A Histological Study of Intrasplenic Transplanted Neonatal Rat Pancreas and of Adjacent Adipose Tissue Proliferation

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Summary. Proliferation of adipose tissue adjacent to intrasplenic transplants of whole isogeneic neonatal rat pancreas has consistently been noted. In this study over a period of 18 months there was a progressive increase in the amount of fatty tissue in the vicinity of surviving transplants. Immunohistochemistry demonstrated the presence of insulin, glucagon, somatostatin and pancreatic polypeptide within islet cells in long term grafts. Electron microscopy demonstrated a close association between islets and lipid droplets. Ductal elements within the transplants survived and showed close association with endocrine cells, but exocrine pancreatic tissue degenerated rapidly. Radioimmunoassay of extracts from surviving transplants in isogeneic rats confirmed the presence of high levels of insulin and glucagon after transplantation. In contrast, allogeneic intrasplenic transplants of rat pancreas failed to survive and showed no evidence of adipose tissue proliferation. Furthermore, isogeneic intrasplenic transplants of both adult rat fat and adrenal gland also failed to demonstrate adipocyte proliferation. It would appear that the presence of both adipocytes and pancreatic endocrine cells, particularly B cells, are required for the proliferation of adipocytes at the graft site.

Key words: Intrasplenic rat pancreas transplantation, insulin, glucagon, adipose tissue.

non-pancreatic tissue. In this study an attempt has been made to follow the development of the transplanted neonatal pancreas and the effects of pancreatic transplantation on adipose tissue proliferation at the implant site.

Methods

The adult AS or PVG/G isogeneic or Wistar allogeneic rats used as recipients were first rendered mildly diabetic by streptozotocin 50 mg/kg administered IP, as the hyperglycaemic state is reported to have a trophic effect on insulin secreting cells [1]. The animals were then left in metabolic cages for 3 weeks and were allowed food and water ad libitum before and after transplantation. Urine was tested daily for glycosuria and blood glucose levels were estimated every 14 days using Ames Dextrostix and an Ames Eyetone reflectance meter.

A total of 250 animals were used as donors or recipients. Less than 1% died following streptozotocin administration. Initially, post-operative survival rates were as low as 75%, but later improved to over 90% with the severely diabetic recipients accounting for most of the fatalities. The severity of diabetes induced by the streptozotocin varied, possibly as a result of the site of administration (IP). The diabetic state was defined in terms of basal fasting blood glucose levels and daily urine production as normal (3–5 mmol/l, < 10 ml of urine), mild (6–8 mmol/l, 10–100 ml) or severe (> 8 mmol/l, > 100 ml) (Tables 1 and 2).

Following laparotomy three whole neonatal rat pancreases (1–3 days postpartum) were inserted into the long axis of the host spleen using a wide bore cannula and blunt probe. The development of the transplants was assessed between 1 and 540 days later using both electron microscopy and light microscopy, including immunohistochemistry for insulin, glucagon, somatostatin and pancreatic polypeptide secreting cells (Table 1). In addition we used radioimmunoassay to investigate tissue content of glucagon-like immunoreactivity and insulin-like immunoreactivity, in whole spleens of individual animals not included in the histology investigation, up to 280 days after isogeneic and 360 days after allogeneic transplantation (Table 2).

Similar intrasplenic transplantation of isogeneic adrenal or adipose tissue was carried out, each in a group of ten rats. The transplant sites were examined histologically up to 6 months after transplantation.

The transplantation of pancreatic endocrine tissue is a possible means for the treatment of diabetes mellitus. Whilst there is general agreement that this may improve the metabolic abnormalities in the recipient, there is little knowledge about the long-term effects of high local concentrations of pancreatic hormones on

