Syngeneic Transplantation of Fetal Rat Pancreas

III. Effect of Insulin Treatment on the Growth and Differentiation of the Pancreatic Implants After Reversal of Diabetes

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SUMMARY

Eight 18-day fetal pancreases were transplanted to syngeneic alloxan diabetic male rats. Some of the recipients were treated with insulin for a 7-day period immediately after transplant. By previously published clinical criteria, three groups of recipients could be identified after reversal of diabetes by the transplanted tissue: insulin-treated rapid reversal; insulin-treated slow reversal; and control (not treated with insulin). Five animals in each group were sacrificed after glucose tolerance testing for morphologic and hormonal analysis of the transplanted tissue. The insulin-, glucagon-, and somatostatin-positive islet cell masses of the fetal pancreatic implants were quantitated.

There was a correlation between the beta cell mass of of the implants and the glucose tolerance exhibited by the host animals. The rapid response insulin-treated recipients had significantly greater implant beta cell mass and insulin content compared with the other groups. There was no difference in implant alpha cell mass among the groups, but the insulin-treated implants had a significantly greater glucagon content. The delta cell mass of insulin-treated rapid response was less than that of the other two groups.

The results are discussed in relation to previously reported morphometric analysis 15 days after transplantation. The relationships of transplanted beta cell mass, beta cell differentiation, transplant site, and cell-to-cell interactions within the transplanted islet to the control of glucose homeostasis are also discussed. DIABETES 28:141–146, February 1979.

Pancreatic transplantation has been extremely successful in reversing experimental diabetes in syngeneic laboratory animals. Translation of these laboratory experiments to human diabetes has been moderately successful.[1] However, immunologic and other problems involved in these preliminary human trials have prevented widespread use of transplantation as an ameliorative procedure in humans with diabetes. Regardless of these difficulties, pancreatic transplantation using experimental laboratory models has been of value. The controlled environment of the laboratory has allowed detailed study of the effects of tissue preparation, amount of tissue transplanted, and transplant site on the rate and degree of reversal of experimental diabetes. In addition, preliminary studies have examined the possible efficacy of pancreatic transplantation in the arrest or reversal of the secondary vascular complications of diabetes. A detailed description of these studies is beyond the scope of this report, but the area has recently been reviewed in detail.[2–4]

We used syngeneic transplantation of fetal pancreas to the renal subcapsular site as a model for the study of the growth and differentiation of the islet cells. In the first paper of this study,[5] the clinical course of a group of 69 fetal pancreatic transplant recipients was presented in detail. The results can be summarized as follows: 100% of the surviving recipients were reversed of diabetes. The period of time between transplant and reversal of diabetes had a negative correlation with glucose tolerance; i.e. those animals that required a longer period for return to normoglycemia had a reduced glucose tolerance. There was no overall effect of a period of insulin treatment (with rapid return to normoglycemia) immediately after transplant on time to reversal or glucose tolerance. However, the insulin-treated animals could be divided into two subgroups. The first group was reversed within 1 month posttransplant; this group had excellent glucose tolerance, fasting hyperinsulinemia, and elevated insulin levels during glucose tolerance testing as compared with normal controls and transplant recipients that did not receive insulin. The second group required longer than 2 months for return to normoglycemia, and showed subdiabetic glucose tolerance accompanied by reduced plasma insulin levels.

In the second paper of the series,[6] the effects of insulin treatment on the growth and differentiation of the fetal pan-

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TABLE 1
Pretransplant clinical parameters of the transplant recipients chosen for sacrifice in both the control and the insulin-treated groups

Group	N	Wk post alloxan	Blood glucose (mg/dl)	Urine glucose (g/24 h)	Body weight (g)	Weight change post alloxan (g)
Control	5	8.4 ± 0.4*	434 ± 26	7.3 ± 0.4	161 ± 6	−7.0 ± 5.4
Insulin-treated						
Rapid response	5	8.6 ± 1.0	412 ± 33	7.2 ± 0.7	186 ± 20	+8.8 ± 13.1
Slow response	5	8.8 ± 0.6	461 ± 20	6.2 ± 0.7	179 ± 7	−8.4 ± 6.0

There are no significant differences in these parameters between the animals sacrificed and the groups as a whole (5).
* All values are mean ± SEM.

creatic implants 15 days after transplant were reported. Insulin treatment resulted in a threefold greater insulin-positive beta cell mass compared with control transplant recipients or normal animals that received a similar fetal pancreatic transplant. Since there was no overall difference between the control and insulin-treated groups in terms of reversal rate or time to reversal, the difference in the beta cell masses was remarkable. It was also of interest that, although the insulin-treated animals had a relatively high percentage (20–25%) of the normal beta cell mass at the kidney site, none were normoglycemic at the time of sacrifice.

