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Foetal Rat Pancreatic Transplantation: Posttransplantation Development of Foetal Pancreatic Iso- and Allografts and Suppression of Rejection With Mycophenolate Mofetil (MMF) and Cyclosporine Based Immunesuppression

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ABSTRACT Provided engraftment can be ensured, vascularization promoted, and ischaemic damage due to storage prevented, foetal pancreatic transplantation (FPT) has the potential to ameliorate the endocrine and metabolic disturbances in diabetic animal models including hyperglycaemia. In a syngeneic Wistar rat substrain (WAG) model (WAG → WAG), FPT was capable of restoring normoglycaemia in diabetic rats rendered diabetic by streptozotocin (STZ). Post-transplantation growth and development of the foetal tissue was characterised by acinar atrophy, preservation of islet tissue, and development and proliferation of fat accumulations at the site of engraftment. B- and A-cell staining and distribution on ICC appeared normal after 12 months. Mycophenolate mofetil (MMF) together with cyclosporine (CSA) was selected to suppress rejection of foetal rat pancreatic allografts in a strong responder allogeneic model (WAG → Sprague-Dawley). MMF, a novel immunosuppressive agent that selectively inhibits de novo purine synthesis, was administered in combination with subtherapeutic doses of CSA (2 mg/kg/day) to prevent rejection after allogeneic foetal rat pancreatic transplantation. Although CSA monotherapy in this model can partially suppress rejection, the combination of CSA and MMF results in significant inhibition of acute allograft rejection and mononuclear cellular (MNC) infiltration as assessed by sequential histology post-operatively. Although the follow-up period of allografts was restricted to 30 days of treatment, histology showed low graft infiltrate scores (1.2+) and preservation of islets and immunocytochemical staining. The results in this animal transplantation model confirm that sub-therapeutic doses of MMF and CSA therapy are effective in preventing acute rejection of foetal rat pancreatic allografts in the short-term, thus allowing preservation of vital endocrine components of the foetal pancreas such as islets. *Microsc. Res. Tech. 43:343–355, 1998.* © 1998 Wiley-Liss, Inc.

INTRODUCTION

Dys-synchronous pancreas and simultaneous pancreas/kidney transplantation has become an acknowledged treatment option for the management of select Type-I (IDDM) diabetics who present with end-stage renal disease (Abecassis and Corry, 1993; Stratta et al., 1995). The benefits of pancreatic transplantation, amongst others, include abrogation of hyperglycaemia, rendering Type-I diabetics insulin independent, and normalizing many of the subtle disorders of carbohydrate metabolism induced by diabetes (Bartlett et al., 1996; Sutherland, 1992). In contrast to the successes documented after vascularized whole pancreatic transplantation in man, the application of allogeneic islet and foetal pancreatic engraftment has been discouraging and bedevilled by technical problems, together with rapid rejection of the grafts before they become viable and functional after engraftment. On the other hand, transplantation of foetal pancreata or free foetal/neonatal tissue is in itself appealing in the clinical and experimental setting because of the potential theoretical advantages of safety, ease of transplantation, reproducibility, and growth and maturation potential of the transplanted foetal tissue (Beattie et al., 1997; Lafferty et al., 1989; Lafferty and Hao, 1993). The potential for the application of, and the ability to reverse hyperglycaemia by, this form of sophisticated and novel endocrine replacement therapy in acknowledged laboratory models has previously been reported by numerous groups (Brown et al., 1984; Eloy et al., 1980; Mandel et al., 1982; Mullen et al., 1976). Acute rejection of newly engrafted, free allogeneic pancreatic grafts currently remains a major obstacle to successful clinical implementation of this modality. Possibly the introduction of new immunosuppressive strategies may rectify this problem.

Recently the immunosuppressive properties and therapeutic potency of cyclosporine (CSA) and mycophenolate mofetil (MMF), used as induction and rescue

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therapy in human and experimental transplantation, has been reported by numerous researchers (Beckstein et al., 1993; Bullingham et al., 1996; Hamilton et al., 1982; Morris et al., 1991; Platz et al., 1991; Qi et al., 1996; Wennberg et al., 1996). The purpose of this study, using a laboratory rodent model, was to assess the early (10 days) and intermediate (1 month) term morphological, histological and endocrine graft development following allogeneic foetal rat pancreatic transplantation (FRPT) in non-diabetic Sprague Dawley (SD) rats immunesuppressed with combinations of cyclosporine and mycophenolate mofetil.

