Decreased Vascular Density in Mouse Pancreatic Islets After Transplantation

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An adequate revascularization is crucial for islet survival and function after transplantation. Previous studies have suggested that islet revascularization is concluded within 14 days after transplantation. We investigated if the vascular density of transplanted islets and endogenous pancreatic islets differs. Cultured islets were syngeneically transplanted into the kidney, liver, or spleen of C57BL/6 mice. One month later, the graft-bearing organ was removed, and histological specimens were prepared and stained for endothelium with the lectin *Bandeiraea simplicifolia*. Pancreata from nontransplanted control animals were prepared similarly. Uniform staining of endothelium within the grafts and endogenous islets was obtained. The vascular density was markedly decreased in transplanted islets at all implantation sites, but preferentially in islets implanted into the spleen. The vascular density in the connective tissue surrounding the transplanted islets was very high compared with that of graft intra-islet capillaries. A much lower vascular density was detected in connective tissue surrounding implanted microspheres of a size similar to the islets, which suggests that the islets per se induced blood vessel formation in their vicinity. We conclude that the vascular density in revascularized transplanted islets is markedly decreased compared with endogenous islets. This has potential implications for islet graft metabolism and function. *Diabetes* 51:1362–1366, 2002

The recent application of a new treatment regimen, the so-called Edmonton protocol, has markedly improved the outcome of clinical islet transplantation (1,2). When applying this protocol, however, transplantation of a large number of islets (>9,000 islet equivalent [IEQ]/kg body wt) has been shown to be necessary to achieve insulin independence. Because of the limited availability of islet tissue, this severely restricts the number of patients who may be treated with islet transplantation. Methods to reduce the number of islets needed to cure a diabetic individual are therefore warranted.

Endogenous pancreatic islets have a unique glomerular-like angioarchitecture with a high blood perfusion of 5–7 ml · min^{-1} · g^{-1} tissue (3,4). This secures an optimal delivery of oxygen and nutrients to islet cells and ensures an adequate dispersal of secreted hormones. When islets are isolated and cultured before transplantation, the islet endothelium dedifferentiates or degenerates (5). A rapid revascularization is therefore crucial for islet function after transplantation, and this has been shown to occur within 7–14 days (6,7). However, the extent of revascularization has not been thoroughly studied, and recent experiments on islets transplanted to the renal, splenic, or hepatic subcapsular space have suggested that this process is insufficient to achieve optimal oxygenation of the transplanted islets (8–10).

The aim of the present study was to compare the vascular density of endogenous pancreatic islets to that of mouse islets syngeneically implanted into different organs. Measurements were performed 1 month posttransplantation, i.e., at a time point when transplanted islets have become fully revascularized. Connective tissue was found to constitute a substantial part of the transplant and to surround individual islets in grafts consisting of several islets (islets implanted to the kidney and spleen). Therefore, the vascular densities in this connective tissue and the transplanted islets of these grafts were determined separately.

RESEARCH DESIGN AND METHODS

Animals. Inbred, male C57BL/6 mice weighing 25–30 g were purchased from B&K Universal (Sollentuna, Sweden). The animals had free access to tap water and pelleted food throughout the course of the study. All experiments were approved by the local animal ethics committee at Uppsala University.

Islet isolation, culture, and transplantation. Pancreatic islets were prepared by collagenase digestion, as previously described (11). The isolated islets were cultured free-floating in groups of 150 for 3–4 days in 5 ml culture medium consisting of RPMI 1640 (Sigma-Aldrich, St. Louis, MO) supplemented with L-glutamine (Sigma-Aldrich), benzylpenicillin (100 units/ml) (Roche Diagnostics Scandinavia, Bromma, Sweden), streptomycin (0.1 mg/ml; Sigma-Aldrich), and 10% (vol/vol) fetal calf serum (Sigma-Aldrich). The culture medium was changed every second day. After culture, 250–350 islets were either packed in a braking pipette and implanted beneath the renal capsule of the left kidney or packed in a butterfly needle (25 gauge) and injected into the splenic parenchyma or portal vein (12). All recipients were anesthetized intraperitoneally with avertin: 0.02 ml/g body wt of a 2.5% (vol/vol) solution consisting of 10 g of 97% (vol/vol) 2,2,2-tribromo-ethanol (Sigma-Aldrich) in 10 ml 2-methyl-2-butanol (Kemila, Stockholm, Sweden).

