Experimental Studies on the Vasculature of Endogenous and Transplanted Islets of Langerhans

BY

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Abstract

The blood vessels of the pancreatic islets are of crucial importance for oxygen and metabolite supply as well as dispersal of secreted hormones. In addition to this, endothelial cells have an important role in the revascularization process after islet transplantation. Previous studies have reported signs of poor engraftment of transplanted islets, presumably due to impaired revascularization. The aims of this thesis were to investigate the revascularization process of transplanted islets and to examine the role of islet endothelial cells. In this context, the lectin Bandeiraea simplicifolia was found to stain endothelium of both endogenous and transplanted pancreatic islets. By using this lectin we investigated the vascular density of both endogenous and islets transplanted syngeneically beneath the renal capsule, into the spleen or intraportally into the liver of normoglycemic C57BL/6 mice. One month post-transplantation, a time point when the grafts are assumed to be completely revascularized, the vascular density was decreased at all three implantation sites when compared to endogenous islets. Furthermore, most of the blood vessels were located in the graft connective tissue stroma. Similar results were obtained when islet transplant vascular density was determined six months post-transplantation and in cured diabetic animals after one month. In order to evaluate the function of intraportally transplanted islets, we developed a method to retrieve such islets. We treated the implantation organ (liver) first enzymatically (collagenase) and then mechanically, thereafter we could re-isolate the transplanted islets for further in vitro studies. The retrieved islets had a decreased insulin release, insulin content and glucose oxidation rate when compared to non-transplanted control islets. To understand the role of islet endothelium in the revascularization of transplanted islets we performed angiogenesis GEArray studies on islet endothelial cells, from non-cultured, cultured and transplanted islets. We found that the islet endothelium expressed mRNA for both inhibitors and inducers of angiogenesis, and that this expression differed with time. The functional consequences of this remain to be determined.

In summary, the results presented above provide a useful platform for future studies of the morphology and function of islet endothelial cells, especially with a view for elucidating changes induced by islet transplantation.

Keywords: islets of Langerhans, endothelial cells, islet transplantation, vascular density, diabetes mellitus

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List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


V. Mattsson, G., Danielsson, A., Kriz, V., Carlsson, P-O., Jansson, L. Endothelial cells in endogenous and transplanted pancreatic islets: differences in the expression of angiogenic peptides and receptors. (manuscript)

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Vi är alla cellbiologins fångar

H.B. Pearl
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### Abbreviations

<table>
<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>Ang</td>
<td>Angiopoietin</td>
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<tr>
<td>ACE</td>
<td>Angiotensin-converting enzyme</td>
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<tr>
<td>BS-1</td>
<td>Bandeiraea simplicifolia-1</td>
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<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
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<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<tr>
<td>Dil-Ac-LDL</td>
<td>1,1´-dioctadecyl-3,3,3,3´-tetramethylindocarbocyanine perchlorate acetylated low density lipoprotein</td>
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<tr>
<td>EC</td>
<td>Endothelial cell</td>
</tr>
<tr>
<td>ECGS</td>
<td>Endothelial cell growth supplement</td>
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<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
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<tr>
<td>FCS</td>
<td>Fetal calf serum</td>
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<tr>
<td>GAPDH</td>
<td>Glyceraldehyde 3 phosphate dehydrogenase</td>
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<tr>
<td>HBSS</td>
<td>Hanks´ balanced salt solution</td>
</tr>
<tr>
<td>IAPP</td>
<td>Islet amyloid polypeptide</td>
</tr>
<tr>
<td>MPC</td>
<td>Magnetic particle concentrator</td>
</tr>
<tr>
<td>NGS</td>
<td>Normal goat serum</td>
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<tr>
<td>PP</td>
<td>Pancreatic polypeptide</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PEDF</td>
<td>Pigment epithelium-derived factor</td>
</tr>
<tr>
<td>PTN</td>
<td>Pleiotrophin</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris-buffered normal saline</td>
</tr>
<tr>
<td>Tie1</td>
<td>Tyrosine kinase receptor 1</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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<tr>
<td>vWF</td>
<td>von Willebrand factor</td>
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1. Introduction

