Low Revascularization of Experimentally Transplanted Human Pancreatic Islets

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Pancreatic islets are avascular immediately after transplantation. Although the islets are rapidly revascularized, it is uncertain whether the revascularization produces an adequate oxygenation of the transplanted islet tissue. We measured pO2, blood flow and vascular density in mouse or human islets 1 month after transplantation to nude mice. Tissue pO2 was measured with Clark microelectrodes. Blood perfusion was measured with laser-Doppler flow cytometry, whereas vascular density was determined in histological specimens stained for the lectin Bandeiraea simplicifolia (BS-1). Both the transplanted mouse and human islets had a pO2 15–20% of that in endogenous mouse islets. Moreover, the vascular density of the transplanted islets was decreased compared with that of endogenous mouse and human islets. Graft blood perfusion was approximately 5% of renal cortex blood flow. A negative correlation was found between donor age and blood perfusion of the human islet grafts. A similar correlation was seen between donor age and the total vascular density of these grafts. In conclusion, transplanted human islets had a markedly decreased vascular density and pO2 compared with endogenous islets. This has potential implications for clinical islet transplantations, because poor vascular engraftment may significantly increase the number of islets needed to obtain insulin independence. (J Clin Endocrinol Metab 87: 5418–5423, 2002)

THE RECENTLY INTRODUCED Edmonton Protocol suggests that insulin independence can be achieved in a majority of islet-transplanted type 1 diabetic patients by strictly adhering to certain criteria, e.g. a steroid-free immunosuppressive regimen (1, 2). However, a major obstacle in islet transplantation is the limited availability of human islet tissue. It is therefore of great importance to optimize engraftment of islets in the implantation organ to reduce the number of islets needed to cure a diabetic individual. Despite this, few studies have been concerned with whether an adequate revascularization of transplanted islets occurs. Normally, the pancreatic islets have a complex glomerular-like angioarchitecture, which ensures that no portion of the islet is more than one cell away from arterial blood (3). Furthermore, animal studies have demonstrated that pancreatic islets have a markedly higher blood supply than the exocrine pancreas and that it is similar to that seen in the renal cortex (4–7 ml/min/g) (4–7). This unique capillary network and high blood perfusion is necessary for a high delivery of oxygen and nutrients to the islet cells and for optimizing the dispersal of the secreted hormones to their target organs (8). However, when pancreatic islets are isolated and cultured before transplantation, the islet vasculature disrupts and degenerates (9). In the immediate post-transplantation period, the islets are therefore supplied with oxygen and nutrients solely by diffusion from blood vessels in the surrounding tissues (10). The transplanted islets are revascularized within 7–14 d (11, 12), but questions have been raised after animal studies of whether the newly formed blood vessels have the capacity to sufficiently meet the demands of the highly metabolically active islet cells (13–17). We have previously recorded markedly decreased oxygen tension and blood perfusion in syngeneically transplanted rat islets (15, 16, 18, 19). Whether this low oxygen tension and blood perfusion also apply to transplanted human islets is unknown. The aim of the present study was to measure tissue oxygen tension and vascular density in mouse and human islets transplanted to athymic nude mice. In addition, islet graft blood perfusion was measured to investigate whether there was a correlation between the vascular density and/or blood perfusion of the human islet grafts and donor age.

Materials and Methods

Animals

Male C57BL/6 and C57BL/6 (nu/nu) mice purchased from Bomholtgaard Research and Breeding Center A/S (Ry, Denmark) and weighing approximately 25 g were used. The animals had free access to water and pelleted food throughout the course of the study. All experiments were approved by the animal ethics committee for Uppsala University.