Preparation of histological sections and staining procedures. One month posttransplantation, the transplanted animals were killed by cervical dislocation, and the graft-bearing organ was removed. Separate, nontransplanted C57BL/6 mice of the same age were also killed, but only their pancreas was removed. The organs were fixed in 10% (vol/vol) neutral buffered formalin for ~24 h, dehydrated in ethanol, and embedded in paraffin. Sections, 5 μm thick, were mounted on glass slides. The slides were pretreated with neuraminidase type X (Sigma-Aldrich), and the microvascular endothelium was stained with the lectin *Bandeiraea simplicifolia* (BS-1) (Sigma-Aldrich), as previously described (13). Briefly, the sections were incubated with normal goat serum (NGS) (Dakopatts, Glostrup, Denmark) for 1 h at room tempera-

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BS-1, *Bandeiraea simplicifolia*; BSA, bovine serum albumin; NGS, normal goat serum; TBS, Tris-buffered saline.

ture (20°C). Biotinylated BS-1 was applied to the sections, which were incubated at 4°C overnight. The slides were washed in Tris-buffered saline (TBS) (3 × 5 min) and incubated with StreptABComplex (Dakopatts) for 30 min at 20°C. The slides were washed again, and new fuchsin substrate system (Dakopatts) was applied to the slides and developed. To exclude endogenous alkaline phosphatase activity in the new fuchsin substrate system, 1 mol/l levamisole (Sigma-Aldrich), dissolved in redistilled water, was added before development. The slides were counterstained with hematoxylin. Negative control slides were incubated with NGS (Dakopatts) diluted in TBS containing 0.1% bovine serum albumin (BSA) (dilution 1:20) instead of BS-1.

To find the islet grafts in the liver sections, the latter were also stained with antibodies against insulin. The slides were washed, incubated for 10 min with 8% (wt/vol) hydrogen peroxide in TBS, and washed again. The sections were thereafter incubated with normal swine serum (Dakopatts) diluted 1:20 with TBS containing 0.1% (wt/vol) BSA for 1 h. Primary antibodies against insulin (ICN Biomedicals, Aurora, OH), diluted in TBS containing 0.1% (wt/vol) BSA, were applied to the slides for 1 h. The slides were washed (TBS; 3 × 5 min) and incubated for 30 min with secondary swine anti-rabbit antibody (Dakopatts) diluted 1:100 in TBS. The slides were then washed again. Rabbit peroxidase anti-peroxidase antibody (Dakopatts), diluted 1:100 in TBS, was applied to the slides for 30 min. The slides were washed and developed with Vector SG substrate kit (Immunkemi, Järfälla, Sweden) for ~8 min, washed again (TBS; 3 × 5 min), stained with BS-1 as above, and counterstained with the nuclear stain methyl green (Immunkemi) according to the supplier's instructions. Negative control slides were incubated with normal rabbit serum instead of primary antibody and lectin. A protocol similar to that used for the insulin immunostaining of islet grafts in the liver was used to stain pancreatic sections with antibodies against insulin (ICN Biomedicals), glucagon (Novo Nordisk, Copenhagen, Denmark), and somatostatin (Dakopatts). The same pancreatic sections were also stained for BS-1.

Evaluation of vascular density. In each animal, ≥12 tissue sections from all parts of the pancreas or islet transplants were randomly chosen and evaluated. The number of blood vessels in endogenous or transplanted islets in each section was counted at a magnification of ×600 in a stereo microscope. In the multi-islet grafts implanted into the kidney or spleen, connective tissue surrounded the individual islets in the grafts, and the number of blood vessels in the endocrine and connective tissue parts was counted separately. All connective tissue in the islet grafts was then evaluated; the demarcation of an islet graft was considered to be the parenchyma of the graft-bearing organ. The fractions of endocrine and connective tissue were determined by a direct point-counting method (14,15). For this purpose, the number of intersections overlapping connective tissue and endocrine cells within the islet grafts was counted (at magnification ×600). Approximately 12 fields (corresponding to ~1,500 intersections) were counted in each islet graft. The areas of the investigated endogenous islets and grafted islets were determined by using a computerized system for morphometry (MOP-Videoplan; Carl Zeiss, Stockholm, Sweden). Vascular density, i.e., the number of blood vessels per measured islet or graft area (mm^2), was then calculated.