1.1 Islets of Langerhans
The human pancreas contains approximately 1-2 million islets of Langerhans constituting 1-2% of the gland (1). Even though the number of pancreatic islets is species dependent, their size is usually in the order of 100-400 µm, representing 2-3000 endocrine cells per islet. The islets are surrounded by a thin connective tissue capsule and consist of several different types of cells. The islet core contains mainly beta cells, whereas the other endocrine cells, namely alpha-, delta- and pancreatic polypeptide (PP) cells are located to the periphery. In addition, the islets contain nerves, fibroblasts, macrophages, dendritic cells and endothelial cells (EC), which are interspersed among the endocrine cells. The function of the endocrine cells is to produce the hormones which regulate especially carbohydrate metabolism. Thus, the beta cells produce insulin, which lowers blood glucose concentrations by suppression of gluconeogenesis and promotion of glucose uptake and storage. In addition to insulin, also islet amyloid polypeptide (IAPP), as well as chromogranin-derived peptides are released from the beta cells. The exact functions of these peptides still remain to be determined. The alpha cells produce glucagon, which increases glucose concentrations by promoting glycogen breakdown in the liver, and thereby protect the body against hypoglycemia. Delta cells produce somatostatin, which is an inhibitor of the release other hormones, including insulin and glucagon. Finally, the product of PP cells is mainly released through vagal mediation, and seems to inhibit the exocrine secretions of the pancreas (2, 3).

1.2 Islet vascular network
The pancreatic vasculature constitutes a complex network, which is adapted to the special needs of the exocrine and endocrine parts of the gland, respectively. The islet blood vessels are of major importance for supplying the endocrine organ with nutrients and oxygen, as well as to transport the
secreted hormones into the blood stream. The islet vasculature is connected both in series and in parallel to the exocrine blood vessels, and differs morphologically and functionally from that in the exocrine parenchyma (4, 5). The islet microvascular organization is dependent on the size of the islets (4). Thus, smaller islets receive blood from one arteriole, and drain through small venules traversing the exocrine parenchyma. It has been noted that some of the islet efferent capillaries connect with acinar and/or ductular microvessels, thereby forming a so called insulo-acinar portal system. The larger islets, on the other hand, have 1-3 arterioles entering the islet, and the islet capillaries drain mainly into postcapillary venules at the edge of the islets, which then empty into intralobular veins.

The endocrine capillaries are wider and their EC possess 10 times as many fenestrae as those in exocrine microvessels (6). These fenestrae are probably important for the high permeability of the islets capillaries. So far, no direct role of the islet vasculature in the pathogenesis of diabetes mellitus has been proven, except for the role of EC in recruitment of immune competent cells in insulitis (7). Furthermore, changes in the blood perfusion (8), permeability (9) and islet capillary blood pressure have been described (10). However, it is at present unknown to what extent these changes are secondary to damage to or impaired function of the beta cells per se.

1.3 Endothelial cells

The EC are lining all blood and lymph vessels and they have several unique functions, e.g. contributing to local blood flow regulation, coagulation and thrombolytic processes, serving as a mechanical and immunological barrier between tissues and blood as well as participation in angiogenesis (11). The EC are adapted to the functional needs of the surrounding tissues, and thereby constitute a heterogeneous group of cells. EC differ functionally between large and small blood vessels, as well as between different organs and species (12, 13). Due to this heterogeneity it is difficult to find markers which are specific for all EC. At present several techniques are used to detect EC in vitro or in histological slides, e.g. antibodies (14), lectins (15) and Dil-Ac-LDL (16). However, none of these markers consistently labels EC from all parts of the body in every application. In line with this, specific markers for the islet endothelium have been difficult to find, especially in formalin-fixed and paraffin-embedded material.