Islet isolation and culture

Human islets from seven heart-beating donors were isolated at the Central Unit of β-Cell Transplant (Brussels, Belgium) and transported by air to Uppsala. The average age (±SEM) of the organ donors was 40 ± 5 yr (range, 19–53). Islet isolation and characterization regarding cell viability by electron microscopy (86 ± 3%), islet cellular composition (57 ± 4% insulin-positive cells), and amount of exocrine tissue (<1%) were performed in Brussels as previously described (20). On arrival in Uppsala, the islets were kept in culture for 4–6 d in RPMI 1640 medium (Sigma-Aldrich, St Louis, MO) supplemented with 5.6 mmol/liter glucose, 10% (vol/vol) fetal calf serum, 0.17 mmol/liter benzylpenicillin, and 0.17 mmol/liter streptomycin. This composition of the medium has previously been found to be favorable for culturing human islets (20, 21).

Pancreatic islets from male C57BL/6 mice were prepared by collagenase digestion and cultured free-floating for 4–7 d in RPMI 1640 medium supplemented with 11 mmol/liter glucose, 10% (vol/vol) fetal calf serum, 0.17 mmol/liter sodium benzylpenicillin, and 0.17 mmol/liter streptomycin.

Abbreviations: BS-1, Bandeiraea simplicifolia.
mmol/liter streptomycin (22). The medium was changed every second day.

**Islet transplantation**

After culture, 200–250 islets from C57BL/6 mice or 0.6 μl human islets were packed in a braking pipette and implanted beneath the capsule of the left kidney in C57BL/6 (nu/nu) mice anesthetized with avertin (a 2.5% (vol/vol) solution of 10 g 97% (vol/vol) 2,2,2-tribromoethanol (Sigma) in 10 ml 2-methyl-2-butanol (Kemila AB, Stockholm, Sweden). Two mice received islets from the same human donor.

**Measurements of oxygen tension in endogenous and transplanted islets**

The animals were anesthetized with avertin (see above), placed on an operating table maintained at body temperature (37°C), and tracheostomized. Polyethylene catheters were inserted into the right carotid artery and left jugular vein. The former catheter was connected to a Statham P23dB pressure transducer (Statham Laboratories, Los Angeles, CA) to monitor mean arterial blood pressure, whereas the latter catheter was used for continuous infusion of Ringer solution (5 ml/kg/h) to substitute for loss of body fluid. A left subcostal flank incision was performed, and the graft-bearing left kidney was immobilized in a plastic cup. The kidney was embedded in cotton wool soaked in Ringer solution and covered with mineral oil (Apoteket, Gothenburg, Sweden) to prevent evaporation and keep the tissue moist and at body temperature. Oxygen tension was measured in the islet graft and the adjacent renal parenchyma with modified Clark microelectrodes (Unisense, Arhus, Denmark) (15, 23). The electrodes (outer tip diameter, 2–6 μm) were inserted into the tissues by the use of a micromanipulator under a stereo microscope. At least 10 measurements were performed in both the islet graft and the renal cortex. The mean of all measurements in each tissue and animal was calculated and considered to be one experiment. For oxygen tension measurements in endogenous pancreatic islets, the pancreas was exposed and immobilized, and its islets were visualized in a similar manner as previously described in rats (7, 15). Measurements of oxygen tension were performed in three or more superficial pancreatic islets and in the surrounding exocrine parenchyma of each animal. Multiple measurements were usually performed in the same islet; the mean was calculated to obtain the oxygen tension value for one islet. The mean of the islet oxygen tension values in one animal was then considered to be one experiment. During the oxygen tension measurements, blood pressure, body temperature, and tissue temperature were continuously recorded with a MacLab Instrument (AD Instruments, Hastings, UK) connected to a Macintosh Power-PC 6100 (Apple Computers, Cupertino, CA).

**Measurements of blood flow in transplanted islets**

The blood perfusion of the islet graft and the adjacent renal cortex was measured by laser-Doppler flow cytometry (PF 4001-2, Perimed, Stockholm, Sweden) with a needle probe (411 mm tip; outside diameter, 0.45 mm; Perimed). The flow probe was positioned perpendicular to the immobilized tissue surface by the use of a micromanipulator, and care was taken not to cause any compression of the tissue. At least three blood flow measurements were performed in the transplanted islets and renal cortex in each animal. The mean of these measurements from each animal was calculated and considered to be one experiment. Because it is difficult to calibrate the instrument in physical units of blood flow, all blood flow values are given as arbitrary tissue perfusion units. The blood perfusion of endogenous islets cannot be determined by laser-Doppler due to their small size.