The following study presents the islet cell masses and hormone content of the pancreatic implants after reversal of diabetes. The purpose of the study was to define more clearly the effects of insulin treatment on the growth and differentiation of the islet beta cells as well as to ascertain the possible role of the alpha and delta cells on functional response of transplanted beta cells.

MATERIALS AND METHODS

Male inbred Fischer 344 rats (ARS/Sprague Dawley) were used. Diabetes was induced by a single i.v. injection of alloxan (32 mg/kg body wt, as a 2% aqueous solution). After at least 4 wk of established diabetes (i.e. blood glucose greater than 300 mg/dl) animals to be transplanted received eight 18-day fetal pancreases at the renal subcapsular site. Insulin-treated animals were given seven daily i.p. injections of 2–4 U protamine zinc insulin beginning on the day after transplant. Control animals were those transplant recipients that were not treated with insulin. Details of the transplantation method, insulin treatment regimen, and the clinical course of the entire group of transplant recipients have been reported.[5] Based on these previous clinical data, three groups were designated for this study: I, control animals; and II, insulin-treated animals: A, rapid response (reversal of diabetes in less than 4 wk; B, slow response (more than 8 wk between transplantation and reversal of diabetes).

Four weeks after reversal of diabetes all reversed recipients were given an intravenous glucose tolerance test (IVGTT) (3 g/kg body wt). Glucose tolerance was expressed as the diabetic index (I_D).[7] At the time of reversal of diabetes certain animals were arbitrarily chosen to be included in this study. Two wk after the GTT these animals were sacrificed by decapitation. Plasma was obtained for hormonal analysis and the pancreatic implants were removed from the transplantation site on the kidney. Eight implants were grossly identified in all but three animals (all insulin-treated slow response), in which only seven distinct implants were found. The implants were trimmed of adherent perirenal adipose tissue, weighed, and either fixed in Bouin's solution for morphologic analysis or homogenized and extracted for estimation of hormone content.

Morphological analysis. The Bouin's fixed implants were embedded in paraffin, serially sectioned (4 μm), and stained with Gomori's aldehyde fuchsin with Ponceau de xylidine counterstain. The method of mounting the tissue sections on the slides[8] allowed adjacent sections at a fixed interval through the entire implant (every 18th section) to be used for the immunocytochemical localization of the insulin-, glucagon-, and somatostatin-containing cells. Our modification[8] of the unlabeled antibody enzyme method of Sternberger[9] was used.

Primary antisera to insulin and glucagon were similar to those used for radioimmunoassay. Guinea pig anti-somatostatin antisera was the generous gift of Dr. Robert Elde (Department of Anatomy, University of Minnesota). Sheep anti-rabbit gammaglobulin was purchased from Antibodies, Inc. Peroxidase-anti-peroxidase (PAP) conjugate was obtained from Cappel Labs and the capturing reagent, 3,3′-diaminobenzidine-tetrahydrochloride (DAB), was purchased from Sigma Chemical (St. Louis, Missouri). Immunocytochemical specificity controls included substitution of normal sera for the primary antisera and/or absorption of the diluted primary antisera with excess specific antigen (1 μg/ml or greater).

After immunocytochemical staining, the masses of insulin, glucagon, and somatostatin-positive cell populations were morphometrically estimated by using the linear scanning method.[10] Quantitation of every 18th section at a transverse interval of 75 μm allowed comparison with previously obtained data on fetal and adult pancreases. The results of scanning technique are expressed in millimeters of scan. The linear distance obtained by the method has been empirically demonstrated to be proportional to the tissue wet weight. The implant hormone-positive cell masses for each of the three hormones were compared with those of 18-day fetal pancreas prior to transplantation[11] and with normal adult rat pancreas.[8] Usually four implants were quantitated from each of the sacrificed animals. The mean millimeters of scan per implant was multiplied by the total number of implants found on the kidney (seven or eight) to give the total transplanted islet cell masses.