Implantation of microspheres. Approximately 300 green cross-linked polystyrene-divinylbenzene microspheres (diameter 200 μm; E-Z Trac, San Diego, CA) were packed in a braking pipette and implanted, using the same technique as for the islets, beneath the renal capsule of separate animals. One month later, the kidneys implanted with microspheres were retrieved and processed similarly to the islet transplants. The number of blood vessels in the connective tissue surrounding the implanted microspheres was determined as in the islet grafts.

Statistical analysis. All values are given as means ± SE. Multiple comparisons between data were performed by ANOVA (Statview; Abacus Concepts, Berkeley, CA) with correction of P values using the Bonferroni method (16). When only two groups were compared, unpaired or paired Student's t test was used. $P < 0.05$ was considered to be statistically significant for all comparisons.

RESULTS

Staining and morphology of endogenous and transplanted islets. Endothelium in all tissue samples was stained with the lectin BS-1 (Fig. 1). No structures corresponding to unstained microvascular blood vessels could be identified in any of the investigated sections. The endothelial cells of endogenous pancreatic islets and islets transplanted under the renal capsule or into the liver were strongly stained. However, endothelium of the intrasplenic islet grafts was less intensely stained. The insulin-, glucagon-, or somatostatin-positive cells in endogenous islets did not stain with BS-1.

The fraction of the islet grafts consisting of connective tissue was similar in the kidney (36 ± 2%; $n = 6$) and spleen (27 ± 2%; $n = 6$). The intraportally transplanted islets were also surrounded by connective tissue. Because most of these islets were entrapped in periportal areas, it was impossible to decide whether the connective tissue belonged to the graft or to the liver stroma. Connective tissue was also formed around the microspheres implanted to the renal subcapsular space and constituted 57 ± 2% ($n = 6$) of these grafts.

Vascular density. The vascular density of the transplanted islets was decreased compared with that of the endogenous pancreatic islets, irrespective of the implantation organ (Fig. 2). Islets transplanted into the spleen had a lower vascular density than islets transplanted beneath the renal capsule or into the liver (Fig. 2). In the connective tissue stroma of grafts implanted into kidney or spleen, the number of capillaries was markedly higher than in the endocrine parts of these grafts (Fig. 3). The density of intra-graft stromal capillaries appeared to be higher in the immediate vicinity of the islets at all three implantation sites.

A markedly lower number of capillaries was found in the connective tissue surrounding microspheres implanted to the renal subcapsular space compared with connective tissue surrounding implanted islets (185 ± 30 vs. 3,854 ± 516 capillaries/mm^2; $n = 6$ in both groups; $P < 0.05$).

DISCUSSION

Previous studies have shown that revascularization of transplanted islets is initiated within 2–4 days and is completed within 14 days after implantation (6,7,17–20). Although vascular density has been estimated in transplanted islets (6,20,21), there have been no quantitative studies comparing the actual number of blood vessels in transplanted and endogenous islets. The present study shows that vascular density in transplanted islets is decreased compared with endogenous islets 1 month after implantation. This decrease is present at all investigated implantation sites, although it is more pronounced in islets implanted into the spleen than in islets implanted into the liver or kidney. Our data therefore suggest that the revascularization of transplanted islets is impaired, at least quantitatively, irrespective of the implantation site.

We have recently evaluated various markers for microvascular endothelial cells in tissue samples from rodents and found that the lectin BS-1, which binds to α-D-galactosyl residues on endothelial cells (22), enabled us to detect these cells in all tissues examined (13). Of particular interest was the finding that BS-1 consistently stained endothelium in endogenous pancreatic islets and in syngeneically transplanted rat and mouse pancreatic islets irrespective of implantation site. As found previously, the microvessels of the intraportal and renal subcapsular islet grafts were distinctly stained with BS-1 in the present study. The blood vessels within the islet grafts implanted to the spleen were, however, more faintly stained. The reason is unknown, but may reflect revascularization from the unique vascular system that occurs in the spleen. Unknown confounding factors for the staining technique