The islet endothelium is highly adapted to the functional needs of the islets. They have fenestrae for rapid delivery of hormones (6), and the blood flow is high compared to that in the exocrine pancreas (8). Previous studies have described a technique for isolation and culture of islet endothelium in
rats (14) and humans (17). Such in vitro studies have confirmed that there are biochemical and functional differences between endothelium from exocrine and endocrine blood vessels.

1.4 Diabetes mellitus

Diabetes mellitus is a syndrome characterized by metabolic aberrations due to an absolute or relative lack of insulin. It is commonly divided into type 1 and type 2 diabetes, both of which are likely to be disorders with a heterogeneous etiology. Type 1 diabetes mellitus (insulin-dependent diabetes mellitus) is characterized by a loss of the insulin-producing beta cells, due to their destruction in an autoimmune process. The reasons for the specific targeting of the beta cells are at present unknown. However, the number of diabetes patients is rapidly increasing and is estimated to be 150-220 millions world wide in the year 2010, most of which will be type 2 diabetes (18).

Type 2 diabetes is due to a combination of an impaired beta cell function and peripheral insulin resistance. It is more frequently seen in older people, and is often associated with obesity. However, this form of diabetes is becoming increasingly frequent, and also younger age groups are affected today. The number of islets in the pancreas can be normal, although it is usually decreased, albeit never to the same degree as seen in type 1 diabetes.

At present, the only known cure for type 1 diabetes is transplantation of insulin-producing cells. Type 2 diabetes patients can often achieve reversal of symptoms if increasing their physical activity in combination with weight reduction, even though glucose intolerance often remains. From now on the discussion will focus on type 1 diabetes, where replacement of beta cells has become a treatment of choice for selected patients. Beta cells can be substituted either by implantation of a whole pancreas, or as isolated pancreatic islets. Whole pancreas transplantation is a major surgical procedure which carries significant risks for the patient (19). Islet transplantations involve minor surgery, and their use will be discussed more in detail below.

1.5 Transplantation of islets

Arguments in favor of choosing isolated islets for clinical transplantations include the technical simplicity, reduced risks for the patients and the lower medical costs associated with the procedure. However, the results of islet transplantations have mainly been poor (20) until the recent application of
the so called Edmonton Protocol. This encompasses changes in patient selection and choice of immunosuppressive drugs, and has led to a markedly improved outcome of clinical islet transplantation (21, 22). However, also when applying this protocol, transplantation of a large number of islets (>9,000 islet equivalents/kg BW) is necessary to achieve insulin-independence. Due to the limited availability of islet tissue, this severely restricts the number of patients that may be treated. Methods to reduce the number of islets needed to cure a diabetic individual are therefore warranted.

1.6 Engraftment and revascularization of transplanted islets

Engraftment is the adaptation of the transplanted islets to the new environment in the implantation organ. An adequate engraftment process is of major importance for the islet graft survival and function, and constitutes a possible target for interventions to improve the outcome of islet transplantations. There are several processes involved in the engraftment, e.g. revascularization, reinnervation and reorganization of stromal and endocrine cell interactions. A rapid revascularization is crucial for islet endocrine function after transplantation, and this has been shown to occur within 7-14 days (23, 24). However, the extent of the revascularization has not been thoroughly studied in detail. Recent experiments on islets transplanted to the renal, splenic or hepatic subcapsular space have suggested that the angiogenic process is insufficient to achieve optimal oxygenization of the transplanted islets (25-27). However, no measurements comparing the vascular density in endogenous islets versus islets transplanted into these implantation sites were performed. Clinically, most islet transplantations are performed intraportally (20-22), and such intrahepatic islets are difficult to visualize or retrieve, which makes post-transplantation studies difficult to perform. Therefore, the development of techniques for retrieval of intraportally implanted islets would be crucial for functional studies.

