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ARTICLE

Improved vascular engraftment and function of autotransplanted pancreatic islets as a result of partial pancreatectomy in the mouse and rat

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Abstract
Aims/hypothesis The few patients subjected to autotransplantation of pancreatic islets after pancreatectomy usually become normoglycaemic after using islets from the resected organ only, whereas allogeneic recipients usually require at least two grafts to retain normoglycaemia. Previous experimental studies have demonstrated that islets transplanted to non-pancreatectomised recipients acquire a markedly decreased blood vessel density, which leads to a hypoxic microenvironment. The aim of the present study was to test the hypothesis that autotransplanted islets have better vascular engraftment and function as a result of the pancreatic surgery involved.
Materials and methods In the present study, athymic mice and inbred rats were subjected to a 60% pancreatectomy and transplanted with human or rat islets, respectively, 4 days later. Control animals underwent sham surgery. Blood flow, oxygen tension, vascular density and endocrine volume in the islet grafts were measured 1 month after transplantation. Separate grafts were used for perfusion experiments and for assessment of beta cell proliferation and endocrine cellular apoptosis at different time periods after transplantation.
Results Islet grafts in partially pancreatectomised recipients had an increased blood flow, oxygen tension, blood vessel density and endocrine mass 1 month post-transplantation compared with control animals. They also exhibited increased insulin release in perfusion experiments performed 1 month post-transplantation, and decreased cellular apoptosis early after transplantation.
Conclusions/interpretation The present study shows that the pancreatectomy procedure itself has beneficial effects on the engraftment of transplanted human and rat islets. Our results provide an additional explanation, besides diminished immunological responses, of the much better outcome of islet autotransplantations compared with allogeneic transplantations in the clinic.

Keywords Blood flow · Insulin release · Islet graft · Islet transplantation · Oxygen tension · Revascularisation

Abbreviations

BS-1	*Bandeiraea simplicifolia*
GLP-1	glucagon-like peptide-1
REG	regenerating gene protein
TBS	Tris-buffered saline
TUNEL	terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling
UE	*Ulex europaeus*
VEGF	vascular endothelial growth factor

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Introduction

Pancreatic islet allogeneic transplantation is a tempting strategy for the treatment of patients with type 1 diabetes. Using the 'Edmonton protocol' [1], a 1-year insulin inde-

pendence rate of 80% [2], i.e. comparable to that for whole pancreas transplantation [3], can be obtained. However, whereas one whole pancreas is sufficient to reverse hyperglycaemia [3, 4], two or more pancreases are generally needed to achieve this when isolated islets are transplanted. Moreover, there seems to be a steady decline in the function of islet grafts, with very few patients remaining insulin-independent beyond 4 years after transplantation [5]. Since the histocompatibility barrier, the underlying autoimmune disease, and the immunosuppressive agents used are the same for both transplantation procedures, it is likely that differences in engraftment between whole pancreas and islets can at least partly account for the divergence in the results.

When islets are isolated for transplantation they become disconnected from their vascular supply. Revascularisation of transplanted islets has been shown to occur within 1–2 weeks [6, 7]. However, the vascular density of the graft remains lower than that of native islets [8, 9], and is associated with impaired oxygenation [9, 10] and function [11–13] of the islet tissue.

Clinically, autotransplantation of islets is performed in a few situations e.g. necessity of pancreatectomy for severe chronic pancreatitis or benign pancreatic tumours [14, 15]. The results after implantation of autologous islets have been strikingly better than those for allogeneically transplanted islets [14, 15]. In fact, only a third of the number of islets seems to be needed to attain a similar degree of insulin independence in the former case [14, 15]. Long-term graft survival is also markedly better for autotransplanted islets [16].

The marked difference in results between autologous and allogeneic islet transplantation has been interpreted to be due to immunological barriers and treatment with immunosuppressive drugs in the allogeneic setting. However, when performing autologous transplantation, nearly the whole pancreas is removed to isolate the islets [14]. In experimental studies, such pancreatic surgery has been shown to be associated with the regeneration of both the exocrine and the endocrine pancreas, because of the induction of a vast number of growth and survival factors, e.g. vascular endothelial growth factor (VEGF), regenerating gene protein (REG) and glucagon-like peptide-1 (GLP-1) [17–21]. Several of the growth factors shown to be induced following pancreatic resection also affect angiogenesis [22–24]. The present study therefore aimed to investigate the hypothesis that autotransplanted rat islets and human islets transplanted into partially pancreatectomised recipients become better engrafted than corresponding control islets.

Materials and methods

Animals Inbred male Wistar–Furth rats and C57BL/6 (*nu*/*nu*) mice, 10–15 weeks of age, were used in this study. The animals were purchased from Scanbur (Sollentuna, Sweden) and M&B (Ry, Denmark), respectively. All experimental procedures were approved by the animal ethics committee of Uppsala University and followed the 'Principles of laboratory animal care' (NIH publication no. 85–23, revised 1985).