MATERIAL AND METHODS

Transplantation Technique

The assessment of graft histology in control, syngeneic, and allogeneic recipients was performed in an established rodent transplantation, diabetic laboratory model (Brown, 1984). All surgical procedures were performed under general ether and ketamine hydrochloride anaesthesia to ensure that animals did not suffer unnecessarily at any stage of the experiments. Time bred foetal pancreases ranging from 16–21 days (20–30 mm crown rump size) were harvested from anaesthetized, pregnant female WAG (substrain of Wistar rat) rats (ketamine hydrochloride, Parke-Davis, Sandy, UT, 0.1–0.2 ml and inhalation ether) via laparotomy and hysterotomy. By means of microsurgical techniques and magnification (Zeiss Operating Microscope, Carl Zeiss, Germany) the foetal pancreases were meticulously separated and removed from the duodenal loop, stomach and spleen of the donors. The transplanted pancreases measured from 0.25–0.5 mm in diameter. Before transplantation (which was usually effected within 30 minutes after removal of the organ), the pancreases were temporarily stored at 4°C in RPMI 1640 medium (Gibco, Scotland). Engraftment of between 4–8 foetal pancreases (without separation of the exocrine component by culturing), into the renal subcapsular space was effected in anaesthetized syn- and allogeneic recipients according to an ethically approved experimental protocol, 87/072, at the University of Stellenbosch. The recipient kidney(s) were exposed under general anaesthesia via a 3–4 cm midline laparotomy incision. A vertical incision, varying in length from 5–7 mm, was made thereafter on the ventral aspect of the kidney capsule. Through the capsular incision, a subcapsular pocket was created on the lateral aspect of the cut by carefully undermining the capsule at the interface between the capsule and the underlying kidney cortex. Haemostasis was obtained in the pocket by gentle exterior pressure with a small surgical dissecting swab for a few seconds. Immediately prior to engraftment of the tiny foetal pancreases, the pocket was gently rinsed with 0.5 ml physiological saline solution or RPMI-medium at 4°C. The grafts were placed together and carefully located in the deepest portion of the subcapsular pocket to avoid spillage. In some cases, the capsule immediately overlying the grafts was intentionally fenestrated by tiny accurately placed perforations to allow excess plasma to escape and to prevent fluid buildup. The capsular incision was left unsutured, as we have found it unnecessary to close it to prevent the grafts from becoming dislodged (unpublished data). The midline laparotomy incision was closed in layers (Dexon-4/0, Davis and Geck) and the recipient was returned to a warm environment, insulated with cotton wool lining. Immediately post-operatively, the recipients had free access to water and chow ad-lib. The ischaemic time of donor tissue was kept as short as possible. Engraftment was effected in syngeneic diabetic and non-diabetic controls (WAG to WAG, 150–200 g), WAG to non-diabetic Sprague-Dawley (SD, 200–500 g) non-diabetic, allogeneic recipients. In all cases donor pancreata were from WAG rats, a substrain of Wistar rodent. Chemically induced diabetes in control syngeneic inbred or isogeneic WAG rodents was induced by a single tail vein injection of Streptozotocin (Upjohn, Kalamazoo, MI; 50 mg/kg). Transplantation was performed in the latter isogeneic WAG group when the random whole blood glucose levels (Ames; Glucometer) exceeded 10 mmol/l. Following transplantation, subcutaneous insulin (Actraphane, Novo) was deemed temporarily necessary in this group in doses varying from 2–5 U given subcutaneously on a daily basis until normoglycaemia was restored and the grafts and islets became functional. Prophylactic, parenteral antibiotics were administered (Amikin, Squibb; and Penicillin, Novo) as a short course postoperatively to prevent intra-abdominal infection.

Immunesuppression

WAG to WAG isogeneic transplant recipients received no immunesuppression due to the inbred nature of the strain. CSA was administered alone or in combination with MMF (50 mg/kg/d per gavage) at a dose of 2.0 mg/kg/d imi, commencing at the time of transplantation and indefinitely thereafter until the grafts were harvested for histological assessment thereof (see Experimental Groups).

Experimental Groups

Experimental groups are reflected in Table 1. Group 1: Non-transplanted, 16–21-day foetal rat pancreases, served as histological controls; group 2: normal, non-transplanted, adult histological and endocrine controls (n = 21); group 3: syngeneic diabetic WAG to WAG transplant control recipients followed for 12 months (n = 8); group 4: allograft recipients with grafts, no immunesuppression (n = 10); group 5: allograft recipients (WAG to SD, n = 8) immunesuppressed with CSA 2 mg/kg/d for 10 days; group 6: allograft recipients (WAG to SD, n = 5) immunesuppressed with MMF alone; allograft recipients (WAG to SD, n = 5) immunesuppressed with CSA (2 mg/kg/d) and MMF (50 mg/kg) for 10 days; group 7; allograft recipients (WAG to SD, n = 5) immunesuppressed with CSA (2 mg/kg/d) and MMF (50 mg/kg) for 30 days (n = 5); group 8. Observations from this laboratory indicate that transplantation of foetal rat pancreata from WAG to SD reflects transplantation across a strong histocompatibility barrier.

Statistical Analysis

Non-parametric statistical analysis was applied to verify results (Mann-Whitney U Test) and analysis were deemed statistically significant if $P < 0.05$.

Flow Cytometry

Flow cytometry was performed before and after transplantation at designated intervals in allograft recipi-

*TABLE 1. Experimental groups**

Group	Treatment	Number
Group 1	Non transplanted foetal pancreatic controls	5
Group 2	Non-transplanted adult pancreatic controls	21
Group 3	Syngeneic diabetic control transplants (WAG → WAG)	8
Group 4	Untreated allograft controls (WAG → SD)	10
Group 5	Allografts plus CSA alone 2 mg/kg/d × 10 days (imi)	8
Group 6	Allografts plus MMF alone (50 mg/kg/d) per gavage for 10 days	5
Group 7	Allografts plus combination CSA (2 mg/kg/d) and MMF 50 mg/kg/d for 10 days	5
Group 8	Allografts plus combination CSA (2 mg/kg/d) and MMF 50 mg/kg/d for 30 days	5