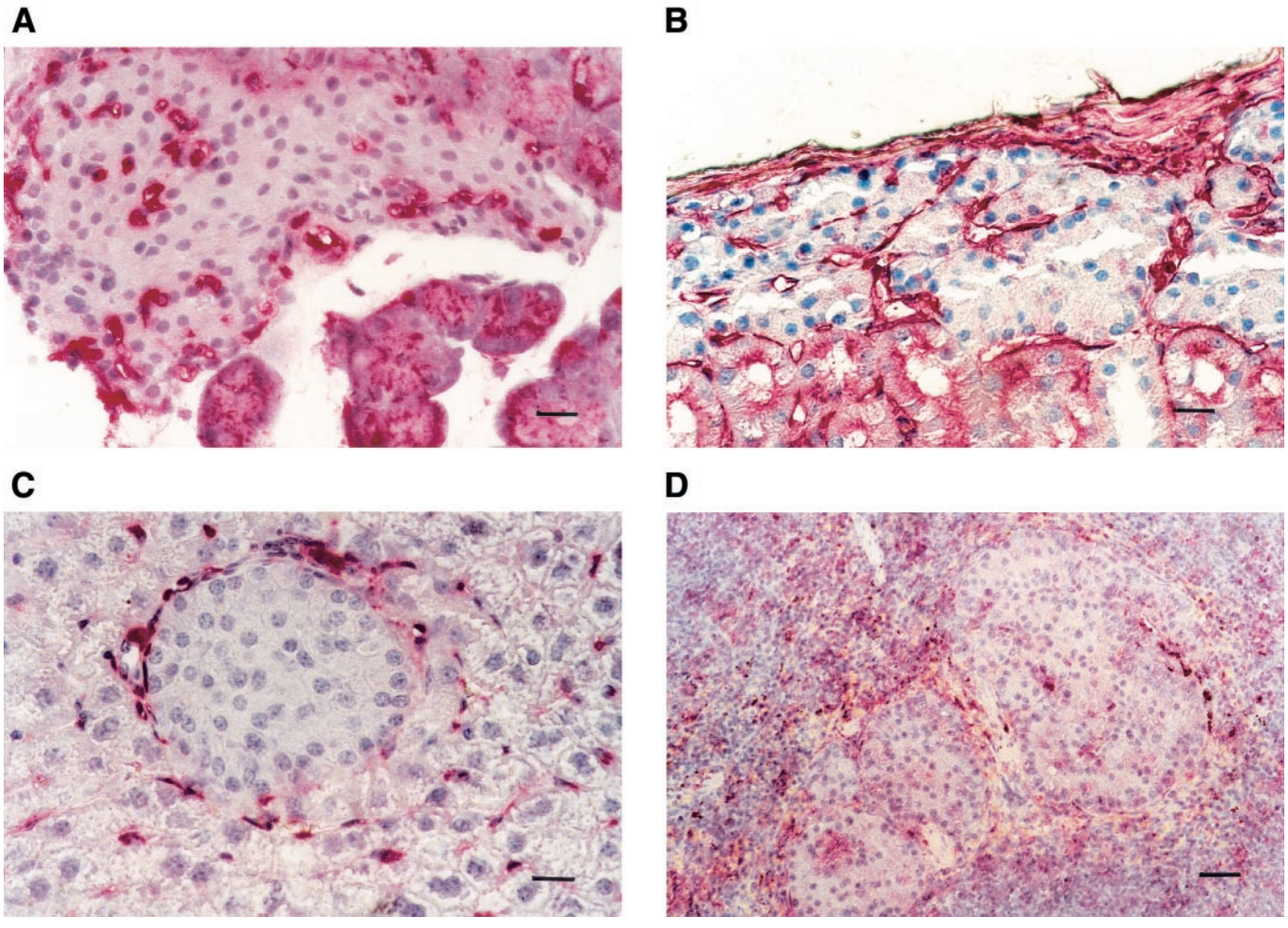

FIG. 1. Microvascular endothelium (red) in endogenous and transplanted pancreatic islets stained with the lectin *Bandeiraea simplicifolia*. *A*: Endogenous pancreatic islets (scale bar 17 μm). *B*: Pancreatic islets transplanted into the renal subcapsular space (scale bar 8.5 μm). *C*: Pancreatic islets transplanted intraportally into the liver (scale bar 17 μm). *D*: Pancreatic islets transplanted into the spleen (scale bar 8.5 μm).