Partial pancreatectomy Wistar–Furth rats were anaesthetised with sodium pentobarbital (Apoteket, Göteborg, Sweden; 60 mg/kg i.p), whereas C57BL/6 (*nu*/*nu*) mice were anaesthetised with avertin (0.02 ml/g i.p. of a 2.5% [vol/vol] solution of 10 g 97% [vol/vol] 2,2,2-tribromoethanol [Sigma-Aldrich, St Louis, MO, USA] in 10 ml 2-methyl-2-butanol [Kemila, Stockholm, Sweden]). The animals were placed on a heated operating table. All parts of the pancreas not attached to the stomach or duodenum, hereafter referred to as the splenic part of the gland, were removed [25]. The splenic part of the pancreas constitutes approximately 60% of the whole gland [25]. Different animals underwent sham surgery, that is, the splenic portion was mobilised, but otherwise not treated.

Islet isolation, culture and transplantation Pancreatic islets were isolated by collagenase digestion [26] from the extirpated part of the pancreas or from the whole pancreas of control Wistar–Furth rats. In some additional control animals, islets from the splenic and duodenal parts of the pancreas were isolated separately. The rat islets were cultured in RPMI 1640 (Sigma-Aldrich) supplemented with 11 mmol/l D-glucose, 2 mmol/l L-glutamine (Sigma-Aldrich) and 10% (vol/vol) fetal calf serum (Sigma-Aldrich) in 95% air/5% CO_2 at 37°C [26] for 4 days. The culture medium was changed every 48 h.

Human islets from nine heart-beating donors (age 51± 4 years; four men, five women; BMI range 18.4–27.8 kg/m^2; HbA_{1c} international standard range 5.0–5.7%) were isolated at the Human Islet Isolation Core Facility for the Nordic Countries (Uppsala, Sweden), and were kindly provided by O. Korsgren. Insulin release from the preparations was investigated by islet perifusion prior to delivery, and the mean glucose stimulation index was found to be 11.1±1.6 (range 6.7–15.6, n=9). The human islets were kept in culture for 4–7 days in RPMI 1640 medium (Sigma-Aldrich) supplemented with 5.6 mmol/l D-glucose, 2 mmol/l L-glutamine (Sigma-Aldrich) and 10% fetal calf serum prior to transplantation [27]. All experiments involving human islets were approved by the human ethics committee for Uppsala University.

At transplantation, groups of 250 Wistar–Furth islets, or 200 human islets, were packed into a braking pipette and implanted beneath the left renal capsule of syngeneic pentobarbital-anaesthetised (see above) Wistar–Furth rats or avertin-anaesthetised (see above) C57BL/6 (*nu*/*nu*) mice, respectively. In five cases, two mice received islets from the

same human donor, but were then allocated to different groups. Some of the islet recipients had been partially pancreatectomised or sham operated 4 days before transplantation. The different experimental groups are summarised in Table 1.

Oxygen tension and blood flow measurements The Wistar–Furth rats were anaesthetised with thiobutabarbital (Research Biochemicals, Natick, MA, USA; 120 mg/kg body weight i.p.), whereas the C57BL/6 (*nu*/*nu*) mice were anaesthetised with avertin (see above). The animals were then prepared for the islet oxygen tension and blood flow measurements [9, 28].

Oxygen tension was measured in the islets using modified Clark-type microelectrodes (Unisense, Aarhus, Denmark), as previously described [28]. At least five measurements were performed in all islets.

Islet graft blood perfusion was measured by laser-Doppler flowmetry (PF 4001-2; Perimed, Stockholm, Sweden) using a needle probe (411; Perimed) [28]. At least three blood flow measurements were performed in the transplanted islets in each animal. The blood perfusion of native islets cannot be determined by the laser-Doppler technique because they occur singly and not in clusters.

During the oxygen tension and blood flow measurements, blood pressure, body temperature and tissue temperature were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, UK).

Measurements of blood parameters Blood glucose concentrations were determined using test reagent strips (Medisense; Baxter Travenol, Deerfield, IL, USA). Once the blood flow and oxygen tension measurements had been completed, a blood sample was collected for analysis of packed cell volume and blood gases. Animals were excluded if one or more of the following pre-set exclusion criteria were met: packed cell volume <40, pH <7.30, pO_2 <10 kPa (75 mmHg) and pCO_2 >6.8 kPa (51 mmHg).

Morphological studies The graft-bearing kidneys or the pancreases from control animals were removed after the oxygen tension and blood flow measurements, fixed in 10% formaldehyde, and then embedded in paraffin. Samples of apparently normal human pancreas, obtained following pancreatectomies, were treated in the same manner. Consecutive 5-μm sections were stained for the lectins *Bandeiraea simplicifolia* (BS-1; both human and rat islets) or *Ulex europaeus* (UE; human islets only) and counterstained with haematoxylin [9]. We have previously shown that BS-1 produces consistent staining of all blood vessels in rat, mouse and human islets, whereas UE only stains blood vessels of human origin [9]. Moreover, we have previously observed that quantification of BS-1- and UE-positive blood vessels provides identical estimates of the vascular density of native human islets [9]. Hence, we were able to determine the fraction of blood vessels that originated from the donor by comparing the estimated