Immunesuppressants were administered at the time of transplantation.

ents (groups 5–8). Tubes containing 100 μl heparinized peripheral whole blood, collected via the tail vein, was incubated for 45 minutes at room temperature with 60 μl of either CD2 (OX34), CD4 (W3/25), or CD8 (OX8) tissue culture supernatant. The incubated blood was washed, centrifuged, and resuspended in PBS. Thereafter the blood was reincubated at room temperature in the dark with 50 μl FITC conjugated goat antimouse IgG (Kpl, Gaithersburg), dilution 1:100, for 30 minutes. Lysing of the labeled whole blood was effected with FACS Lysing Solution (Becton Dickinson, San Jose, CA). After further washing, centrifuging, and resuspension in PBS, the specimens were analyzed on a FACScan (Becton Dickinson) flow cytometer using Forward scatter (FSC) vs. Side scatter (SSC) to gate the lymphocyte populations. Positive staining of the lymphocytes within the lymphocyte gate was determined by log fluorescence intensity using FLI (Fluorescence channel 1). Histograms obtained were analyzed using Lysys II (Becton Dickinson) software.

Endocrine Studies

WBG (whole blood glucose) was measured on peripheral blood (Ames Glucometer) and insulin release (IRI) determined by radioimmunoassay (Coat-A-Count, California) on specimens obtained during IVGTT from isografts (using 0.5 g 50% dextrose). C-peptide was not determined. Glucose disappearance constant (K-values) was calculated from the 10 to 60 minute IVGTT blood specimens.

Histological and Morphological Assessment

Engrafted pancreases were harvested for histological analysis from syngeneic and allogeneic recipients. Multiple sections of the graft/kidney interface were prepared to obtain representative tissue for histological analysis. Tissue was obtained for light-microscopy (haematoxylin and eosin), immunocytochemistry, immunophenotyping, and electron microscopy. The histological changes in allograft recipients were graded after the scoring method of Guymer and Mandel (1993). Immunocytochemistry was performed on formaldehyde fixed paraffin sections. Initially endogenous peroxidase was blocked with 3% hydrogen peroxide. Primary antisera raised against insulin, glucagon, pancreatic peptide, or somatostatin (DAKO, Glostrup, Denmark), diluted in TBS 1:200, was applied to the sections and incubated for 30 minutes. After 3 washes in TBS, biotinylated anti-rabbit anti-mouse immunoglobulin link-antibody (DAKO) was applied in dilutions of 1:250 for 30 minutes. Incubation with horse anti-rabbit peroxidase conjugated streptavidin-biotin complex (ABC) (DAKO) followed for 30 minutes. Positive labelling was demonstrated using 0.05% 3,3-diaminobenzidine tetrachloride (DAB) in TBS. Immunotyping of pancreatic graft tissue was performed on frozen sections cut on an American Optical Cryo-cut Microtome. Either CD2 (OX34), CD4 (W3/25), CD8 (OX8), or IL2R (OX39) was used to cover the sections. FITC conjugated goat antimouse IgG (Kpl, Gaithersburg) was used as secondary antibody. Sections were mounted in PBS buffered glycerine and the slides were viewed with a Zeiss fluorescent microscope fitted with a mercury vapor lamp and a 450–490 (blue) exciter filter. For electron microscopy, tissue was fixed in 2.5% glutaraldehyde. Pre-staining and fixation was effected using 2% uranyl acetate (Merck, Darmstadt, Germany) in 50% ethanol. After dehydration and impregnation with 50% ethanol and 50% Spurr's resin, blocks were embedded into Spurr's resin filled gelatin capsules and polymerized at 70°. A Reichert TM90 trimmer was utilized to expose the tissues within the blocks. Survey semi-thin 1 μm sections were cut on a LKB (Bromma, Sweden) Ultratome III and stained with 1% toluidine blue in 1% borax. Thin gold sections (100 nm) were cut of selected blocks with the same ultratome, mounted on G200 grids (Bio-Rad, Richmond, CA) and stained with uranyl acetate and Reynold's lead citrate. The stained grids were viewed on a Hitachi (Tokyo, Japan) H600 transmission electron microscope.

RESULTS

Light-microscopic histological evaluation of control non-transplanted 16–21-day gestation foetal pancreases (group 1) revealed proliferating and developing exocrine tissue (Fig. 1) together with visible islet tissue (at 16 days) that stained faintly for B- and A-cells. Electron microscopy (EM) confirmed the presence of sparsely granulated islets consistent with the presence of B-cells. Further histological assessment of foetal and normal adult pancreases (groups 1 and 2) revealed a normal variation of lipid distribution by Oil-Red-O staining and a conventional distribution of perinephric and extra-capsular fat. Intraparenchymal fat deposits were distinctly absent in foetal pancreases as well as in young adult pancreases. Occasional fat accumulations were present in the pancreases of adult rats older than 1 year. Endocrine studies revealed random whole blood glucose (WBG) values of 5.6 ± 1.27 mmol/l in normal WAG adult controls (group 2). K-values (glucose disappearance) during IVGTT were 2.48 ± 0.73% per min. The administration of STZ (50 mg/kg) resulted in 90% of group 3 WAG recipients developing clinically overt diabetes (i.e., hyperglycaemia, polyuria, and polydipsia) within 5–7 days and the majority recorded ketones in the urine (Multistix Ames). An interval top-up dose of STZ, 10 mg/kg, rendered the remainder diabetic. Blood glucose values were elevated to 13.61 ± 6.46 mmol/l and k-values reduced to 0.43 ± 0.1% per minute (range 0.01– 1.15% per minute) ($P < 0.05$ group 2 cf group 3). Renal subcapsular transplantation of between 4 and 8 foetal pancreases (WAG → WAG isografts; group 3) rendered 80% of rats normoglycaemic within 7–14 days at