1.7 Effects of culture of isolated islets

When islets are isolated prior to transplantation they are disconnected from the vascular network, and during the subsequent culture period they depend on diffusion of oxygen and nutrients for survival. Studies have suggested that during culture, islet EC disappear or dedifferentiate (28). This means that the islet vasculature has to be re-built after transplantation. There are
only few studies on islet endothelium (14, 29; see 1.3), and the knowledge
on this cell type is therefore scarce. In order to investigate the islet
endothelium of transplanted islets, developments of methods to provide
access to these cells are mandatory.

1.8 Immune suppressive drugs and rejection
When evaluating islet allotransplantation, both the effects of rejection,
immune suppressive drugs as well as possible recurrence of the autoimmune
disease must be considered. Many of the drugs used to prevent the immune
system from attacking the graft are toxic for the beta cells, and may even by
themselves participate in the destruction of grafted islets (30). In the present
studies, we have consistently used syngeneically transplanted islets, or have
used athymic recipients, to circumvent these confounding factors.
2. AIMS

The aims of the study were to:

1. compare and evaluate the use of different endothelial markers for visualization of rodent microvascular endothelium in formalin-fixed, paraffin-embedded samples of endogenous and transplanted pancreatic islets

2. compare the vascular density of endogenous mouse pancreatic islets to that of islets syngeneically implanted into liver, spleen or beneath the kidney capsule

3. compare the vascular density of endogenous mouse pancreatic islets to that of islets syngeneically implanted beneath the kidney capsule of cured diabetic recipients and in islet grafts late post-transplantation

4. retrieve transplanted islets from the liver for morphological and functional studies

5. isolate and study EC from endogenous and transplanted islets of Langerhans
3. MATERIALS AND METHODS

3.1 Animals (I-V)
Adult, male Wistar-Furth rats (B&K Universal, Sollentuna, Sweden), Sprague-Dawley rats (a local colony bred at the Biomedical Centre, Uppsala, Sweden), C57BL/6 mice (B&K Universal; Bomhaltgaard Research and Breeding Center, Ry, Denmark), C57BL/6 (nu/nu) (Bomhaltgaard Research and Breeding Center) and YC3 mice constitually expressing enhanced yellow fluorescent protein (kindly provided by Professor Per-Olof Berggren; Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden) were used. 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 and human ethics committees for Uppsala University.

3.2 Diabetic animals (III-IV)
Some of the recipient mice were injected intravenously with alloxan (75 mg/kg BW) 5 days prior to transplantation, and had blood glucose concentrations ≥14 mM at this time. Blood from the cut tip of the tail was used to measure blood glucose concentrations by means of glucose reagents strips (MediSense Sverige, Sollentuna, Sweden). Chemicals used in the experiments were purchased from Sigma-Aldrich (St Louis, MO, USA) if nothing else is mentioned.

3.3 Islet isolation and culture (I-V)
Pancreatic islets from rats and mice were isolated according to a previously described collagenase digestion method (31, 32). Briefly, the pancreatic glands were distended by injections of Hanks’ balanced salt solution (HBSS; The National Bacteriological Laboratory, Stockholm, Sweden) and cut into pieces. The tissue was transferred to vials containing collagenase (from
Clostridium histolyticum; Boehringer Mannheim, Mannheim, Germany) and shaken until the tissue had disintegrated. The digest was sedimented in HBSS and pancreatic islets were subsequently picked under a stereo microscope using a braking pipette. Groups of 150 islets were maintained free-floating in culture medium RPMI 1640 supplemented with L-glutamine, benzylpenicillin (100 U/ml; Roche Diagnostics Scandinavia, Bromma, Sweden), streptomycin (0.1 mg/ml) and 10% foetal calf serum (FCS). The islets were usually cultured for 3-4 days, but in some cases 7 days, and the culture medium was changed every second day. Isolated human islets were kindly provided by Professor Olle Korsgren (Department of Radiology, Oncology, and Clinical Immunology, Uppsala University Hospital, Uppsala, Sweden).