Table 1. Histology of pancreatic transplants

Allogeneic transplants

Implantation time (days)	Diabetic condition ^a	Types of cell present in graft ^b							
		Adipose cells	Endocrine cells	Acinar cells	Ductal cells	A cells	B cells	D cells	PP cells
0	M	—	+	+	+	+	+	+	+
0	M	—	+	+	+	+	+	?	?
0.3	N	—	+	+	+	+	+	+	+
1	N	—	+	+	+	+	+	?	—
1	M	—	+	+	+	+	+	—	—
2	M	—	+	+	+	+	+	—	—
3	N	—	+	—	+	+	+	?	—
3	N	—	+ / —	—	+	?	—	—	—
4	N	—	+	—	+	—	+	—	—
4	N	—	+	—	+	?	?	?	?
5	M	—	—	—	—	—	—	—	—
6	M	—	+	—	+	?	?	?	?
6	M	—	+	—	+	?	?	?	?
7	S	—	+	—	+	—	+	?	?
7	S	—	+	—	+	+	+	?	?
7	S	—	—	—	—	—	—	—	—
8	M	—	+ / —	—	+	?	?	?	?
9	N	—	+	—	+	+	+	?	?
11	N	—	—	—	+	—	—	—	—
13	M	—	—	—	—	—	—	—	—
20	N	— / +	+	—	+	+	+	+	+
20	M	—	—	—	—	—	—	—	—
20	S	—	—	—	+ / —	—	—	—	—
21	M	—	—	—	+ / —	—	—	—	—
21	M	—	—	—	—	—	—	—	—
21	M	—	—	—	—	—	—	—	—
23	N	—	—	—	+	—	—	—	—
31	N	—	—	—	—	—	—	—	—
330	N	—	—	—	—	—	—	—	—

Isogeneic transplants

Implantation time (days)	Diabetic condition ^a	Types of cell present in graft ^b							
		Adipose cells	Endocrine cells	Acinar cells	Ductal cells	A cells	B cells	D cells	PP cells
7	N	—	+	—	+	+	+	?	?
8	M	—	—	—	+	—	—	—	—
16	M	+	—	—	—	—	—	—	—
30	S	—	—	—	—	—	—	—	—
46	N	+	+	—	+	+	+	+	—
63	N	+	+	—	+	+	+	—	+
90	N	+	+	—	+	+	+	+	—
90	N	+	+	—	+	+	+	+	—
90	S	+	+	—	+	+	+	+	—
90	N	+	+	—	+	+	+	+	—
90	N	+	+	—	+	+	?	+	—
120	N	+	+	—	+	+	+	?	+
120	M	+	+	—	+	+	?	+	—
120	N	+	+	—	+	+	+	?	+
150	S	+	+	—	+	?	?	?	?
150	S	+	+	—	+	?	?	?	?
180	M	+	+	—	+	+	?	—	—
190	S	+	+	—	+	+	+	+	—
190	S	+	+	—	+	+	+	+	—
190	S	+	+	—	+	+	+	+	—
220	M	+	+	—	+	+	?	+	—
300	N	+	+	—	+	+	+	+	+
340	N	+	+	—	+	?	?	?	?
340	N	+	+	—	+	+	+	+	—
360	N	+	+	—	+	+	+	+	—
400	N	+	+	—	+	+	+	+	—
400	N	+	+	—	+	+	+	+	—
540	N	+	+	—	+	+	+	+	—

^a N – normal, M – mildly diabetic, S – severely diabetic^b + / — very small numbers observed, ? no attempt or inconclusive results

Table 2. Amounts of insulin-like and glucagon-like immunoreactivity detected by radioimmunoassay after extraction from individual spleens at various intervals following implantation of pancreas

Allogeneic transplant (<i>n</i> = 13)				Isogeneic transplant (<i>n</i> = 20)			
Implant period (days)	Diabetic condition ^a	Glucagon-like immuno-reactivity (ng)	Insulin-like immuno-reactivity (mU)	Implant period (days)	Diabetic condition ^a	Glucagon-like immuno-reactivity (ng)	Insulin-like immunoreactivity (mU)
1	N	16.0	18.0	2	N	0.8	—
2	N	0.6	0.8	9	N	2.4	—
4	N	1.2	6.0	13	N	16.0	40.0
7	N	2.8	14.0	14	N	7.8	17.4
9	N	0.1	4.7	16	N	2.0	—
20	N	8.7	13.5	20	N	1.8	—
39	N	0.3	0	30	S	4.5	0
40	N	1.3	—	30	S	0	0
53	N	0.3	0	30	S	76.5	4.0
62	N	0	0	41	N	12.5	—
75	N	0	0	55	N	17.5	7.5
150	N	0	0	90	N	7.5	8.5
360	N	0	0	90	N	18.0	88.5
				90	N	51.0	4.6
				150	M	28.5	5.2
				150	N	1.0	—
				250	M	43.5	67.5
				250	M	45.0	3.4
				250	M	285.0	34.5
				280	N	540.0	3.4

^a N – normal, M – mildly diabetic, S – severely diabetic

Control non-implanted spleens: mean glucagon-like immunoreactivity = 0.005 ng; mean insulin-like immunoreactivity = 0.015 mU

Transmission Electron Microscopy

Tissue for electron microscopy was fixed for 4–24 h at 4 °C in 2.5% glutaraldehyde buffered to pH 7.4 with Millonig's phosphate buffer 0.1 mol/l. The tissue was dissected into 1-mm cubes and rinsed for 4–24 h in phosphate buffer 0.1 mol/l containing sucrose 0.2 mol/l. Following this the blocks were post-fixed in S-collidine buffered osmium tetroxide for 90 min at room temperature. After several brief rinses in water to remove excess osmium, the blocks were stained in 2% uranyl acetate for 30 min before dehydration and embedding in Spurr's resin.