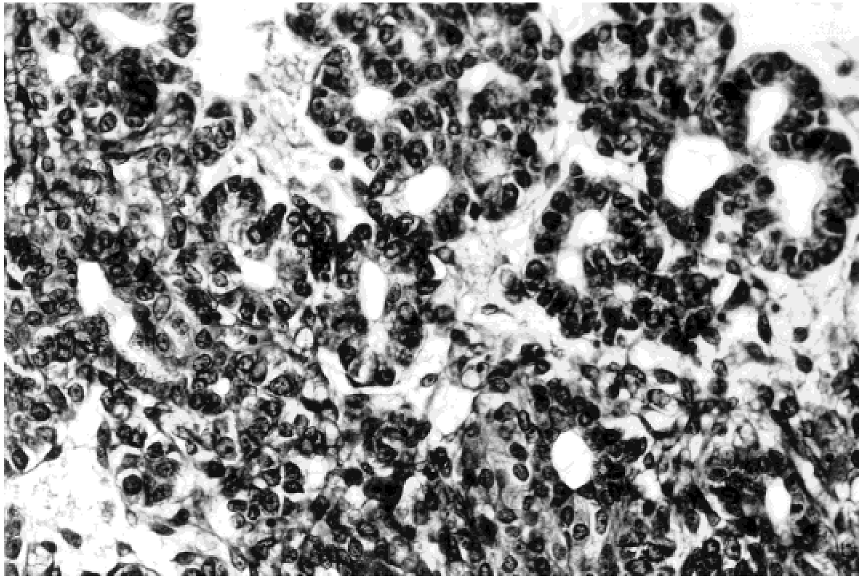

Fig. 1. Sixteen-day gestation foetal rat pancreas showing established acinar pattern and ductules (H & E, ×100).

which stage supplemental insulin (Actraphane, Novo) was stopped. In the short term, all the clinical features of diabetes (i.e., glycosuria, ketosis) were quickly abrogated by foetal pancreatic transplantation. During IVGTT, WBG values of 7.32 ± 1.39 mmol/l (range 5.2 –9.7 mmol/l) and k-values of 1.73 ± 0.68 were recorded. Histological assessment of engrafted tissue, harvested at intervals of 7, 14, 21, 90, and 365 days after transplantation, showed that, in all syngeneic recipients (group 3) studied, the natural course of the transplanted pancreatic tissue was to undergo progressive atrophy or involution of the exocrine (acinar) element, dilatation of the ducts with flattening of the ductal epithelium with time, and growth and maturation of the islets of Langerhans (Figs. 2 and 3). Within the first 90 days a mild chronic lymphocytic cellular tissue reaction was present in the periacinar spaces and adjacent to the exocrine component. All group 3 isogeneic transplanted recipients, developed macro and microscopic fat deposition at the site of engraftment provided the graft took and survived. Evidence of fat deposition was present in 70% of cases as early as 10 days after transplantation but especially prominent from 3 months (Fig. 3). With time the fat, of a white adipose nature, accumulated under the kidney capsule, adjacent to the transplant eventually embedding the engrafted tissue (Fig. 3). By 12 months, large macroscopic, but discrete fat deposits marked the site of transplantation. Measurement of the fat accumulations (pads) showed a variation in size from 9 ×10 mm to 24 ×10 mm. Diabetic recipients of grafts transplanted 12 months previously remained normoglycaemic and the random whole blood glucose levels were within the normal range. Fifty percent of graft recipients showed modest hyperinsulinaemia. Light microscopic assessment of the grafts at 12 months, revealed between 5 and 10 large islets per highfield. The islet counts did not differ from assessments performed at intervals of 1, 3, 6, and 9 months. Overall the morphology appeared normal and no peri-islet fibrosis was present. Compared to grafts assessed at 3–4 months after transplantation, the perigraft lymphocytic infiltrate had disappeared completely at 12 months. Extensive atrophy of the exocrine component was present and in some grafts only remnants of the acinar tissue could be identified. Ducts with flattened epithelium and absent acinar granules was a consistent finding in all grafts. In a few grafts, however, focal islands of well-granulated exocrine tissue could still be detected. Islets were clumped together or free-lying in the surrounding fat pads and occasionally appeared to coalesce with one another. Immunocytochemistry of the islets showed normal staining for B- and A-cells but reduced staining for D- and PP cells. Re-innervation of the islets was specifically investigated but could not be confirmed. Immunohistochemistry, using a streptavidin biotin complex technique, showed no immunoreactivity for, or staining of, S100 protein, neuron specific enolase (NSE) or neural filaments. EM confirmed the presence of well-granulated B- and A-cells at 12 months post-transplantation in the isogeneic recipients (group 3).