3.4 Islet transplantation and implantation of microspheres (I-V)

Recipient mice were anesthetized with an intraperitoneal injection of 0.02 ml/g body wt of Avertin [a 2.5% (vol/vol) solution of 10 g 97% 2,2,2-tribromo-ethanol in 10 ml 2-methyl-2-butanol (Kemila, Stockholm, Sweden)]. Cultured syngeneic islets, 200-500 per graft, were either packed in a braking pipette and implanted beneath the left kidney capsule, or packed in a butterfly needle (25 G) and injected into the splenic parenchyma or intraportally into the liver (33). Approximately 300 microspheres (diameter 200 µm; E-Z TRAC, San Diego, CA, USA) were implanted beneath the kidney capsule, with the same technique as used for the islets, in separate animals. One month later, the kidneys implanted with microspheres were retrieved and processed similarly to the islet transplants (see 3.11-12). The number of blood vessels in the connective tissue surrounding the implanted microspheres was determined as in the islet grafts (see 3.17).

3.5 Retrieval of intraportally transplanted islets (IV-V)

The transplanted animals were anesthetized with avertin (see 3.4) and an incision through the abdominal skin and muscle was made. The viscera were displaced to expose and allow for ligature of the inferior vena cava cranially to the renal veins. The diaphragm was opened and a ligature was placed around the inferior vena cava immediately below the heart. A polyethylene catheter was inserted caudally into this vein, fixed with a ligature and connected to an infusion pump. The portal vein was incised and the liver was perfused retrogradely. The liver was initially perfused with 7.5 ml buffer (pH
7.7; 8.3 g sodium chloride, 0.5 g potassium chloride, 2.4 g HEPES/l) followed by 300 μl neutral red (20 mg/ml; Kebo Grave, Stockholm, Sweden). After this, an additional perfusion with 7.5 ml perfusion buffer was performed, followed by collagenase dissolved in 8 ml buffer (3.125 mg/ml; Clostridium histolyticum; Boehringer Mannheim). The liver was then excised, cut into smaller pieces and placed in a vial containing HBSS. The liver pieces were further dispersed into clusters of cells by gentle flushing with HBSS using a syringe. The dispersed tissue was washed with fresh HBSS. Pancreatic islets could then be identified and picked under a stereo microscope using braking pipettes. Retrieved islets were either used directly or cultured for 3-4 days as given above before further experiments took place. Some of these islets were used for islet EC culture as mentioned below.

3.6 Binding of the lectin BS-1 to uncoated Dynabeads (V)

Dynabeads M-450 Uncoated (Dynal, Oslo, Norway) were pre-washed in phosphate buffered saline (pH 7.4; PBS) according to the manufacturers instructions. The Dynabeads were thereafter stored in PBS/0.1% bovine serum albumin (BSA; ICN Biomedicals, Aurora, OH, USA) at 4°C.

Bandeiraea simplicifolia (BS-1) was coated onto the Dynabeads by a modification of a previously described method (manufacturers advice; 34). Briefly, BS-1 was resuspended in borate buffer (1 mg BS-1/ml buffer) and washed. Equal volumes of BS-1 (1 mg/ml in 0.1 M borate buffer) and Dynabeads (4x10^8 Dynabeads/ml) were then incubated at 4°C for 24 hours with end-over-end rotation. The Dynabeads were collected with a magnetic particle concentrator (MPC; Dynal) and the supernatant was removed. The Dynabeads were washed, and incubated overnight with end-over-end rotation at 4°C in PBS/0.1% BSA. The Dynabeads were collected and resuspended in HBSS, supplemented with benzylpenicillin (100 U/ml), streptomycin (0.1 mg/ml) and 5% FCS to a final concentration of 4x10^8 BS-1 coated Dynabeads/ml.