A one-micron survey section was cut from each block and stained with 0.5% Toluidine blue in 1% sodium borate. Areas were selected for further study and ultra-thin sections 80 nm thick were examined following staining with lead citrate.

Immunohistochemistry

The spleen was removed, sliced and fixed in modified Susa fixative [2] for 4 h, washed in 70% alcohol and processed for wax embedding. Immunohistochemistry was carried out on dewaxed 4-μ paraffin-embedded sections for insulin, glucagon, somatostatin and pancreatic polypeptide. The Sternberger specific indirect antibody peroxidase method was applied [3]. The following antisera were used: GP9 for insulin, raised in guinea-pigs; YY118 for glucagon, raised in rabbits; OB2 for somatostatin, raised in rabbits; and pancreatic polypeptide PP204, raised in rabbits. Antisera were incubated with an excess of the relevant hormone to act as specific blocking controls, and the antisera were used at dilutions of 1:10 to 1:100. Normal adult rat pancreas and non-implanted spleen were used as positive and negative controls respectively.

The specificity of the antibodies was checked in radioimmunoassay. The insulin antibody cross-reacted with extracts of rat pancreas and also with proinsulin. The glucagon antibody showed negligible cross-reactivity with secretin, vasoactive intestinal polypeptide and gastric inhibitory polypeptide. The somatostatin antibody did not react with any of these gastrointestinal hormones, nor did the antibody to pancreatic polypeptide.

Extraction and Hormonal Content of Spleen

The whole spleen from test and control animals was chilled immediately after excision and all subsequent manipulations were carried out at 4 °C. The spleen was finally sliced and extracted overnight by eight volumes of a mixture of ethanol and HCl 0.7 mol/l (3:1 v/v). Then the mixture was centrifuged at 3,000 g for 30 min, the pH was adjusted to 7.5 with ammonia solution and the resultant precipitate was removed by centrifugation. The extract was then dried in a jet of air. The samples were reconstituted for radioimmunoassay in sodium phosphate buffer 0.4 mol/l, pH 7.4.

Insulin Radioimmunoassay

The antibody (GP25) was raised in guinea-pigs against porcine insulin and was used at a final dilution of 1:280,000 in the assay tube. The antibody cross-reacted with porcine proinsulin to approximately 50% on a weight basis. Insulin was labelled with ¹²⁵I (Radiochemical Centre, Amersham, Bucks, UK) and purified on micro-fine silica. The assay can detect 0.5 mU/l of insulin.

Fig. 1. Light micrograph: 64-day isogeneic neonatal pancreas transplant, immunohistochemically stained for insulin. There is no regeneration of exocrine tissue. The small islets contain strongly staining B cells (*closed arrow*). There are two positively stained cells within the epithelium of one ductal element (*open arrow*). D = duct. Immunoperoxidase and Methylene blue counterstain ($\times 560$)

Fig. 2. Low power light micrograph ($\times 19.3$): transverse section of host spleen (S) with a 350-day isogeneic neonatal pancreatic transplant, immunohistochemically stained for somatostatin. Immunoperoxidase and Methylene blue counterstain. The central region of the spleen is occupied by the graft which is composed mainly of adipocytes (Ad) and small islets (*arrows*). The graft is well vascularised. SC = splenic capsule. Islet indicated by open arrow shown at high power *Inset* ($\times 580$)

Glucagon Radioimmunoassay

Antibodies raised to pancreatic glucagon were used in the glucagon assay, namely YY89 at a final dilution of 1:45,000. YY89 reacts with the C-terminal region of glucagon [4]. The assay can detect glucagon 10 ng/l. No cross-reaction has been noted with other gut and islet hormones, including insulin.

Results

Blood Glucose Levels

Three weeks following streptozotocin administration, the blood glucose levels of the animals ranged from 5–12 mmol/l.