All recipients of non-immunosuppressed grafts (WAG SD; group 4) showed consistent rejection of the grafts by day 10 post-transplantation. Rejection could be detected as early as 4–5 days after engraftment. At 10 days, graft scores were 0–1+ (grafts) and 4+ (lymphocytic infiltrate) as determined by the evaluation of Guymer and Mandel (1993). Graft and MNC infiltrate scores are reflected in Table 2. At 10 days, virtually no acinar or islet elements were visible due to the advanced stage of rejection.

Immunesuppression with CSA 2 mg/kg/day indefinitely (group 5; WAG → SD) of non-diabetic allograft recipients rendered graft scores of 3–4+ (Ave 3.4) (grafts) and 1–3+(Ave 1.6) (MNC infiltrate). In 90% of grafts studied, visible islets were present (5–10 /hf). Flow cytometry performed on a sequential basis showed a gradual reduction of $CD4^+$ cells from 68.8 ± 5.1% to 61.7 ± 7.3% (CD4 expressed as a percentage of CD2); P = NS compared to normal controls.

Graft evaluation of MMF monotherapy recipients (group 6) revealed histology inferior to that of group 5

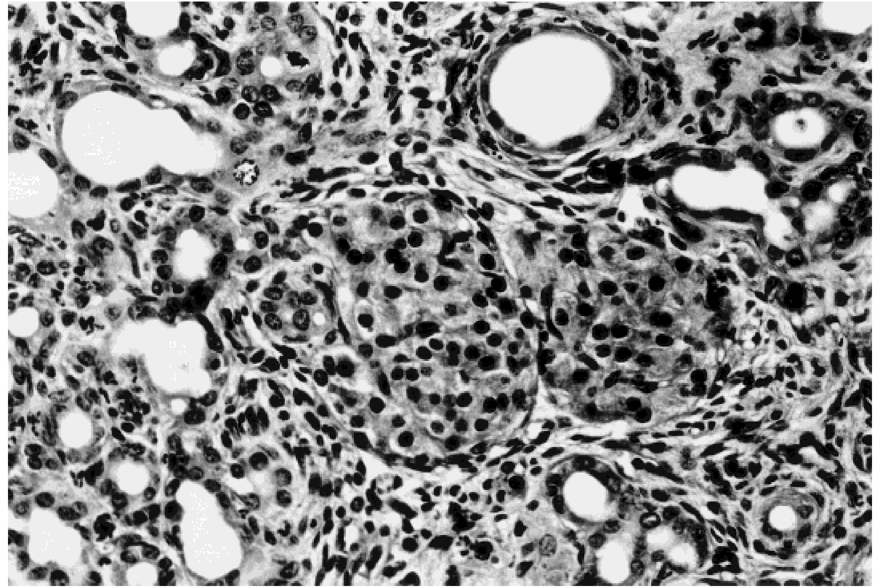

Fig. 2. Post transplantation isograft pancreas at 3 months showing intact well-developed islet tissue (H & E, ×200).

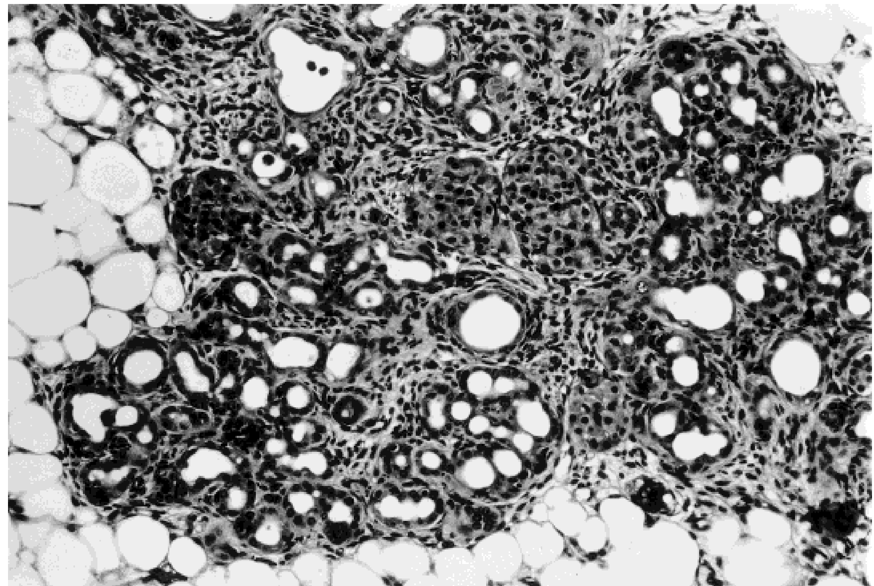

Fig. 3. Isografted pancreas at 3 months showing typical peripancreatic accummulations of fat. Note acinar atrophy and dilated pancreatic ducts (H & E, ×100).

(CSA) and graft scores of 0–2+ (Ave 1.0) (grafts) and 3–4+ (Ave 3.6) (infiltrate) were recorded. Occasional islets were detected but were undergoing rejection. MMF given alone did not result in an alteration of the peripheral blood lymphocytes in the short-term in this rodent model.