Assays. Blood and urine glucose concentrations were determined by the methods of Hoffman[12] and Somogyi,[13] respectively. Insulin[14] and glucagon[15] were estimated in acid alcohol extracts of the implants by using polyethylene oxide (PEO) precipitation[16] to separate bound antigen. Plasma in-

TABLE 2
Data obtained from glucose tolerance testing of transplant recipients chosen for sacrifice after reversal of alloxan diabetes

Group	N	Wk to recovery	Fasting glucose (mg/dl)	Diabetic index	Fasting insulin (μU/ml)	Peak insulin during GTT (μU/ml)	Fasting glucagon (pg/ml)
Control	5	10.8 ± 0.8*	69 ± 5	2.4 ± 0.7	20 ± 5	69 ± 12	192 ± 28
Insulin-treated							
Rapid response	5	2.0 ± 0.4†	64 ± 5	0.5 ± 0.1††	36 ± 5†	151 ± 16††	173 ± 24
Slow response	5	21.4 ± 3.3††,‖	68 ± 3	2.6 ± 0.1‖	20 ± 7§	43 ± 11‖	278 ± 58

There are no significant differences between these animals and the group as a whole (5).
* All values are mean ± SEM.
† Significantly different from control, Student's *t* test, $P < 0.01$.
†† $P < 0.001$.
§ Significantly different from rapid response, $P < 0.01$.
‖ $P < 0.001$.
All others are not significantly different, $P < 0.05$.

sulin[17] and glucagon[18] were determined by double antibody methods. The insulin assays used guinea pig anti-bovine-porcine insulin antisera,[17] crystalline rat insulin standards (Novo) and ^{125}I-porcine insulin (New England Nuclear). Glucagon assays employed an antisera specific for pancreatic glucagon,[15] highly purified porcine pancreatic glucagon standards (Novo), and ^{125}I-porcine glucagon (New England Nuclear). Aprotenin (Novo, 1000 U/ml) was added to the plasma samples before glucagon assay. Precipitating antibodies for the double-antibody methods were obtained from Antibodies, Inc. normal serums from International Scientific Industries, and PEO from Polysciences. All insulin samples were diluted to the portion of the standard curve where crystalline rat insulin standards and dilutions of extracted rat pancreas give comparable results.[19,20]

RESULTS

Tables 1 and 2 detail pretransplant clinical parameters and glucose tolerance data of the animals sacrificed for morphologic analysis in this study. There are no significant differences between these animals and the data for any of the groups as a whole. As shown in Table 1, all the animals had well-established diabetes (more than 8 wk) of moderate severity (blood glucose greater than 400 mg/dl, urine glucose excretion greater than 6 g/24 h). The animals were relatively small, having had little change in weight after alloxan administration. It should be noted that the slow response insulin-treated animals had a significantly lower body weight than those insulin-treated animals that had rapid reversal of diabetes. Although not significantly different, the rapid reversing animals had tended to gain weight following alloxan administration, while the slow reversing animals had tended to lose weight.

Although fasting glucose was similar in all three groups, the glucose tolerance as estimated by the I_D was significantly better in the rapid response insulin-treated animals than in the other two groups. These rapid response animals had slightly elevated fasting plasma insulin, which was not accompanied by hypoglycemia and a significantly elevated insulin response to the intravenous glucose challenge. The control and slow response insulin-treated animals had glucose tolerance similar to nontransplanted normal rats. The plasma glucagon was not significantly different among the transplant groups and was similar to that of nontransplanted controls (mean 161 ± 8 pg/ml, N = 63). This represents a significant change from the hyperglucagonemia of untreated alloxan diabetic rats (407 ± 44 pg/ml, N = 31).

At sacrifice, there was a noticeable difference in the size of the implants between the control and the insulin-treated groups. As shown in Table 3, this difference is reflected in the wet weight of the implants. The control implants weighed more than twice as much as the rapid response insulin-treated implants. The slow response implants were intermediate in weight. It should be noted that in all groups

TABLE 3
Hormone analysis of implants from control and insulin-treated animals compared with 18-day fetal pancreas (before transplant) and to normal adult rat pancreas