may also be present within the spleen. Despite the more faint staining, the endothelium within the intrasplenically grafted islets and their surrounding connective tissue could be discerned. A further evaluation of the staining specificity of BS-1 was added in the present study, by applying staining with BS-1 and antibodies for insulin, glucagon, or somatostatin in the same pancreatic slides. The rationale for this was that expression of α-galactosyl epitopes on neonatal porcine islet cells has previously been shown (23). No staining of any of these islet endocrine cells with BS-1 was detected, which is consistent with the findings that these epitopes are not expressed in adult pig (24) or adult mouse (22) pancreatic tissue.

We did not quantitatively compare the vascular density in the surface and interior regions of the renal subcapsular grafts, but there were no obvious regional differences in vascular density within these grafts. This result is in line with previous findings of an absence of oxygen-tension gradients in such islet grafts (8). We have previously observed that both blood perfusion and oxygen tension are similar in renal subcapsular islet grafts curing a diabetic animal and in islet grafts implanted to normoglycemic recipients (10). It is therefore anticipated that similar results would have been obtained if cured diabetic recipients had been investigated in the present study rather than normoglycemic recipients. However, this awaits further study.

Besides their main endocrine constituent, islet grafts consist of blood vessels, connective tissue, and, at least initially, a few contaminating exocrine cells. The amount of connective tissue found within the grafts varies between implantation sites, and most likely depends on, at least partially, a foreign body reaction. Both the endocrine and stromal compartments of islet grafts contribute to graft blood flow as measured by, for example, laser-Doppler flowmetry. The oxygenation of the hormone-producing cells, however, depends only on closely adjacent capillaries, i.e., mainly (or only) blood vessels in the endocrine parts of the grafts. We therefore decided to evaluate the endocrine and stromal compartments separately for vascular density. Surprisingly, whereas the vascular density in transplanted islets per se was low, a large number of blood vessels were found in the connective tissue surrounding the renal subcapsular and intrasplenic islets. To elucidate whether this reflected inherent properties of a normal foreign body reaction or a compensation for the low revascularization of the endocrine parts of the grafts, plastic microspheres, rather than islets, were im-

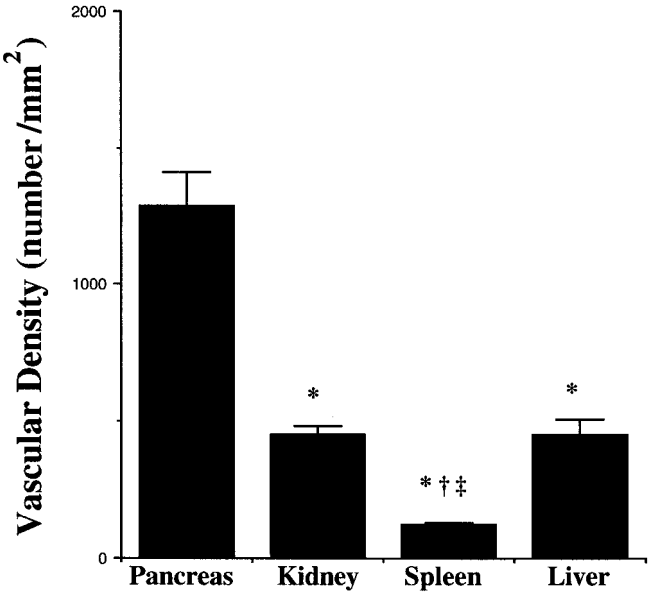

FIG. 2. Vascular density in endogenous pancreatic islets and in islets implanted into kidney, liver, or spleen 1 month posttransplantation. Values are expressed as means ± SE for six animals in each group. *P < 0.05 vs. endogenous pancreatic islets; †P < 0.05 vs. renal subcapsular grafts; ‡P < 0.05 vs. grafts implanted to the liver.

planted in separate animals. Interestingly, no accumulation of newly formed blood vessels was seen in the foreign body reaction of connective tissue surrounding the implanted microspheres. Thus, an angiogenic response initiated by the cells within the transplanted islets seems to induce a compensatory increase in vascular density in the surrounding connective tissue. Indeed, the predominant location of capillaries in the connective tissue stroma to the immediate vicinity of the endocrine tissue is also consistent with this. To our knowledge, this preferential distribution of graft blood vessels to the connective tissue stroma has not been previously described.