Table 1 Schematic overview of surgical procedures and parameters investigated

	Surgical procedures					Parameters investigated post-transplantation
	n	Day –4 Pancreatectomy	Day 0 Transplantation	Day 2	Day 5	Day 30
Rat islets						
AutoTx	8	Yes	Yes			Oxygen tension, blood flow, graft volume, blood vessel density
PxTx	33	Yes	Yes	Ki67, TUNEL, plasma VEGF	Ki67, TUNEL, graft volume	Oxygen tension, blood flow, Ki67, TUNEL, graft volume, blood vessel density
ShamTx	35	Sham surgery	Yes	Ki67, TUNEL, plasma VEGF	Ki67, TUNEL, graft volume	Oxygen tension, blood flow, Ki67, TUNEL, graft volume, blood vessel density
Tx	13	No	Yes			Oxygen tension, blood flow, blood vessel density
Px	16	Yes	No			Non-transplanted control
Non-operated	8	No	No			Blood vessel density
Human islets						
PxTx	8	Yes	Yes			Oxygen tension, blood flow, blood vessel density
ShamTx	8	Sham surgery	Yes			Oxygen tension, blood flow, blood vessel density

AutoTx pancreatectomy followed by autotransplantation of retrieved islets, *Px* only pancreatectomised animals, *PxTx* syngeneic transplantation with previous pancreatectomy, *ShamTx* syngeneic transplantation with previous sham surgery, *Tx* syngeneic transplantation without pancreatectomy

blood vessel density using BS-1 and UE. The blood vessel density in pancreatic islets and the surrounding stroma was determined using a direct point-counting method on histological sections at ×400 magnification [29]. A total of five sections, or 620±25 intersection points, were counted in each transplant or pancreas. Sections were also investigated for infiltration of inflammatory cells in the islets. The total graft volumes and the proportions represented by endocrine cells were estimated using a computerised system for morphometry [30], using updated software (Scion Image; Scion, Frederick, MD, USA).

Ki67 and insulin staining In separate experiments, graft-bearing rat kidneys were removed at 2, 5 or 30 days post-transplantation, prepared for histology, and then stained for the cell proliferation marker Ki67 according to the manufacturer's instructions (Lab Vision, Fremont, CA, USA). Briefly, antigen retrieval was performed by boiling for 20 min with citrate buffer (10 mmol/l, pH 6.0) in a microwave oven (750 W). The sections were washed in Tris-buffered saline (TBS) and thereafter incubated for 1 h with normal swine serum (Dako, Glostrup, Denmark) diluted 1:20 with TBS containing 0.1% (wt/vol) BSA. Primary monoclonal rabbit antibodies directed against Ki67 (1:200 dilution; clone SP6; Lab Vision) diluted in TBS were applied to the slides overnight at 4°C. The slides were washed (in TBS; 3×5 min) and incubated for 1 h with a swine anti-rabbit antibody (Dako) diluted 1:100 in TBS, and finally developed with 3,3′-diaminobenzidine-tetrahydrochloride (Sigma-Aldrich). The sections were thereafter stained for insulin [29] and visualised with Vectastain ABC (Vector Laboratories, Burlingame, CA, USA).

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling staining Separate slides of sections from rats at 2, 5 or 30 days post-transplantation were prepared for terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) staining, as previously described [31], for detection of apoptotic cells. Briefly, sections were incubated for 15 min with proteinase K (20 μg/ml; Sigma-Aldrich), followed by a 5-min exposure to 2% hydrogen peroxide. The sections were then incubated with terminal deoxynucleotidyl transferase (10 enzyme units/50 μl; Boehringer-Mannheim, Mannheim, Germany) and biotinylated deoxyuridine (0.5 nmol/50 μl; Boehringer-Mannheim) in transferase buffer (30 mmol/l Tris, pH 7.2, 140 mmol/l sodium cacodylate and 1 mmol/l cobalt chloride) for 60 min at 37°C. After washing and blocking with 2% BSA, the sections were incubated with Vectastain ABC (Vector Laboratories) for 30 min at room temperature and developed in 3-amino-9-ethylcarbazole solution.

Islet graft perfusion Grafts from some of the Wistar–Furth rats that received transplants were investigated 1 month after transplantation for glucose- and arginine-stimulated insulin secretion [32]. The insulin concentrations of the effluent samples were measured by ELISA (Mercodia, Uppsala, Sweden) and multiplied by the flow rate, giving values of insulin expressed as pmol/min. The AUC was then determined from these values.

Plasma VEGF measurement Blood samples were taken from partially pancreatectomised and sham-operated rats investigated at day 2 post-transplantation. The blood samples were kept on ice, and the protease inhibitor Trasylol (10,000 KIE/ml; Bayer Healthcare, Leverkusen, Germany) was added. Plasma was stored at −70°C. The VEGF-A contents in the plasma were determined using a rat VEGF ELISA (R&D Systems, Minneapolis, MN, USA).

Statistical analysis All values are given as means±SEM. Multiple comparisons of normally distributed data with control values were performed using ANOVA and Dunnett's post-hoc test, whereas non-parametric values were compared using non-parametric ANOVA and Dunn's post-hoc test. For pairwise comparisons of normally distributed data, the Student's *t* test was used. For all comparisons, a p value of <0.05 was considered to be statistically significant.