**Measurements of blood and urine parameters**

Blood glucose concentrations were determined with test reagent strips (Medisense, Baxter Travenol, Deerfield, IL) from samples obtained from the cut tip of the tail. At the end of blood flow and oxygen tension measurements, a blood sample was collected for analysis of hematocrit and blood gases. Animals with pH less than 7.30, pO2 less than 10 kPa, pCO2 more than 6.8 kPa, or hematocrit less than 40 were excluded from the study.

**Light microscopic evaluation**

The graft-bearing left kidneys or pancreata from control animals were removed after the oxygen tension and blood flow measurements, fixed in 10% (vol/vol) formaldehyde, and embedded in paraffin. Samples of apparently normal human pancreas were obtained in association with pancreatectomies and treated in the same manner. The average age (±SEM) of these pancreas donors was 58 ± 9 yr (range, 26–74). Consecutive sections (5 μm thick) of the islet grafts and pancreata were prepared and stained for the lectins Bandeiraea (Griffonia) simplicifolia (BS-1) or Ulex europaeus, as described in detail previously (24). The slides were counterstained with hematoxylin. Positive control slides were comprised of paraffin-embedded rat lungs for BS-1 and paraffin-embedded human lungs for Ulex europaeus. Only BS-1 produced consistent staining of blood vessels in all mouse and human samples and was therefore chosen in the subsequent protocol. In each animal, 12 or more tissue sections stained with BS-1 from all parts of the pancreas or islet transplants were randomly chosen and evaluated. The numbers of stained blood vessels in endogenous and transplanted islets were quantified under a stereomicroscope (magnification, ×600). In the islet grafts connective tissue surrounded the individual islets in the grafts. The numbers of blood vessels in the endocrine and connective tissues were therefore counted separately. The fraction of endocrine and connective tissue was determined by a direct point-counting method (25, 26). For this purpose the number of intersections overlapping connective tissue stroma and endocrine cells within the islet grafts was counted at a magnification of ×600 in a light microscope. Approximately 12 fields (corresponding to ~1500 points) were counted in each islet graft. The area of the investigated endogenous and grafted islets was determined by using a computerized system for morphometry (MOP-Videoplan, Carl Zeiss, Stockholm, Sweden). Vascular density, i.e., the number of stained blood vessels found per measured islet or graft area (square millimeters), was then calculated.

**Statistical analysis**

All values are given as the mean ± SEM. Multiple comparisons between data were performed using ANOVA (StatView, Abacus Concepts, Inc., Berkeley, CA) with correction of P values using the Bonferroni method (27). Correlation analysis was obtained by simple linear regression. With regard to the two mice receiving islets from the same donor, the mean vascular density, pO2, and blood perfusion of these two grafts were calculated and thus represented only one observation in the following statistical evaluation. For all comparisons, P < 0.05 was considered statistically significant.

**Results**

**Body weights and blood glucose concentrations**

The investigated animals had a mean weight of 27.5 ± 0.4 g (n = 22). All studied mice were normoglycemic, and when performing oxygen tension and blood flow measurements, the blood glucose concentration was 6.1 ± 0.3 mmol/liter (n = 22). There were no differences in body weight and blood glucose concentrations between the study groups.

**Tissue oxygen tension**

In endogenous mouse pancreatic islets tissue pO2 was approximately 40 mm Hg, whereas in the neighboring exocrine pancreas a slightly lower tissue oxygen tension (~25 mm Hg) was recorded (Fig. 1). Both transplanted mouse and human islets had markedly decreased tissue oxygen tension compared with endogenous mouse islets (Fig. 1). The tissue pO2 of the superficial renal parenchyma adjacent to the grafts was about 15 mm Hg (Fig. 1).

**Arterial blood pressure and blood flow measurements**

All animals had a mean arterial blood pressure of approximately 85 mm Hg. Both the transplanted mouse and human
islets had a blood perfusion approximately 50% of renal cortex blood flow (Fig. 2). The human islets were obtained from donors ranging from 19–53 yr old. A strong negative correlation was found between donor age and blood perfusion of the grafted islets (Fig. 3).