Graft recipients of combined CSA and MMF (group 7) exhibited good overall histological appearance at 10 days and graft scores of 3–4+ (Ave 3.4) (grafts) and 0-1+ (Ave 0.4, MNC infiltrate) were detected. Five to 10 visible and normal appearing islets were counted per high field (Fig. 4). Early fat accumulation was detected in this group, as in other groups, but also occurred in 20% of CSA recipients. Immunocytochemistry revealed, as in the case of isografts, normal or near normal granulation of B- and A-cells with reduced staining of D- and PP-cells. Anti-insulin staining for B-cells at 10 and 30 days post-transplantation, showed well-granulated cells (Fig. 5). Flow cytometry in the group receiving combined CSA and MMF also revealed reduced $CD4^+$ levels at 10 days (CD4 expressed as a percentage of CD2) and values were diminished from 71 ± 5.7 % (baseline) to 60%, results not significantly different from CSA controls (group 5). However, in a small group receiving both agents for 30 days (group 8) the values were reduced from 71 ± 5.7% to 46.6 ± 9% values ($P < 0.05$, group 8 compared to group 5). E.M. confirmed the presence of well-granulated B- and A-cells in group 7 (Fig. 6) recipients. Histological parameters in group 8 were similar to group 7 ($P =$ NS). Histological immunotyping of graft infiltrates by immunofluorescence, in groups 5–7, showed mixed graft lymphocyte infiltrates consisting of CD4 and CD8 cells. Differences could not be accurately detected within these groups.

TABLE 2. Light-microscopic point scoring of graft and mononuclear cellular infiltrate after Iso- and Allograft Transplantation (after Guymer and Mandel 1993)

Group	Treatment/ immune suppression	Graft score	Ave	Infiltrate	Ave
Group 3	Isografts	4, 4, 4, 4, 4	4	No rejection	0
Group 4	Non-treated allografts	0–1+	0	4+	4
Group 5	CSA 2 mg/kg/d for 10 days	4, 3, 4, 3, 3	3.4	1, 2, 1, 2, 2	1.6
Group 6	MMF alone for 10 days	1, 0, 1, 1, 1, 2, 1	1.0	4, 4, 4, 3, 4, 2, 4	3.6
Group 7	MMF & CSA for 10 days	3, 3, 4, 3, 4	3.4	0, 1, 0, 1, 0	0.4
Group 8	MMF & CSA for 30 days	3, 2, 3, 3, 3	2.8	0, 3, 1, 1, 1	1.2

Group 8 versus group 5, P = NS.
Group 8 versus group 7, P = NS.

DISCUSSION

From an organogenesis point of view, the development and maturation of the denervated, duct-disrupted, foetal pancreas differs considerably from the natural growth of the foetal pancreas in-situ. In the normal anatomical position, the foetal rat pancreas develops into three discrete components consisting approximately of 98% acinar tissue, 1% ductular tissue, and 1% islets. This is the characteristic pattern of the normal adult rat pancreas. In the case of transplanted foetal rat pancreas engrafted into the renal subcapsular site in isogeneic models, vast morphological differences are observed in organogenesis, development, and maturation of exocrine and endocrine tissue compared to the development of the pancreas in normal rats. Instead of the normal orderly expression of exocrine and endocrine components, the transplanted foetal rat pancreas undergoes gross morphological developmental changes characterized by partial to complete atrophy of the exocrine tissue and ductal elements, preservation of islets, and excessive proliferation or accumulation of white adipose tissue within and around the transplanted tissue. The exact cause of this unique growth disorder is unknown but it is tempting to speculate that factors such as denervation of the graft, prolonged cold ischaemic time, transposition of the foetal tissue from the customary entero-insular axis location to a peripheral developmental site, drainage of insulin into the systemic circulation as opposed to the portal system, and reduced availability of growth factors in the adult recipient rat compared to a growing rat, may be contributing factors. Disruption of the anatomical drainage of the exocrine element and ductular system in the foetal graft following harvesting may also be responsible. Despite the unusual appearance of the retrieved grafted tissue, characterized by the macroscopic development of white-yellow blobs under the kidney capsule of isogeneic recipient rats after 12 months, a significant, but definite proportion of functional islet tissue does survive and is capable of the reversal of chemically induced diabetes in syngeneic rat recipients. The exact mass of islet tissue that develops from the grafted tissue, compared to normal expected organogenesis, is not answered by this study. However, sufficient islet tissue does develop with maturation of the grafts and this is capable of long term restoration of normoglycaemia in diabetic rats. The development of a viable mass of islet tissue in this model is of critical importance if hyperglycaemia is to be reversed and the changes are to be sustained for prolonged periods of time. In our experience, the transplantation of a single foetal rat pancreas of 16–20-day gestation period is unable to reverse hyperglycaemia in this model although the graft actually exhibits growth and development (data not shown). Our data indicate that from 4–8 pancreases are needed for transplantation to induce hyperglycaemia, and this strongly suggests that a substantial number of islets do not develop or become dysfunctional in the isogeneic model. This has been the finding in most reported studies although some have reported reversal of diabetes by the use of a single foetal pancreas (Mullen et al., 1976). Possibly the process of attrition and atrophy of the acinar tissue or scarring after transplantation induces a negative effect on the further organogenesis of the developing islet because of the loss of the intimate islet-acinar morphological configuration. Although these growth aberrations in relation to the islet are present, our studies do indicate that, at least in the isogeneic model, reasonably good graft function (as assessed by IVGTT, K-values, and insulin release) can be expected in about 65–70% of recipients, with a primary non-function rate of about 10%, the causes of which are unknown. As in the case of kidney transplantation, early allograft loss remains problematical, the cause of which may be attributed to acute rejection, vessel thrombosis (either technically induced or related to the use of cyclosporine), or ischaemic-induced injury during organ storage (Sollinger, 1996). Clearly the etiology is multifactorial and some of the important causes are avoidable and need to be addressed when applying this mode of transplantation. The results in syngeneic rats (WAG → WAG) were encouraging in that the transplanted tissue could improve the k-value of STZ-induced diabetic rats, but could not entirely reach the values of normal rats. The exact cause of the impaired glucose tolerance values and reduced k-values, following syngeneic transplantation in about 20% of recipients, is probably multifactorial and factors such as defective organogenesis after engraftment, transplantation of an insufficient islet cell mass, graft denervation, systemic drainage of insulin, ischaemic graft damage, diabetogenic effect of cyclosporine, and reduced GLUT2 may be important (Zangen et al., 1997). The modest systemic hyperinsulinaemia detected after transplantation is attributed to the systemic venous drainage of insulin secreted by the graft. We have been encouraged by these endocrine findings and it is our expectation that similar studies will be possible in allogeneic recipients, once early rejection can be optimally suppressed. This would prevent premature destruction of the tiny grafts before they become viable and established and allow maturation of the islet tissue, keeping in mind that a critical time is needed for the grafts to become functional. In our studies, a period of about 10–12 days is needed for reversal of hyperglycaemia, a time in which the acute rejection process is particularly prominant.