3.7 Freshly isolated islet EC (V)

Some of the isolated islets were transferred directly to an Eppendorf tube and washed in PBS. Trypsin (Gibco, Invitrogen, Stockholm, Sweden) was added to the islets, which were thereafter incubated at 37°C. The islets were disrupted into a cell suspension and washed. The EC were extracted from the
cell suspension by a Dynabead method previously described (17, 34). Briefly, BS-1 coated Dynabeads were applied to the cell suspension and incubated at 4°C. The Dynabeads attached to the EC and these cells were retrieved by the use of MPC. The remaining cells were saved as negative control. The EC were washed and then stored at -70°C until the RNA was extracted from the cells.

3.8 Islet EC culture (V)

Freshly isolated or seven days pre-cultured islets were used for EC culture. The islet EC were prepared by a modification of a previously described protocol (14). Briefly, islets were transferred onto a collagen matrix (Collagen GmbH, Nutacon, Leimuden, Netherlands) and cultured in RPMI 1640 supplemented with L-glutamine, benzylpenicillin (Roche Diagnostics Scandinavia), streptomycin, 20% FCS and endothelial cell growth supplement (30 µl/ml; ECGS) in an incubator at 37°C. The islets were then removed, and the remaining cells were detached with 0.25% (wt/vol) trypsin (Invitrogen). The cell suspension was centrifuged and washed. The cells were reseeded in collagen-coated plastic culture dishes in the culture medium given above. Cells were subcultured for up to 2 passages, and removal from each passage was achieved by trypsin treatment as given above. The viability of cells was evaluated by a Trypan Blue exclusion test.

3.9 Retrieval of endothelium from islets transplanted beneath the kidney capsule (V)

The transplanted animals were anesthetized with avertin (see 3.4) and the islet graft was removed with a modification of a previously described method (35). Briefly, the kidney capsule surrounding the graft was cut and the capsule with the adherent islet graft was lifted from the kidney cortex. The capsule surrounding the graft was removed and the remaining grafted islets, connective tissue and parts of the capsule were placed on a collagen matrix and cultured to promote EC outgrowth as described above (see 3.8).
3.10 Purification of islet endothelium from cultured islets with BS-1 coated Dynabeads (V)

EC outgrown from freshly isolated islets, pre-cultured islets or transplanted islets retrieved from the kidney capsule or the liver, together with contaminating non-EC, were suspended in HBSS/FCS (≤5x10^5 cells/ml) and incubated with end-over-end rotation at 4°C with BS-1 coated Dynabeads (see 3.7). The concentration of Dynabeads was chosen to provide at least 10 Dynabeads per EC. After incubation, the BS-1-coated Dynabeads containing bound EC were washed. The contaminating cells obtained from the washing medium were used as negative controls. Some of the EC were stored at -70°C until the RNA was extracted from the cells. Other cells were instead fixed for histological studies (see 3.15).

3.11 Preparation of histological sections (I-V)

One or six months post-transplantation, transplanted animals were killed and the graft-bearing organ was removed. Separate, non-transplanted 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 and thereafter dehydrated in ethanol and embedded in paraffin. Samples from brown and white adipose tissue, small intestine, kidney, pancreas and lung were taken from non-transplanted Sprague-Dawley, Wistar-Furth rats and C57BL/6 mice. The tissues were fixed and embedded as given above. Some of the islets isolated from Wistar-Furth and Sprague-Dawley rats were fixed in formalin immediately after isolation, and then embedded in paraffin. The embedded samples were sectioned (5 μm) and mounted on glass slides. To remove blocking sialic acid residues when staining with the lectin BS-1, sections of the endogenous pancreas, isolated islets and islet grafts from C57BL/6 mice and Sprague-Dawley rats were pre-treated with neuraminidase Type X before further staining procedures. Samples from other species and organs were directly stained, without any pre-treatment, according to one of the protocols given below. When it is stated that slides were washed, it means washing 3x5 min in Tris-buffered normal saline (TBS) unless given otherwise. All lectins or primary antibodies were diluted with TBS, usually containing 0.1% BSA, and secondary antibodies were diluted with TBS if nothing else is given. All incubations were performed in room temperature (RT; 20°C) unless otherwise stated.
3.12 Staining with lectins BS-1 and Ulex europaeus and antibodies against CD34 and CD31 (I-V)