Results

Animal data All animals allocated to the study were normoglycaemic (5.6±0.1 mmol/l for the rats, $n=120$; 6.9±0.2 mmol/l for the mice, $n=12$). The Wistar–Furth rats weighed approximately 300 g and the C57BL/6 (*nu*/*nu*) mice weighed 25–30 g. The resected part of the pancreas weighed 477±11 mg and 127±4 mg in the rats ($n=43$) and mice ($n=6$), respectively. All animals subjected to partial pancreatectomy or sham surgery remained normoglycaemic on the day of islet transplantation (5.5±0.1 [$n=31$] vs 5.7±0.1 mmol/l [$n=34$] for the two groups of rats, respectively and 6.9±0.3 [$n=6$] vs 7.0±0.3 mmol/l [$n=6$] for the two groups of mice, respectively), as well as at days 2, 5 and 30 after transplantation (data not shown). The body weight increase was similar in animals subjected to a partial pancreatectomy or sham operation, as evaluated 1 month post-transplantation (18.4±2.6% [$n=9$] vs 15.8±2.7% [$n=8$] for the rats, and 12.5±2.5% [$n=6$] vs 9.0±3.2% [$n=6$] for the mice). At the time of the oxygen tension and blood flow measurements, mean arterial blood pressure was approximately 110 mmHg in the rats and 90–100 mmHg in the mice, and did not differ between the

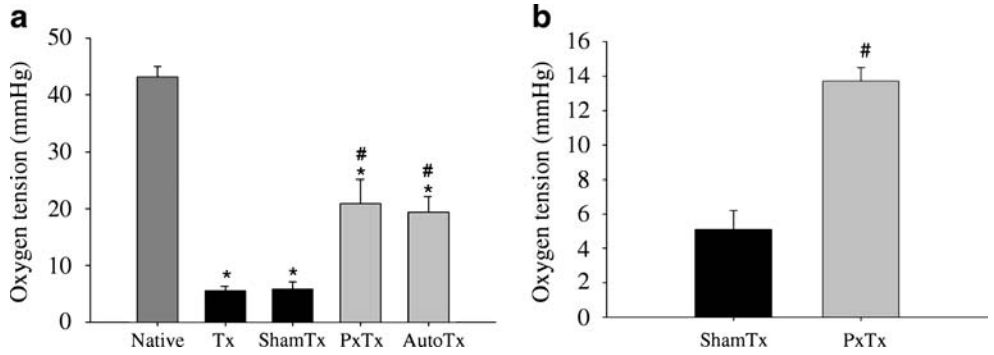

Fig. 1 Oxygen tension in native and transplanted rat islets (**a**) and human islets transplanted into *nu*/*nu* mice (**b**) as measured by Clark microelectrodes at day 30 post-transplantation. Experimental groups: syngeneic transplantation (*Tx*), syngeneic transplantation with previous sham surgery (*ShamTx*), syngeneic transplantation with previous pancreatectomy (*PxTx*), pancreatectomy followed by autotransplantation of retrieved islets (*AutoTx*) $p<0.05$ (*) vs native islets; $p<0.05$ (#) vs ShamTx

experimental groups. There were no signs of inflammatory reactions in the pancreas of any of the investigated partially pancreatectomised or sham-operated animals, as judged from the haematoxylin-stained histological sections, and no animals were excluded from the study based on the pre-set exclusion criteria regarding blood gases and packed cell volumes (pO_2 range 10.2–12.1 kPa and 10.4–11.9 kPa, pCO_2 range 4.1–6.5 kPa and 4.5–6.7 kPa, packed cell volume ranges 44–52% and 47–53% in rats and mice, respectively).

Oxygen tension The oxygen tension was 40–45 mmHg (5.3–6.0 kPa) in native rat islets, but only 4–7 mmHg (0.5–0.9 kPa) in 1-month-old syngeneic control rat islet transplants (Fig. 1a). However, the oxygen tension in the islet transplants of autotransplanted or partially pancreatectomised recipients was three- to fourfold higher than that in the corresponding control animals. The oxygen tension in transplants composed of islets derived from the splenic portion of the donor pancreas did not differ from that in transplants composed of islets derived from the duodenal portion, and was similar to the oxygen tension in the control group (data not shown).

Human islets transplanted into sham-operated C57BL/6 (*nu*/*nu*) mice had an oxygen tension similar to that of transplanted rat islets (control transplanted group) when investigated 1-month post-transplantation (Fig. 1b). When implanted in partially pancreatectomised recipients, the oxygen tension of the transplanted human islets was markedly increased.

Blood flow The blood flow of 1-month-old rat islet grafts in autotransplanted and partially pancreatectomised recipients was increased by ~50% compared with that in syngeneic control islet grafts (Fig. 2a), whereas islets of sham-operated recipients did not differ from transplanted control rats in terms of blood perfusion. The blood perfusion of the islet grafts did not vary according to the location of isolation (the splenic or duodenal portion of the pancreas), and was similar to that observed in the control group (data not shown). The blood perfusion of grafts composed of human islets implanted in partially pancreatectomised