In a previous study, a similar vascular density as in the present study was found in endogenous rat pancreatic islets (25). Because of the use of entirely different techniques to visualize blood vessels, it is difficult to compare our results on vascular density in transplanted islets to previous studies quantitating graft blood vessels (6,20,21). Merchant et al. (20) did not mention whether endocrine and connective tissue parts were evaluated separately, and Menger et al. (6) and Heuser et al. (21) evaluated only single islets implanted into a skinfold chamber preparation.

The islet grafts investigated in the present study contained insulin-producing β-cells at all implantation sites, as confirmed by immunohistochemistry. Previous studies have also shown that islets in similar numbers normalize the hyperglycemia of alloxan-diabetic recipients (11). Thus, despite the decreased vascular density of transplanted islets, functional activity remains. However, we have previously noted a 75% decrease in insulin content of transplanted islets compared with islets cultured in vitro (10). Although β-cell death in the immediate posttransplantation period may explain some of the difference, low revascularization with concomitant decreased tissue oxygen tension may also contribute at later stages by suppressing insulin production (26). Indeed, we have previously observed a markedly decreased tissue oxygen tension in transplanted rat islets compared with endogenous islets up to 9 months after implantation (9). This low tissue oxygen tension of islet grafts seems to be independent of implantation site (10) and related to a high degree of nonoxidative glucose metabolism (27). Also, islet graft blood flow, as investigated by means of nonradioactive microspheres or laser Doppler flowmetry, has been found to be decreased compared with endogenous islets (8–10,28,29). In this context, it should be noted that a laser Doppler flow probe measures whole-blood perfusion, i.e., all moving blood cells within the illuminated tissue. In view of the preferential location of blood vessels to the connective tissue in composite islet grafts, as described in the present study, it may therefore be envisaged that the