Vascular density

Ulex europaeus stained microvascular endothelium in human lung and endogenous human islets, but failed to stain blood vessels in endogenous mouse islets and transplanted mouse and human islets. In contrast, BS-1 stained capillary endothelium in both endogenous and transplanted mouse and human islets (Fig. 4). No structures corresponding to unstained microvascular blood vessels could be identified in any of the investigated BS-1-stained tissue sections. Similar numbers of blood vessels were found in endogenous human islets stained for Ulex europaeus and BS-1 (1139 ± 97 and 1012 ± 60 capillaries/mm²; respectively; n = 5). Evaluation of BS-1-stained sections showed that the vascular density in endogenous mouse islets was higher than that in endogenous human islets, but that capillary numbers in the endocrine tissue of the mouse and human grafts were similar and in both cases were markedly lower than those in endogenous islets (Fig. 5). In the connective tissue stroma surrounding the mouse and human islets in the grafts, a markedly larger number of capillaries was found than in the endocrine parts of the grafts, and the mean vascular density of the grafts was therefore higher (Fig. 5). The connective tissue stroma constituted about 30% of the mouse and human islet grafts. When correlating the vascular density of the human islet grafts to donor age, a negative correlation was found for vascular density in the whole graft (Fig. 6), but not for the endocrine or stroma compartment of the grafts.

Discussion

The pancreatic β-cells normally have a very high demand on metabolic activity and oxygen consumption to meet the varying needs for insulin secretion (28, 29). To meet these demands, an adequate oxygenation of the islet tissue must occur. In previous studies we have found that the tissue oxygen tension in endogenous rat islets is about 40 mm Hg, whereas it is markedly lower in transplanted rat islets (15, 16,
In the present study, a markedly decreased oxygen tension was also found in both mouse and human islets 1 month after transplantation to nude mice. These findings therefore suggest that a low tissue oxygen tension of transplanted islets is not restricted to syngeneically transplanted rat islets, but is also seen in transplanted human islets.

The low oxygenation of the transplanted islet tissue suggests an insufficient revascularization. However, transplanted islets are generally considered to be rapidly revascularized within 7–14 d (11, 12, 30–32). Studies have aimed to quantitate islet graft blood vessels in rodents (11, 32, 33), but until recently (34) there were no quantitative studies comparing the vascular density in transplanted islets to that in endogenous islets. In the present study a lower density of capillaries was found in both mouse and human islets transplanted to the renal subcapsular space compared with endogenous islets. At least for mouse islets, no improved revascularization occurs if the islets instead are transplanted intrasplenically or intraportally into the liver (34). The investigation of vascularity in the mouse and human islet grafts revealed a vast number of capillaries in the connective tissue surrounding the individual islets in the grafts. We have had similar findings in mouse islets transplanted intraportally to the liver or injected into the spleen (34).

**FIG. 4.** Microvascular endothelium (red) stained for the lectin BS. A, Endogenous human pancreatic islets (scale bar, 20 μm). B, Human pancreatic islets transplanted into the renal subcapsular space (scale bar, 20 μm).

**FIG. 5.** Vascular density in endogenous mouse and human pancreatic islets and in mouse and human islets transplanted beneath the renal capsule of normoglycemic nude mice. Values are given both for the endocrine tissue per se and for whole islet graft. Measurements were performed 1 month after transplantation. All values are the mean ± SEM for six to eight animals. *, P < 0.05 compared with endogenous mouse islets; †, P < 0.05 compared with corresponding endogenous islets. All comparisons were made using ANOVA.

**FIG. 6.** Vascular density of the whole islet graft in relation to donor age. Measurements of vascular density in the human islet grafts were performed 1 month after transplantation beneath the renal capsule of normoglycemic nude mice. Analysis was made using simple linear regression (n = 7; r = 0.88; P < 0.05).
(11, 32, 33), because Menger et al. (11) and Heuser et al. (33) only evaluated single islets implanted into the skinfold chamber, whereas Merchant et al. (32) did not mention whether endocrine and connective tissue parts were evaluated separately.