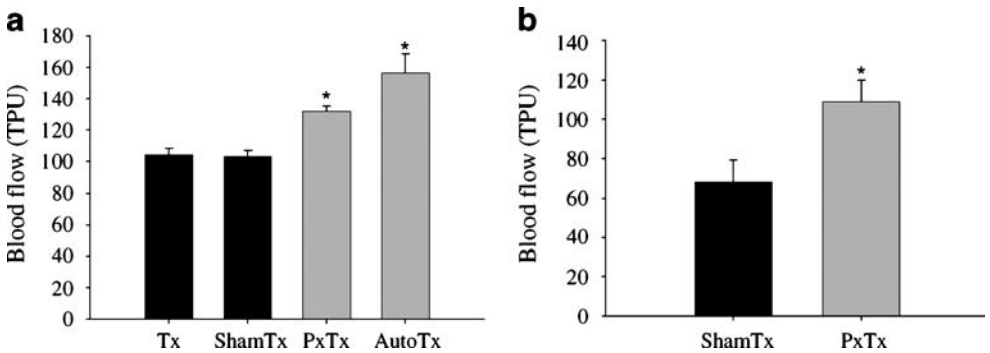

Fig. 2 Blood flow in transplanted rat islets (**a**) and human islets transplanted into *nu*/*nu* mice (**b**) as measured by laser-Doppler flowmetry day 30 post-transplantation. Experimental groups: syngeneic transplantation (*Tx*), syngeneic transplantation with previous sham surgery (*ShamTx*), syngeneic transplantation with previous pancreatectomy (*PxTx*), pancreatectomy followed by autotransplantation of retrieved islets (*AutoTx*). $p<0.05$ (*) vs ShamTx. Flow values are presented as tissue perfusion units (*TPU*)

recipients was also increased when compared with corresponding islets implanted in sham-operated recipients (Fig. 2b).

Blood vessel density Blood vessels represented approximately 12% of rat native islet volume, but only 5–6% in 1-month-old syngeneic control rat islet transplants (Fig. 3). The graft vascular density in autotransplanted and partially pancreatectomised recipients was, however, restored 1 month post-transplantation, and was thus markedly higher than in the control grafts. Sham-operated rats receiving syngeneic islets had values similar to those of transplanted control animals. The vascular density of the islet grafts did not vary according to the location of isolation (the splenic or duodenal portion of the pancreas), and was similar to that observed in the control group (data not shown). The vascular density in the graft connective tissue stroma surrounding each implanted islet was similar in all experimental groups (data not shown).

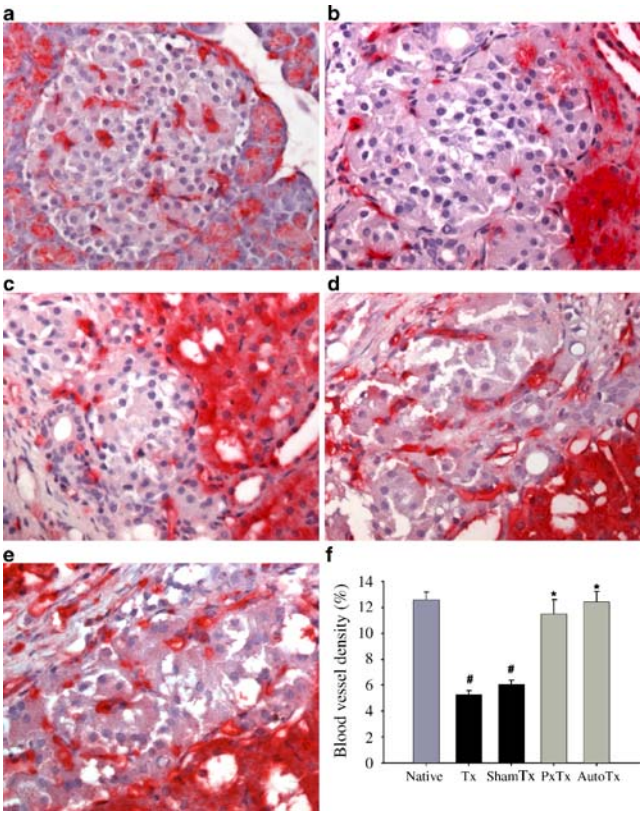

Fig. 3 Blood vessel density in native and transplanted rat islets. The transplanted islets were investigated at day 30 post-transplantation. Representative micrographs for native islets (**a**), syngeneic transplantation (*Tx*; **b**), syngeneic transplantation with previous sham surgery (*ShamTx*; **c**), syngeneic transplantation with previous pancreatectomy (*PxTx*; **d**), pancreatectomy followed by autotransplantation of retrieved islets (*AutoTx*; **e**). **f** Estimated vascular density in the different groups. $p<0.05$ (*) vs ShamTx; $p<0.05$ (#) when vs native islets

The proportion of islet volume taken up by blood vessels in native human islets was approximately 8% when evaluated as BS-1- or UE-positive structures (Fig. 4). In contrast, blood vessels only constituted 4–5% of the islets in the human islet transplants given to sham-operated recipients. The vascular density of human islets implanted into partially pancreatectomised recipients was higher than that in the control islet transplants and was similar to that in native islets. The higher blood vessel density in transplants of partially pancreatectomised recipients seemed to be the result of a combination of increased ingrowth of recipient blood vessels and an expansion of donor endothelial cells, since both the number of BS-1-positive/UE-negative structures (indicative of recipient blood vessels) and BS-1-positive/UE-positive structures (indicative of donor blood vessels) increased, although only the latter increase reached statistical significance.