The fascinating finding of causation and initiation of the white adipose tissue accumulation, adjacent and

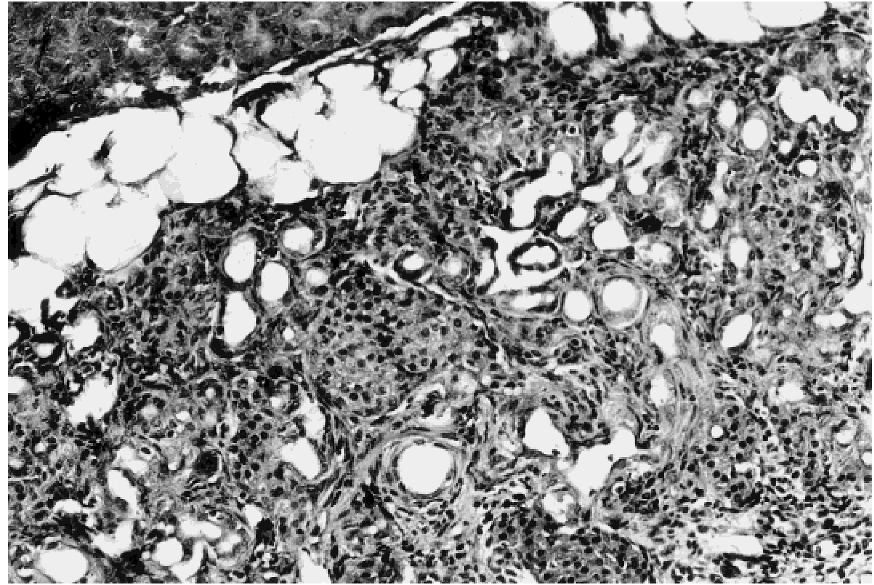

Fig. 4. Allograft pancreas 10 days after transplantation and immunesuppression with MMF and CSA showing intact islets (H & E, ×100).

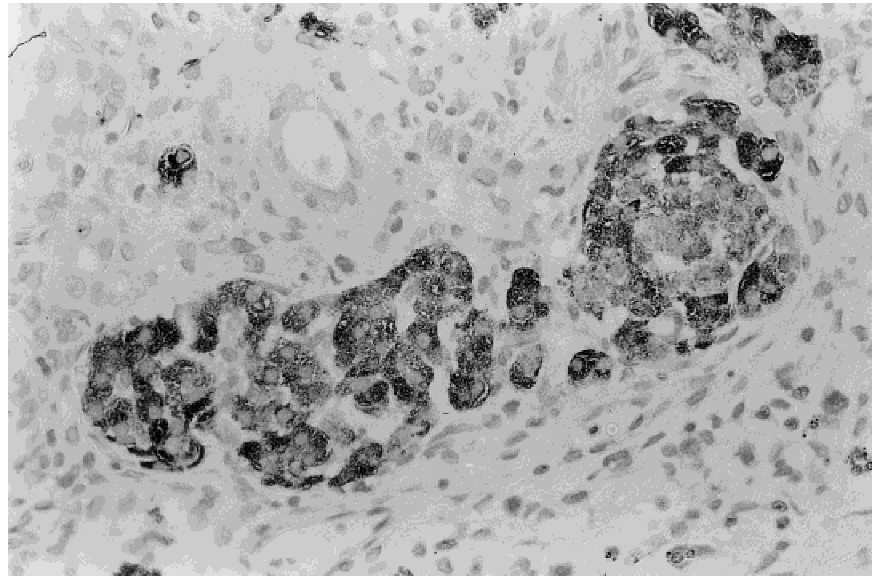

Fig. 5. Allografted pancreas harvested 30 days after engraftment and immunesuppression with MMF and CSA. Anti-insulin stain (for B-cells) shows uniform beta-cell granulation within islets (anti-insulin, ×200).

within grafts after transplantation, remains unexplained although it is a constant finding. It would be due to inadvertant transplantation of perigraft donor fat cells, excised at the time of graft harvesting, differentiation or proliferation of mesenchymal fat precursor cells, or induction of local lipogenesis by the secretion and diffusion of insulin in the vicinity of the graft or it could be a result of, or associated with, atrophy of the exocrine tissue.