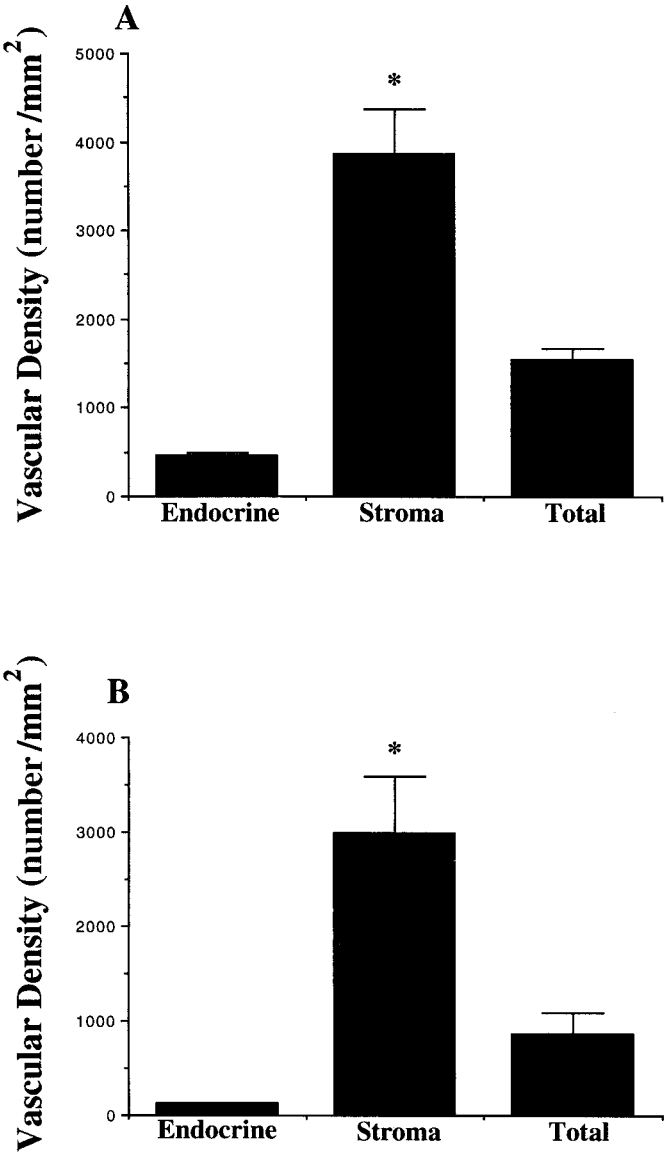

FIG. 3. Vascular density in islet grafts implanted into renal subcapsular space (*A*) or splenic parenchyma (*B*) 1 month posttransplantation. Values are provided separately for the endocrine and stromal components of the graft. The bar for total vascular density denotes the combined vascular density of the connective tissue and endocrine compartments of the graft. Values are expressed as means ± SE for six animals in each group. *P < 0.05 for endocrine tissue vs. connective tissue stroma.

blood flow within the endocrine parts of the grafts is even lower than previous estimates ($\approx$4 ml $\cdot$ min^{-1} $\cdot$ g^{-1}) (29). Indeed, in experiments using a combination of microspheres and an ultrasonic flow probe, a nutritional islet graft blood perfusion of only ~10% of that seen in endogenous islets (5–7 ml $\cdot$ min^{-1} $\cdot$ g^{-1}) was observed (8). An islet blood flow more similar to that of endogenous islets was recorded in islets autotransplanted beneath the renal capsule of partially pancreatectomized animals (30,31). However, in the latter experiments, the influence of the partial pancreatectomy has been difficult to assess.

In conclusion, we found a marked decrease in vascular density of transplanted islets compared with that of endogenous islets. In contrast, the connective tissue surrounding the transplanted islets contained a large number of blood vessels. As suggested by previous studies on blood flow, oxygen tension, and metabolism, the low revascularization of transplanted islets may influence islet graft function.