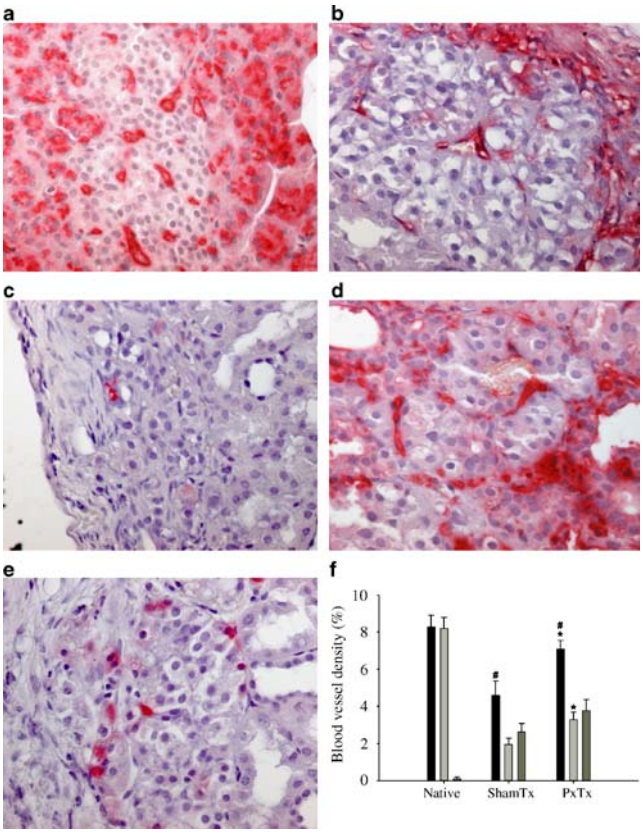

Fig. 4 Blood vessel density in native and transplanted human islets at day 30 post-transplantation. The endothelial cells were stained with BS-1, to visualise all endothelial cells, or UE, for identification of human (donor) blood vessels. Representative micrographs of native islets (**a**), syngeneic transplantation with previous sham surgery (*ShamTx*; **b**, **c**) and syngeneic transplantation with previous pancreatectomy (*PxTx*; **d**, **e**). Blood vessels (*red*) in parts **a**, **b** and **d** were stained with BS-1, and those in parts **c** and **e** were stained with UE. **f** Graph to show the estimated vascular density in the different groups. *Black bars* BS-1-positive vessels; *light-grey bars* UE-positive vessels; *dark-grey bars* UE-negative vessels. $p<0.05$ (*) vs ShamTx; $p<0.05$ (#) vs native islets

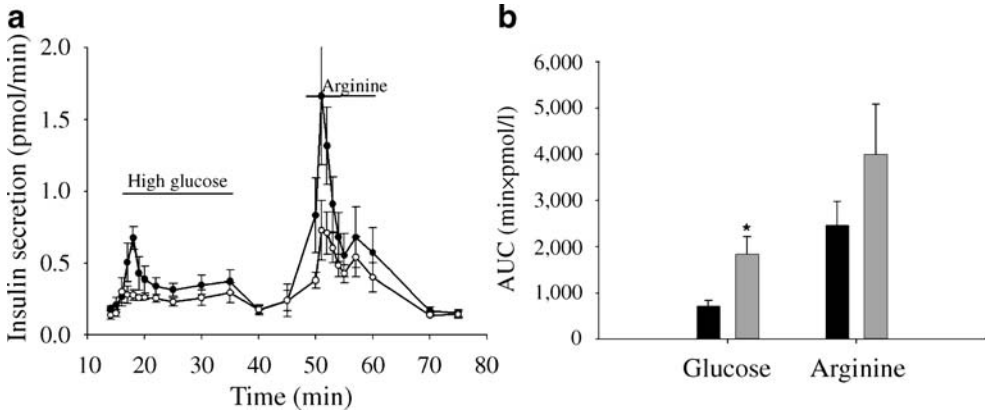

Fig. 5 **a** Insulin secretion in response to high glucose and arginine during perfusions of graft-bearing rat kidneys. **b** AUC for insulin release after stimulation with high glucose or arginine. The transplanted rat islets were investigated at 30 days post-transplantation. Experimental groups: syngeneic transplantation with previous sham surgery (*open diamonds*; *black bars*) and syngeneic transplantation with previous pancreatectomy (*closed diamonds*; *grey bars*). $p<0.05$ (*) vs syngeneic transplantation with previous sham surgery

Graft perfusion A distinct first- and second-phase insulin release was observed in response to high glucose stimulation in 1-month-old rat islet grafts of sham-operated control recipients (Fig. 5a). These grafts also responded to arginine. However, corresponding rat islet grafts implanted in partially pancreatectomised recipients released comparatively more insulin in response to either of these stimuli (Fig. 5b). First-phase insulin release during high glucose stimulation was markedly improved, with peak values more than twice as high as those of control grafts (129±17 vs 275±50 pmol insulin/l, $p=0.016$, for grafts of sham-operated and partially pancreatectomised recipients, respectively).

Beta cell proliferation, apoptosis and islet graft volume Both at day 5 and day 30 after transplantation, partially pancreatectomised recipients of syngeneic rat islets had a higher graft endocrine volume than corresponding sham-operated control animals (Fig. 6a). The endocrine volume of 1-month-old autotransplanted grafts in rats was similar to that in partially pancreatectomised animals, whereas control recipients receiving syngeneic rat islets had an endocrine volume similar to that seen in sham-operated animals (365±45 and 248±15 nl for autotransplanted and transplanted control animals, respectively).