The slides were deparaffinized and washed. The sections were incubated with serum (DAKOPATTS, Glostrup, Denmark). Biotinylated forms of BS-1, Ulex europaeus, CD34 or CD31 (HyCult Biotechnology, Uden, Netherlands) were applied to the sections, which were incubated at 4°C overnight. The slides were washed and incubated with StreptABComplex (DAKOPATTS). The slides were then washed again and New Fuchsin Substrate system (DAKOPATTS) was applied to the slides, developed and counterstained with hematoxylin.

3.13 Antibodies against CD200, Ox43, vWf, insulin, eNOS or ACE (I, II, IV, V)

The slides were deparaffinized, washed, incubated with hydrogen peroxide in TBS and washed again. Sections were incubated with serum (DAKOPATTS). Primary antibodies against CD200 (Serotec, Raleigh, NC, USA), Ox43 (Serotec), von Willebrand factor (vWf; DAKOPATTS), insulin (ICN Biomedicals), endothelial nitric oxide synthase (eNOS; Transduction Laboratories, BD Biosciences, Stockholm, Sweden) or angiotensin converting enzyme (ACE; Chemicon, Chandlers Ford, UK; Autogen Bioclear, Calne, UK) were applied to the slides, which were then incubated at 4°C overnight. The slides were washed and incubated with a secondary antibody (DAKOPATTS), after which the slides were washed again. Peroxidase-anti-peroxidase antibody (DAKOPATTS) was applied to the slides, which were then incubated. Slides were washed, developed with 3,3'-diaminobenzidinetetrahydrochloride, then washed again and counterstained with hematoxylin.

3.14 Double staining with antibodies against vWf and the lectin BS-1 (I)

Slides with isolated rat pancreatic islets were deparaffinized, washed and then stained, as described above, with the antibody against vWf and subsequently with BS-1. The slides were counterstained with hematoxylin.
3.15 Morphological studies of islet EC (V)

The purified EC collected with Dynabeads were transferred onto slides by the use of a cytopsin (Cytospin 3, Shandon, Labex, Helsingborg, Sweden). The slides were then washed with PBS and fixed with 4% paraformaldehyde. The slides were washed and incubated with antibodies against eNOS (Transduction Laboratories) or ACE (Chemicon), or BS-1 diluted with TBS at 4°C over night. The slides were thereafter washed and incubated with StrepABComplex (Vector, Immunkemi, Sweden). The slides were then again washed and Vectastain red (Vector) was applied and developed. The EC were counterstained with hematoxylin.

3.16 Evaluation of staining (I)

At least ten tissue sections from every organ were examined and evaluated. The intensity of the EC staining was scored semiquantitatively: -, negative staining, +/−, positive staining in some cells, +, positive staining and ++, strong positive staining in all cells.

3.17 Evaluation of vascular density (II-III)

In each C57BL/6 mouse, ≥12 tissue sections stained with BS-1 from different (randomly chosen) parts of the pancreas or islet transplants were evaluated. The number of blood vessels in endogenous or transplanted islets in each section was counted in a light microscope. In the grafts implanted into the kidney or spleen, connective tissue surrounded individual islets in the transplants. There was also connective tissue stroma surrounding the islets implanted into the liver, but these islets were often trapped in periportal fields, which made it impossible to distinguish whether the connective tissue belonged to the graft or liver. In the grafts in kidney and spleen, the number of blood vessels in the endocrine and connective tissue parts was counted separately. The amount of 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 (36, 37). For this purpose, the number of intersections of an ocular grid overlapping connective tissue and endocrine cells within the islet grafts was counted at a magnification of 600X. Approximately 12 fields (each corresponding to 121 intersections) were counted in each islet graft. The areas of the investigated endogenous islets and grafted islets were
determined with 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 (mm²), was then calculated.

