreatic beta cell line. *Proc Natl Acad Sci U S A* 90:5781–5885, 1993
34. Kanaka-Gantenbein C, Dicou E, Czernichow P, Scharfmann R: Presence of nerve growth factor and its receptors in an in vitro model of islet cell development: implication in normal islet morphogenesis. *Endocrinology* 136:3154–3162, 1995
35. Sher E, Rosa P, Bassetti M, Zanini A: Immunolocalization of secretogranin II and insulin in a nerve growth factor-differentiated insulinoma cell line. *Eur J Cell Biol* 67:15–22, 1995
36. Edward RH, Rutter WJ, Hanahan D: Direct expression of NGF to pancreatic beta cells in transgenic mice leads to selective hyperinnervation of the islets. *Cell* 58:161–170, 1989
37. Landgraft R, Landgraft-Leurs MM, Weissmann A, Horl R, Von Werder K, Scriba PC: Prolactin: a diabetogenic hormone. *Diabetologia* 13:99–104, 1977
38. Martin JM, Hans KA, Garay G: Insulin secretion in rats with elevated levels of circulating growth hormone due to MtT-W15 Tumor. *Diabetes* 17:661–667, 1968
39. Akerblom HK, Martin JM, Garay GL, Moscatello M: Experimental hypersomatotropism. Metabolic effects in rats bearing the MtT-W15 tumor. *Horm Metab Res* 4:15–21, 1972
40. Garay GL, Akerblom HK, Martin JM: Experimental hypersomatotropism: serum growth hormone and insulin, and pituitary and pancreatic changes in MtT-W15 tumor-bearing rats before and after tumor removal. *Horm Metab Res* 3:82–89, 1971
41. Bates RW, Scow RO, Lacy PE: Induction of permanent diabetes in rats by pituitary hormones from a transplantable mammotropic tumor: concomitant changes in organ weight and the effect of adrenalectomy. *Endocrinology* 78:826–839, 1965
42. Adler RA, Sokol HW: Glucose tolerance in rats with elevated circulating prolactin levels. *Horm Metab Res* 14:307–309, 1982
43. Matsuda M, Mori T: Effect of estrogen on hyperprolactinemia-induced glucose intolerance in SHN mice. *Proc Soc Eng Biol Med* 212:243–247, 1996
44. Matsuda M, Mori T, Park MK, Sassa S, Sakamoto S, Kawashima S: Chronic effect of hyperprolactinemia on blood glucose and lipid levels in mice. *Life Sci* 58:1171–1177
45. Shirasawa N, Kihara H, Yamaguchi S, Yoshimura F: Pituitary folliculo-stellate cells immunostained with S-100 protein antiserum in postnatal, castrated and thyroidectomized rat. *Cell Tissue Res* 231:235–248, 1983
46. Brier G, Albrecht U, Sterrer S, Risau W: Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. *Development* 114:521–532, 1992
47. Ohzato H, Porter J, Monaco AP, Montana E, Maki T: Minimum number of islets required to maintain euglycemia and their reduced immunogenicity after transplantation into diabetic mice. *Transplantation* 56:270–274, 1993