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REFERENCES

1. Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
2. Ryan EA, Lakey JRT, Rajotte RV, Korbutt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AMJ: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–719, 2001
3. Brunicardi FC, Stagner J, Bonner-Weir S, Wayland H, Kleinman R, Livingston E, Guth P, Menger MD, McCuskey R, Intaglietta M, Charles A, Ashley S, Cheung A, Ipp E, Gilman S, Howard T, Passaro E Jr: Microcirculation of the islets of Langerhans. *Diabetes* 45:385–392, 1996
4. Jansson L: The regulation of pancreatic islet blood flow. *Diab Metab Rev* 10:407–416, 1994
5. Parr EL, Bowen KM, Lafferty KJ: Cellular changes in cultured mouse thyroid glands and islets of Langerhans. *Transplantation* 30:135–141, 1980
6. Menger MD, Jäger S, Walter P, Feifel G, Hammersen F, Messmer K: Angiogenesis and hemodynamics of microvasculature of transplanted islets of Langerhans. *Diabetes* 38 (Suppl. 1):199–201, 1989
7. Sandberg JO, Margulis B, Jansson L, Karlsten R, Korsgren O: Transplantation of fetal porcine pancreas to diabetic or normoglycemic nude mice: evidence of a rapid engraftment process demonstrated by blood flow and heat shock protein 70 measurements. *Transplantation* 59:1665–1669, 1995
8. Carlsson PO, Liss P, Andersson A, Jansson L: Measurements of oxygen tension in native and transplanted rat pancreatic islets. *Diabetes* 47:1027–1032, 1998
9. Carlsson PO, Palm F, Andersson A, Liss P: Chronically decreased oxygen tension in rat pancreatic islets transplanted under the kidney capsule. *Transplantation* 69:761–766, 2000
10. Carlsson PO, Palm F, Andersson A, Liss P: Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 50:489–495, 2001
11. Korsgren O, Jansson L, Sandler S, Andersson A: Hyperglycemia-induced B cell toxicity: the fate of pancreatic islets transplanted into diabetic mice is dependent on their genetic background. *J Clin Invest* 86:2161–2168, 1990
12. Mellgren A, Schnell Landström AH, Petersson B, Andersson A: The renal subcapsular site offers better growth conditions for transplanted mouse pancreatic islet cells than the liver or spleen. *Diabetologia* 29:670–672, 1986
13. Mattsson G, Carlsson PO, Olausson K, Jansson L: Histological markers for endothelial cells in endogenous and transplanted rodent pancreatic islets. *Pancreatology* 2:155–162, 2002
14. Weibel ER: *Stereological Methods*, *Volume 1: Practical Methods for Biological Morphometry*. London, Academic Press, 1979, p. 101–161
15. Carlsson PO, Andersson A, Jansson L: Pancreatic islet blood flow in normal and obese-hyperglycemic (*ob/ob*) mice. *Am J Physiol* 271:E990–E995, 1996
16. Wallenstein S, Zucker CL, Fleiss JL: Some statistical methods useful in circulation research. *Circ Res* 47:1–9, 1980
17. Andersson A, Korsgren O, Jansson L: Intraportally transplanted pancreatic islets revascularized from hepatic arterial system. *Diabetes* 38 (Suppl. 1):192–195, 1989
18. Mendola JF, Goity C, Fernández-Alvarez J, Saenz A, Benarroch G, Fernández-Cruz L, Gomis R: Immunocytochemical study of pancreatic islet revascularization in islet isograft: effect of hyperglycemia of the recipient and of in vitro culture of islets. *Transplantation* 57:725–730, 1994
19. Mendola J, Corominola H, Gonzalez-Clemente JM, Esmatjes E, Saenz A, Fernandez-Cruz L, Gomis R: Follow-up study of the revascularization process of cryopreserved islets of Langerhans. *Cryobiology* 33:530–543, 1996
20. Merchant FA, Diller KR, Aggarwal SJ, Bovik AC: Angiogenesis in cultured and cryopreserved pancreatic islet grafts. *Transplantation* 63:1652–1660, 1997
21. Heuser M, Wolf B, Vollmar B, Menger MD: Exocrine contamination of isolated islets of Langerhans deteriorates the process of revascularization after free transplantation. *Transplantation* 69:756–761, 2000
22. Laitinen L: Griffonia simplicifolia lectins bind specifically to endothelial cells and some epithelial cells in mouse tissues. *Histochem J* 19:225–234, 1987
23. Rayat GR, Rajotte RV, Elliott JF, Korbutt GS: Expression of Gal alpha(1,3)gal on neonatal porcine islet beta-cells and susceptibility to human antibody/complement lysis. *Diabetes* 47:1406–1411, 1998
24. Bennet W, Björkland A, Sundberg B, Davies H, Liu J, Holgersson J, Korsgren O: A comparison of fetal and adult porcine islets with regard to Galα (1,3)Gal expression and the role of human immunoglobulins and complement in islet cell cytotoxicity. *Transplantation* 69:1711–1717, 2000
25. Vetterlein F, Pethö A, Schmidt G: Morphometric investigation of the microvascular system of pancreatic exocrine and endocrine tissue in the rat. *Microvasc Res* 34:231–238, 1987
26. Dionne KE, Colton CK, Yarmush ML: Effect of oxygen on isolated pancreatic tissue. *ASAIO Trans* 35:739–741, 1989
27. Carlsson PO, Kiuru A, Nordin A, Olsson R, Lin JM, Bergsten P, Hillered L, Andersson A, Jansson L: Shift to non-oxidative glucose metabolism in transplanted rat pancreatic islets as evidenced by microdialysis measurements (Abstract). *Acta Chir Austriaca* 33 (Suppl. 174):44A, 2001
28. Carlsson PO, Jansson L, Andersson A, Källskog Ö: Capillary blood pressure in syngeneic rat islets transplanted under the renal capsule is similar to that of the implantation organ. *Diabetes* 47:1586–1593, 1998
29. Olsson R, Jansson L, Andersson A, Carlsson PO: Local blood flow regulation in transplanted rat pancreatic islets: influence of adenosine, angiotensin II, and nitric oxide inhibition. *Transplantation* 70:280–287, 2000
30. Sandler S, Jansson L: Blood flow measurements in autotransplanted pancreatic islets of the rat: impairment of the blood perfusion of the graft during hyperglycemia. *J Clin Invest* 80:17–21, 1987
31. Jansson L, Sandler S: Altered blood flow regulation in autotransplanted pancreatic islets of rats. *Am J Physiol* 259:E52–E56, 1990