Beta cell (Fig. 6b,c) and total endocrine cell proliferation (data not shown) were increased in 2-day-old islet grafts of sham-operated rats as compared with both islet grafts of partially pancreatectomised recipients and native islets. However, endocrine cell apoptosis was also markedly increased in 2-day-old grafts of sham-operated control rats when compared with both corresponding grafts of partially pancreatectomised recipients and native islets (Fig. 6d,e). At days 5 and 30 after transplantation, proliferation and apoptosis rates were similar in both groups, and did not differ from native islets.

Plasma VEGF Plasma VEGF was increased in pancreatectomised rats at day 2 after transplantation when compared with sham-operated animals (131±12 vs 95±11 pg/ml, $p<0.05$).

Discussion

This study tested the hypothesis that removal of parts of the pancreas per se would affect the revascularisation and function of islet grafts. Because we wanted to avoid the confounding effects of immunosuppressive drugs or immune reactions on islet revascularisation [7], beta cell survival and proliferation rates [33], we chose to use syngeneic or immunocompromised recipients as control groups. We also chose to use normoglycaemic recipients in the study, and therefore performed only a 60% pancreatectomy instead of a near-total pancreatectomy, as performed in clinical cases [14] and some experimental models [34]. The rationale for this was that we deemed it difficult to reproducibly design a control group with chemically induced diabetes in which blood glucose concentrations and remaining beta cell mass in control and study groups would be the same. Variable blood glucose concentrations between groups may not have influenced the vascular density of the islet transplants [35], but such differences would most probably have affected both islet survival and function [36–38]. We chose the renal subcapsular site to enable us to perform the in vivo studies. However, considering that islets implanted intraportally in the liver are located downstream of the pancreas, any factors released from the gland would be expected to reach such transplanted islets at even higher concentrations, at least prior to islet revascularisation [39]. We therefore deem the results obtained in the present study as pertinent to intraportally transplanted islets.

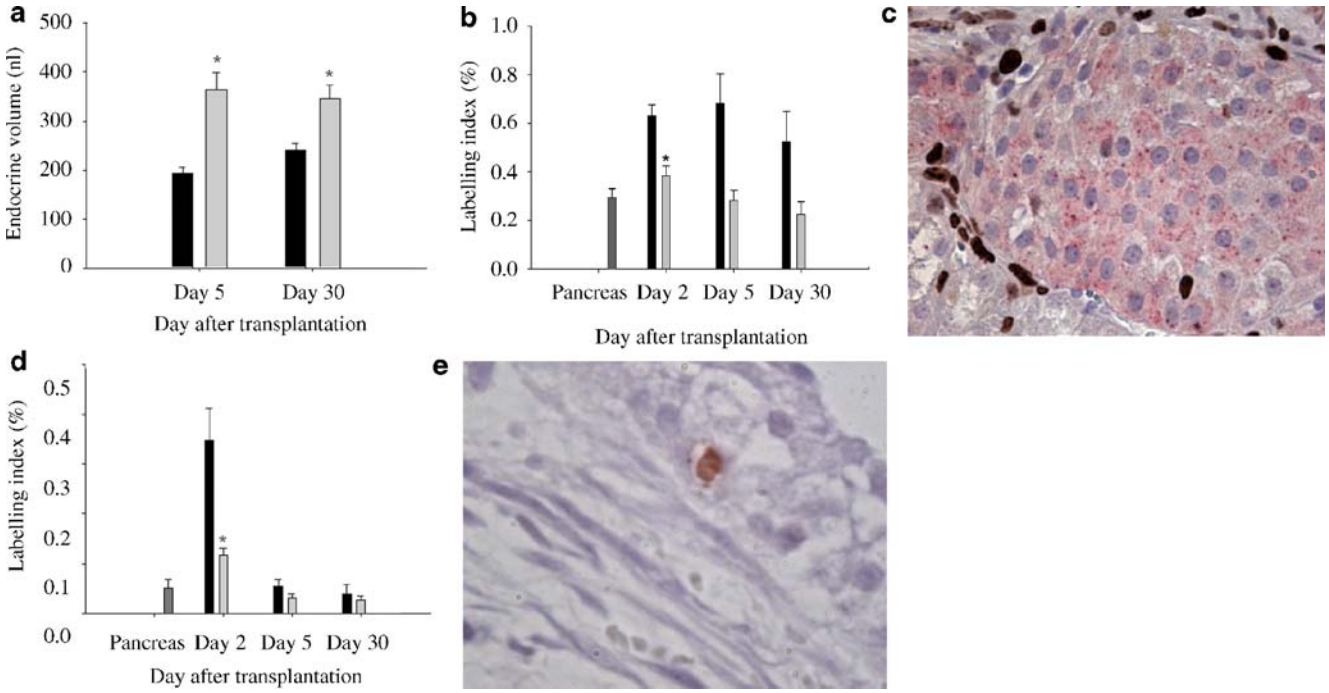

Fig. 6 Endocrine volume (expressed as nanolitre, nl) of rat islet grafts day 5 and 30 post-transplantation (**a**), beta cell labelling index in native rat islets and in rat islet grafts at days 2, 5 and 30 post-transplantation using the cell proliferation marker Ki67 (**b**), micrograph of a 2-day-old rat islet transplant stained for insulin (*red*) and Ki67 (*brown*) (**c**), endocrine cellular apoptosis rates in native rat islets and in rat islet grafts at days 2, 5 and 30 post-transplantation, estimated by TUNEL staining (**d**), micrograph of a 2-day-old rat islet transplant stained with TUNEL (*brown*) (**e**). Experimental groups: syngeneic transplantation with previous sham surgery (*black bars*) and syngeneic transplantation with previous pancreatectomy (PxTx; *grey bars*). $p<0.05$ (*) vs syngeneic transplantation with previous sham surgery