Group	N	Wet wt (mg)	Insulin		Glucagon	
			mU/mg	mU/implant	ng/mg	ng/implant
18-Day fetal	11	1.03 ± 0.13*	0.22 ± 0.04	0.2 ± 0.1	5.26 ± 0.69	5.4 ± 0.5
Control	20	120.0 ± 16.0	0.19 ± 0.03	18.2 ± 2.2	0.64 ± 0.11	57.5 ± 7.8
Insulin-treated						
Rapid response	20	47.1 ± 4.7††	3.38 ± 0.43††	112.0 ± 10.0††	2.64 ± 0.44††	101.1 ± 14.8†
Slow response	16	92.3 ± 13.7‖	0.20 ± 0.05‖	14.9 ± 2.3‖	2.58 ± 0.80†	197.3 ± 42.0††,§
Normal adult	12	1072 ± 55	1.51 ± 0.19	1620 ± 211	4.50 ± 0.60	4824 ± 671

* Mean ± SEM.
† Significantly different from control, Student's *t* test, $P < 0.02$.
†† $P < 0.001$.
§ Significantly different from rapid response, $P < 0.05$.
‖ $P < 0.005$.

TABLE 4
Hormone-positive islet cell masses expressed as mm of scan/implant. Total cell mass/animal is the product of mm scan/implant × number of implants. The percentage of each cell type to the total of the three is given in parentheses.

Group	Implants quantitated	Total mm/implant			Total mm/animal		
		Insulin-positive	Glucagon-positive	Somatostatin-positive	Insulin-positive	Glucagon-positive	Somatostatin-positive
18-Day fetal	10	1.1 ± 0.1* (45.8 ± 4.5)	1.1 ± 0.1 (45.8 ± 4.5)	0.2 ± 0.1 (8.4 ± 4.0)	—	—	—
Control recipients	18	20.0 ± 2.2 (73.3 ± 8.0)	4.6 ± 0.5 (16.8 ± 1.8)	2.7 ± 0.4 (9.9 ± 1.5)	151 ± 17	35 ± 8	20 ± 6
Insulin-treated recipients							
Rapid response	20	50.6 ± 5.7†† (89.4 ± 10.1)	4.9 ± 0.5 (8.7 ± 0.9)	1.1 ± 0.2†† (1.9 ± 0.4)	405 ± 75†	39 ± 6	9 ± 2
Slow response	18	20.8 ± 4.1‖ (62.2 ± 3.0)	6.9 ± 1.0 (22.9 ± 2.2)	4.0 ± 0.5‖ (15.1 ± 1.4)	158 ± 39§	45 ± 10	27 ± 4‖
Normal adult rats	20	—	—	—	928.8 ± 70.2 (79.9 ± 6.1)	164.0 ± 17.9 (14.0 ± 2.1)	73.1 ± 13.0 (6.24 ± 1.2)

* All values are mean ± SEM.
† Significantly different from control, Student's *t* test, $P < 0.005$.
†† $P < 0.001$.
§ Significantly different from rapid response, $P < 0.01$.
‖ $P < 0.001$.
All others are not significantly different, $P > 0.05$.

the implants were composed largely of adipose tissue with small nests of pancreatic epithelial cells between the fat cells.

The implant insulin content (Table 3) changed markedly from the value at the time of transplant. Although the insulin per milligram wet weight of the control implants fell from its level at 15 days after transplant[6] (0.19 ± 0.03 from 2.00 ± 0.24) the total insulin content per implant doubled due to the increase in total wet weight (18.2 from 9.4 mU/implant). This change in insulin content was paralleled by a proportional increase in insulin-positive beta cell mass (Table 4) from 9.3 ± 0.9 15 days after transplant to 20.0 ± 2.2 after reversal of diabetes.

The difference in the implant insulin content between the rapid and slow response animals of the insulin-treated group reflected not only the differences in time to reversal and in glucose tolerance between the subgroups but also the beta cell mass of the implants. The implant insulin content of the rapid response animals on a per milligram wet weight basis fell slightly compared with the content 15 days after transplant (3.38 ± 0.43 from 5.56 ± 1.06 mU/ml) but the increase in implant wet weight resulted in an increase in total insulin/implant of almost fourfold (112 from 28.4 mU/implant[6]). Since there was a doubling in beta cell mass (Table 4) between 15 days posttransplant and reversal of diabetes to 50.6 ± 5.7 mm/implant (compared with 25.3 ± 3.2 at 15 days posttransplant) the insulin content per beta cell mass increased. The total stored insulin in all eight implants at the renal subcapsular site was more than 50% of that in a normal adult rat pancreas.