Allogeneic Transplants

There was rapid degeneration of the exocrine mass within the pancreatic transplant, with few cells remaining 2 days after implantation. The endocrine and ductular tissue were retained for up to 20 days and there were no detectable amounts of extractable peptide 52 days beyond transplantation (Table 2). There was no evidence of any exocrine, endocrine or ductular regeneration for up to 1 year after implantation. Furthermore, there was no evidence of adipose proliferation throughout the transplant period, only a small fibrous scar remaining at the implantation site.

Isogeneic Transplants

There was similar rapid degeneration of the exocrine mass with few cells remaining 2 days after implantation. At 7 days only endocrine and hollow spheroids of ductular tissue remained within the implant. After a marked initial reduction 1 day following transplantation, variable but significant amounts of extractable immunoreactive peptides were obtained from the grafts (Table 2). The amounts of extractable insulin varied considerably and were in general low. However significant amounts were still detectable in grafts up to 280 days old showing that insulin was still being produced. Glucagon levels increased considerably towards the end of the graft period. After transplantation periods in excess of 1 year, glucagon, somatostatin and insulin secreting cells were identified in considerable numbers, usually in small islets often composed of single cell types. Pancreatic polypeptide cells were identified less frequently and were apparently absent from many grafts. Electron microscopy confirmed the presence of these islets. Throughout the transplant period, insulin, glucagon, somatostatin and occasionally pancreatic polypeptide cells were identified immunohistochemically within the ductal epithelium, which was often in close association with small islets (Fig. 1).

A striking feature of the development of the transplants was the proliferation of adipose tissue closely associated with the surviving islets. This was noted after 1 month from implantation and often occupied the major part of the central region of the host spleen after 1 year (Fig. 2). Islets were found in and around the fatty mass, usually associated closely with blood capillaries. The adipocytes were increasingly distended with intracellular lipid throughout the transplant period. Appearances suggestive of lipogenesis were seen in adipocytes in close association with B cells (Fig. 3), a feature not noted adjacent to a heterogenous group of islet cells or to A cells alone.

There was no proliferation of adipocytes in control transplantations of isogeneic adult rat fat or adrenal gland.

Discussion

The development of transplanted isogeneic and allogeneic neonatal pancreas has been investigated by a large number of authors, and the spleen has often been chosen as the site of implantation [5–8]. There is general agreement that while allogeneic tissue fails to survive, isogeneic endocrine tissue is retained for considerable periods [6, 7]. This study demonstrates the ability of ductal elements not only to survive within an

Fig. 3. Electron micrograph: 300-day isogeneic neonatal pancreas transplant. B cells are in close association with a number of capillaries (Ca) and an adipocyte. The adipocyte has a number of small intracellular coalescent lipid droplets (Li); arrow in the cytoplasm adjacent to the B cell (B). Uranyl acetate and lead citrate stain ($\times 600$)

isogeneic host, but also the intimate association of endocrine cells with the duct lumen. The presence of endocrine cells within the duct epithelium of pancreas, whether transplanted, organ cultured or in situ, has been reported by a number of authors [9–12], and it would appear that this capacity is not lost after transplantation. There was an apparent increase in the number of endocrine cells within the grafts and this was supported by significant amounts of immunoreactive polypeptide extracted from the graft site. Ductal production of endocrine tissue would ensure the continued presence of active hormone secreting cells and associated high local concentrations of pancreatic hormones within the surrounding tissue.

A rapid and marked proliferation of adipocytes occurred one month after implantation and this may be linked closely to the continued production of hormones within the graft, as it has been shown that there is a close association between pancreatic hormones and lipid metabolism [13, 14]. The cells may originate from adipocytes within the host spleen but are more likely to be derived from the small number of lipid cells within the original implant and may explain why authors using separated (and presumably adipocyte-free) islets for transplantation fail to record any similar proliferation at the intrasplenic site [5, 8]. Indeed prior organ culture of whole foetal pancreas eliminates or reduces fatty proliferation within renal subcapsular or intraperitoneal transplants [15, 16]. The fact that the neonatal pancreas contains very few adipocytes points to a strong trophic effect within the

graft on lipogenesis and fatty tissue proliferation. Adipocyte proliferation has been reported by a number of authors transplanting whole fetal pancreas to the renal capsule or anterior chamber of the eye [15, 17–20]. Electron microscopic evidence in this study suggests that there may be a localised action of B cell secretions on lipogenesis within closely associated adipocytes, together with a general trophic effect within the graft, an observation which is consistent with the known lipogenic actions of insulin [13]. The morphological appearance of the adipocytes adjacent to B cells within the graft is similar to that of developing fatty tissue [22].

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