We have previously observed consistent staining with the lectin BS-1 for microvascular blood vessels in endogenous and transplanted rodent islets (24). BS-1 stains vascular endothelium in several species, but rarely tissues of human origin (35). However, the present study demonstrates that staining with this lectin is applicable to endogenous and transplanted human islets. Interestingly, the lectin Ulex Europaeus, specific for human endothelium, stained endogenous, but not transplanted, human islets. This lends further support to the view that the newly formed capillary network in cultured transplanted islets is of recipient origin (36).

Laser-Doppler flow cytometry demonstrated an islet graft blood perfusion amounting to only about 50% of the renal cortex blood flow, i.e. approximately 50% of endogenous islet blood flow (18). There were no differences in blood perfusion between grafted human and mouse islets. In rats we have recorded similar low blood flow using the laser-Doppler technique (15, 16, 19, 37). In this context it should be noted that laser-Doppler flow cytometry measures whole blood perfusion, i.e. all moving blood cells, within the illuminated tissue. The present study and a recent report (34) reveal that the majority of intragraft capillaries are stroma capillaries and are therefore likely to substantially contribute to whole graft blood perfusion. However, due to limitations in oxygen diffusion distance (38), it could be expected that blood flow in capillaries in the endocrine parts mainly contributes to the delivery of oxygen to the endocrine cells. This latter blood flow must be expected to be substantially lower than the whole graft blood flow recorded in the present study. Indeed, in experiments using a combination of microspheres and an ultrasonic flow probe, a nutritional islet graft blood perfusion only about 10% of that seen in endogenous islets was observed (18). An islet blood flow similar to that of endogenous islets was recorded in islets autotransplanted beneath the renal capsule of partially pancreatectomized animals (39, 40). However, in these latter experiments the influence of partial pancreatectomy has been difficult to access.

Blood perfusion of the human islet grafts was observed to decrease with the age of the organ donors. Although some caution must be taken in view of the limited number of observations (n = 7), a very strong correlation seemed to exist. Interestingly, a similar negative correlation was obtained for donor age and total vascular density of these grafts. These combined findings suggest that islets from younger donors have a higher capacity to induce blood vessel formation in the islet grafts. The mechanisms for this remain to be determined, but may involve an attenuated production of angiogenic factors, e.g. vascular endothelial growth factor or basic fibroblast growth factor from islets with age. No correlation was observed between donor age and oxygen tension in the transplanted human islets. Tissue oxygen tension in a specific location is dependent on several parameters, i.e. the supply of oxygen to the site by blood perfusion, the diffusion capacity for oxygen, the oxygen extraction (dependent mostly on changes in blood pH according to the Bohr equation), and the cellular oxygen consumption. A simple correlation between tissue oxygen tension and blood perfusion is therefore seldom seen. Despite the decreased vascular density and tissue oxygen tension, the transplanted islets remained functionally active, as confirmed by immunohistochemistry for insulin. However, it is uncertain whether the attained islet function after transplantation is optimal. In clinical islet transplantation, a surprisingly large number of islets (>900,000 islet equivalents) are still needed to obtain insulin independence, even when applying the successful Edmonton Protocol (1, 2). Experiments in an insulinoma cell line, βTC3 cells, have shown that pO2 levels below 25 mm Hg gradually shift these cells from aerobic to more anaerobic metabolism with a concomitant increased lactate production (41, 42). Reduced insulin secretion from the βTC3 cells was observed at pO2, less than 7 mm Hg. Similarly, glucose-stimulated insulin secretion from single rat islet cell aggregates (5–10 cells) was affected by less than 12 mm Hg (38).

In conclusion, a markedly decreased vascular density with a concomitantly low tissue oxygen tension was found in transplanted human islets. Attempts to improve islet vascularization are clearly warranted, as this may significantly decrease the number of transplanted β-cells needed to obtain insulin independence.

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