In contrast with the rapid response animals, those in the slow response group had much lower implant insulin levels that were not significantly different from the control implants. Similarly, there was no difference in the implant beta cell mass between the implants of the slow response insulin-treated animals and the control group. The beta cell mass of both groups was significantly lower than that of the rapid response insulin-treated animals.

There was a highly significant correlation ($P < 0.005$) between the glucose tolerance estimated by the I_D and the estimated total transplanted beta cell mass (correlation coefficient $r = 0.69$ for the 15 animals in this study).

The levels of glucagon (Table 3) in the implants after reversal of diabetes were also markedly changed from those of 15 days after transplant. There was a greater than sixfold increase in total implant glucagon in the control implants over this period (57.5 vs 9.0 ng/implant). In contrast, there was only a three- to fourfold increase in implant alpha cell mass from 1.2 ± 0.2 to 4.6 ± 0.5 (Table 4). Both of the insulin-treated groups had significantly higher implant glucagon levels than the control implants. There were no significant differences, however, in the alpha cell masses among the three groups. In all cases the glucagon content and alpha cell mass were markedly reduced compared with normal rat pancreas.

The somatostatin-positive delta cell mass of the control implants (Table 4) was not significantly different from that of the slow response insulin-treated group. Both of these groups had a significantly greater delta cell mass per implant than did the rapid response insulin-treated animals.

DISCUSSION

In the second of this series of reports on fetal pancreatic transplantation, we noted that insulin treatment resulted in a rapid growth of the beta cells in the transplanted tissue. The total beta cell mass 15 days after transplant was 20–25% of the normal beta cell mass of the adult rat. This was approximately three times the beta cell mass of implants in control animals that had not been treated with insulin. Pancreatectomy studies[21] suggested that only 5–10% of the pancreas was necessary to maintain normoglycemia. None of the insulin-treated transplant recipients sacrificed at 15

days was normoglycemic at transplant. The question of why these animals remained diabetic with a presumably adequate islet mass remained to be answered. We suggested several alternative possibilities. The morphologic and hormonal data in the present report were collected to test which of the alternatives, if any, might be responsible. Each alternative shall be discussed separately in light of this additional information.

First we proposed that perhaps the insulin-treated animals examined in the previous study were close to reversal at the time of sacrifice. As a group they had a significant decrease in blood glucose during the 2 wk posttransplant, although they remained hyperglycemic, hypoinsulinemic, and hyperglucagonemic. The implant beta cell masses of the seven animals examined were not all uniform. Implants of six of the seven animals examined had a mean beta cell mass of 27.0 ± 2.5 mm/implant, while one animal had a mean implant beta cell mass of only 6.4 ± 1.9 mm/implant. Since only 10 of the 24 transplanted animals could be categorized as rapid response, it seemed unlikely that six of seven of the animals sacrificed arbitrarily at 15 days posttransplant would be in the rapid response group. The morphometric data in the present study, however, support this hypothesis. After reversal, the slow response insulin-treated animals had a beta cell mass of only 20.8 ± 4.5 mm/implant. This is actually less than the mean for the group of seven animals quantitated 15 days after transplant. Since the beta cell mass in the control group rose from 9.3 ± 0.9 mm/implant 15 days after transplant to 20.0 ± 2.2 mm/implant after reversal, it seems unlikely that the beta cell mass of some of the insulin-treated animals would actually fall during this interval. If the above hypothesis is correct, then the implant beta cell mass of the rapid response insulin-treated animals doubled to 50.6 mm/implant between 15 days after transplant and reversal and that of the slow response animals increased from 6.4 to 20.8 over a much longer time interval. This hypothesis will remain conjectural. There were no pretransplant clinical parameters that were predictive of a rapid or a slow response to insulin treatment. Consequently, other alternative explanations still must be considered.

We suggested that since the venous drainage from the kidney is into the systemic rather than into the portal circulation, the insulin secretion of the transplanted beta cells may have been ineffective in restoring normoglycemia. The reports of several investigators have suggested that portal venous drainage decreased the amount of tissue required for amelioration of experimental diabetes.[22–24] The beta cell masses presented in this paper indicate that this is not a viable explanation for the lack of reversal in the insulin-treated animals 15 days after transplant. The control and slow response insulin-treated animals had lower mean beta cell masses (20.0 and 20.8 mm/implant, respectively) after amelioration of the diabetic state than did the insulin-treated animals 15 days after transplant (25.3 mm/implant). Clearly this beta cell volume (15–20% of normal) is capable of maintaining normoglycemia and near normal glucose tolerance despite the nonorthotopic venous drainage from the kidney site.