The study unequivocally shows that transplantation of allogeneic pancreas tissue to non-immunosuppressed recipients results in 100% rejection at 6–10 days as confirmed by histology. All grafts were heavily infiltrated with MNC with graft infiltration scores of 4+ (massive intra- and perigraft infiltrate). Immunesuppression with low dose CSA (2 mg/kg/d) prolonged graft survival to 30 days in 70–80% grafts allowing maturation of the islets and the early deposition of fat accumulations at the grafted site by day 10. Graft infiltrate scores of 1–2+ were, however, still present. Unfortunately, the use of MMF alone in subtherapeutic doses, was accompanied by severe rejection after 10 days (100% of cases) and high graft infiltrate scores of 4+. MMF, an inhibitor of purine synthesis and lymphocyte proliferation, was again tested at subtherapeutic doses, but in combination with CSA (2 mg/kg/d), in the same strong responder foetal rat allograft pancreatic transplantation model. This decision was based on the current literature, which illustrates the potency of MMF monotherapy in combination with CSA-based immunesuppression in the prevention of acute rejection of organ allografts in other animal transplantation models (Beckstein et al., 1993; Morris et al., 1991; Platz et al., 1991; Qi et al., 1996; Wennberg et al., 1996). This

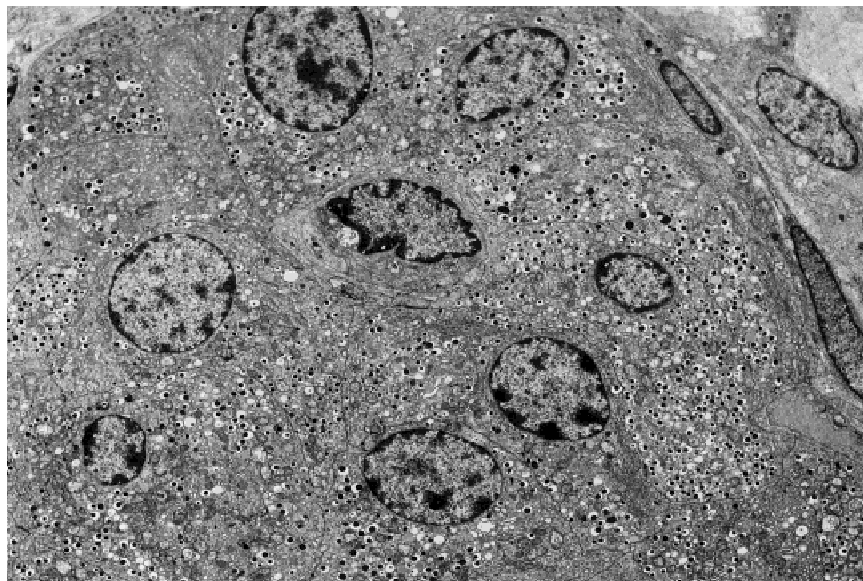

Fig. 6. Electron micrograph of pancreatic allograft immunesuppressed with MMF and CSA showing a normal distribution of granulation within B-cells. The section shows predominantly B-cells (×3,150).

is one of the few experimental studies conducted, in which MMF together with CSA has been tested to prevent rejection of foetal rat pancreatic allografts, the ultimate object being optimal suppression of rejection thus allowing the islets to mature and develop and, theoretically, thereby enabling normoglycaemia to be restored in a diabetic laboratory rat model. Encouraging preliminary results have been observed in this study and the combination of MMF and CSA has provided excellent preservation and staining properties of islets in the short term in the allogeneic non-diabetic rat model. These results have been paralled by noticeable changes in the peripheral blood T-lymphocyte counts as monitored by FACSCAN. Flow cytometric monitoring of the peripheral blood T-lymphocytes showed that while administration of MMF or CSA, on their own, had a modest effect on the percentage $CD4^+$ peripheral blood lymphocytes, the use of the drugs in combination demonstrated a marked propensity to decrease $CD4^+$ lymphocyte percentage. We have been impressed by the preservation of islet architecture, staining properties, and EM configuration following MMF and CSA treatment and are convinced that reversal of hyperglycaemia in diabetic models in future studies will be possible provided rejection is vigorously kept in check. Our enthusiasm stems from the potent immunesuppressive effects provided by CSA and MMF at 30 days (i.e., 3 times greater prolongation of graft survival compared to untreated controls), which is characterized by the preservation of normal appearing islets and islet counts in excess of 8–10 per high-field. Promising results have also been reported by Qi et al. (1996) who studied the effect of MMF and CSA in a pancreaticoduodenal rat model. Data from their laboratory show the importance of combination immunesuppression, if optimal results are to be obtained. This immunosuppressive strategy may prove very important in the future as various groups are exploring the application of pig-to-human islet xenotransplantation as a potential treatment for patients with diabetes (Wennberg et al., 1996).

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