Pancreatic islets normally have a dense capillary network, which is of importance for the provision of oxygen and nutrients, to allow accurate glucose sensing, and for the release of hormones into the systemic circulation. Recent evidence also indicates an important role for islet endothelial cell products in sustaining beta cell function [40] and growth [29, 40]. Following transplantation, islets require rapid and adequate revascularisation for their survival and optimal function. Irrespective of whether implanted into the kidney, spleen or liver of normo- or hyperglycaemic recipients, experimentally transplanted rodent and human islets seem to become insufficiently revascularised [8, 9, 35]. Instead, a rich vascular network is formed in the graft stroma surrounding individual transplanted islets [8]. In the present study, the vascular density within the transplanted islets was selectively increased in partially pancreatectomised recipients. Moreover, the systemic concentration of VEGF was increased 2 days after pancreatectomy. VEGF is an important stimulator of islet angiogenesis [41, 42]; it inhibits beta cell apoptosis and increases insulin secretion of transplanted islets [43]. VEGF has also been shown to mobilise endothelial progenitor cells [44], which increases revascularisation of transplanted islets [13].

An early study indicated that the vascular network of the islet graft is predominantly derived from recipient blood vessels [45]. However, in recent years it has become evident that the remaining donor endothelium may also contribute vessels, at least if freshly isolated islets are used for transplantation [46, 47]. In the present study, the use of not only BS-1 to stain endothelium, but also a lectin specific for human endothelium, UE, enabled us to determine the relative contribution of donor and recipient endothelium to the islet vascular system in the different experimental groups. We deduced that an expansion of the donor endothelial pool occurred in the partially pancreatectomised recipients, but that there also tended to be an increased ingrowth of recipient blood vessels in these recipients as compared with sham-operated recipients. The expansion of donor endothelial cells did, however, seem to be the predominant effect of the pancreatectomy.

The increased vascular density of autologously or syngeneically transplanted islets in partially pancreatectomised recipients was associated with increased graft blood perfusion. The laser-Doppler technique measures the blood flow in both stromal and endocrine compartments of the grafts. Since the majority of the blood vessels are situated in the graft stroma, the changes in blood perfusion were expected to be less prominent than the changes in islet blood vessel density.

The improved vascular density and blood perfusion of islets implanted in partially pancreatectomised recipients

were also reflected by the markedly improved oxygenation of such islets. However, in contrast to the vascular density, the oxygenation of the transplanted islets was not fully restored. This may reflect a less than optimal vascular organisation in transplanted islets relative to native islets. A vascular dysfunction with altered blood flow regulation has also been reported in transplanted islets [48].

Islet graft function was evaluated and compared by perfusion of graft-bearing kidneys. This enabled us to study islet transplant function isolated from the function of the native pancreas in these normoglycaemic recipients. Moreover, the technique allowed the first and second phases of glucose-stimulated insulin release to be evaluated separately. One-month-old grafts obtained from partially pancreatectomised recipients responded to stimulation with either high glucose or arginine with a much better insulin release than grafts obtained from sham-operated control recipients. This increase in total insulin output can most probably be explained by the higher endocrine mass in the former grafts. We did not detect higher rates of beta cell or total endocrine cell proliferation in grafts implanted in partially pancreatectomised recipients; instead, these values were paradoxically decreased as compared with those in sham-operated rats 2 days after transplantation. However, the endocrine cell apoptosis was markedly lower in the grafts of partially pancreatectomised recipients early after transplantation. Thus, the increased endocrine mass in grafts in partially pancreatectomised recipients seemed to depend on the release of survival signals, e.g. VEGF, GLP-1 or REG, from the surgically manipulated pancreas. A better level of revascularisation may contribute to increased islet cell survival, but this is unlikely to be the sole explanation at only 2 days post-transplantation.

The grafts of partially pancreatectomised recipients seemed to have a preferentially increased first phase of insulin release in response to high glucose or arginine as compared with control islet grafts. Interestingly, we recently observed that islets implanted into the kidney and the liver both have a decreased first phase of insulin release in response to glucose as compared with native islets [39]. This impaired insulin release in control islet grafts may reflect disturbances in the secretory machinery of beta cells [49, 50]. It should be noted that islet endothelial cells can directly stimulate beta cell insulin secretion through paracrine effects [40]. Additionally, an acute increase in graft oxygen tension, through stimulation of blood perfusion, of islet renal subcapsular grafts has been shown to improve the first phase of glucose-stimulated insulin release [48].

In conclusion, rat and human islets implanted in partially pancreatectomised recipients become much better revascularised and oxygenated than when transplanted into control or sham-operated recipients. The improved vascular engraftment was also correlated with an improved capacity for insulin release and a larger endocrine volume. Our results provide an additional explanation, besides diminished immunological responses, for the much better outcome of clinically performed islet autotransplantations in which the recipient is pancreatectomised prior to transplantation.

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