The degree of differentiation of the transplanted islet tissue may also be of significance in the eventual reversal of alloxan diabetes. Eighteen-day fetal pancreas has a very low insulin content, and fetal pancreas has been shown to have a reduced insulin secretory responsiveness to glucose stimulation.[25,26] At 15 days posttransplant the implants from the insulin-treated animals exhibited a 100-fold increase in total insulin content, containing approximately 28 mU/implant. However, the total stored insulin at the kidney site was only 10–15% of that of the normal adult pancreas. After reversal of diabetes the rapid response insulin-treated animals had a total insulin content of 112 mU/implant or 50–55% of that of the normal adult. The implant insulin content of the slow response insulin-treated animals after reversal again supports the hypothesis that most of the animals examined 15 days after transplant should be placed in the rapid response group. The total insulin content was 14.9 ± 2.3/implant or only about 50% of that of the insulin treated animals examined at 15 days posttransplant. The difference in the implant insulin content between the rapid response animals and control and slow response animals may help explain the better glucose tolerance and rapidity of recovery in the rapid response animals.

The only parameter testing the physiologic responsiveness of the transplanted islet tissue in these studies was the IVGTT. Several data support the hypothesis that the insulin-treated animals were not responding appropriately to hyperglycemia at 15 days after transplant. The beta cells were well granulated and the insulin content of the tissue was quite high in the face of a blood glucose of 300 mg/dl. The circulating insulin level at that time was correspondingly low and not significantly different than in untreated diabetic animals. In contrast, after the reversal all of the animals, regardless of group, showed a significant and appropriate insulin secretory response to an i.v. glucose challenge. Perhaps the insulin treatment, although stimulating the growth of beta cells in some of the transplant recipients, delays in some way the physiologic maturation of those beta cells. This hypothesis is currently being investigated by in vitro secretory responsiveness testing of pancreatic implants removed from the kidney site at various intervals after transplantation.

Finally, we noted that at 15 days posttransplant the mass of alpha and delta cells in the implants was markedly reduced compared with normal islets. It had been suggested by others that interactions between the cells of the islet may be important in the maintenance of normal glucose homeostasis.[27,28] Therefore, the question remained whether the decreased alpha and/or delta cells may be responsible for the failure of the beta cells, especially in the insulin-treated animals, to control the blood glucose. Unfortunately, the morphologic data presented in this paper do not allow a definitive statement about the role of the nonbeta cells in the transplanted tissue. There was an increase in the alpha and delta cell masses in all groups between 15 days posttransplant and after reversal of diabetes. However, the percentage of glucagon- and somatostatin-positive cells remained lower than that of the normal adult islet. In addition, the group with the best glucose tolerance after reversal, the rapid response insulin-treated animals, had the lowest somatostatin-positive delta cell mass. Although both of the insulin-treated groups had a significantly higher implant glucagon content than did the control animals, there was no significant difference in the alpha cell mass among the three groups. In addition, although there was no difference

in implant glucagon or alpha cell mass between the two insulin-treated groups, there was a significant difference in glucose tolerance between these animals. While there was a significant correlation between glucose tolerance (I_D) and the calculated total beta cell mass of the implants, neither the alpha or delta cell mass of the implants correlated with the degree of control of blood sugar.

The effect of insulin treatment on the developing fetal pancreas, after transplantation, remains of great interest. Although not all animals responded to insulin treatment, those that did had more rapid reversal of diabetes, better glucose tolerance, and greater implant beta cell mass and insulin content. Since the insulin treatment was given for only the first 7 days after transplant, its continued effects weeks later are of particular interest. The period of insulin treatment presumably altered some aspect of beta cell development, which then continued to stimulate beta cell growth and differentiation after exogenous insulin was stopped. As one possibility, the insulin might have stimulated the initial formation of beta cells within the transplanted pancreas whose endogenous insulin synthesis and secretion continued to stimulate additional beta cell growth and differentiation. This hypothesis is currently under investigation in an in vitro organ culture system in our laboratory.

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