3.18 Transmission electron microscopy (TEM; IV-V)
Some of the retrieved transplanted islets were prepared for and studied with TEM, as previously described (38). Briefly, the retrieved islets were fixed over night in glutaraldehyde and cacodylate buffer (Agar Scientific, Stansted, UK). After being washed in cacodylate buffer, the islets were post-fixed in OsO₄ dissolved in cacodylate buffer. A second wash was followed by dehydration in graded series of ethanol, before the islets were finally embedded in Agar 100 Resin (Agar Scientific). Sections were cut, contrasted and examined in a Hitachi H7100 transmission electron microscope (Hitachi, Tokyo, Japan) at 75 kV.

3.19 Scanning electron microscopy (SEM; IV-V)
Islets were fixed and post-fixed as described above for TEM and thereafter washed in cacodylate buffer and distilled water. The islets were mounted on poly-L-lysine-treated cover slips and dehydrated in a graded series of acetone, by means of the critical point drying method. After drying, the coverslips were mounted on holders and the islets were gold sputtered and then examined in a LEO 1530 field emission SEM (LEO, Cambridge, UK) at an accelerating voltage of 5 kV.

3.20 Glucose- and theophylline-stimulated insulin release (IV)
Groups of ten islets, isolated from the pancreas or retrieved from the liver, were transferred in triplicates to vials containing Krebs-Ringer bicarbonate buffer supplemented with HEPES and BSA (hereafter referred to as KRBH buffer). The KRBH buffer contained 1.67 mM D-glucose during the first hour of incubation at 37°C. The medium was then removed and replaced by KRBH supplemented with 16.7 mM glucose and incubated for a second hour. The medium was again removed and the islets were, in some cases, incubated with KRBH supplemented with 16.7 mM glucose and 5 mM theophylline (Apoteksbolaget, Gothenburg, Sweden) for a third hour. After retrieval of this medium, the islets were harvested, pooled in groups of 30,
and homogenised in redistilled water. Two aliquots of the aqueous homogenate were then used for DNA measurements by fluorophotometry (39). A fraction of the homogenate was mixed with acid-ethanol from which insulin were extracted overnight at 4°C. Insulin contents in incubation mediums and homogenates were determined by ELISA (Mercodia, Uppsala, Sweden).

3.21 Glucose oxidation rate (IV)

Islet glucose oxidation rates were determined according to a previously described method (40). Briefly, triplicates of ten islets were transferred to vials containing KRBH supplemented with D-[U-14C]glucose (Amersham-Pharmacia Biotech, Amersham, UK) and nonradioactive D-glucose to a final glucose concentration of 16.7 mM glucose (specific radioactivity 0.5 mCi/mM). After incubation for 90 min at 37°C, the oxidation was terminated by injection of antimycin A into the vials. By the addition of NaH₂PO₄ (pH 6.0) during a 120 min incubation ¹⁴CO₂ generated by cell metabolism was released. The radioactivity in the samples was then measured by liquid scintillation counting.

3.22 RNA extraction and GEArray (V)

RNA extraction was made by use of Oligotex mRNA kits (Kebo, Spånga, Sweden) according to the manufacturers instructions. The mRNA samples were stored at -20°C until use. Angiogenesis-1 GEArray gene expression array systems (Tebu-bio, Le Perray en Yvelines, France) were used and each array consisted of 26 coordinates containing specific cDNA fragments spotted in duplicate as well as control sequences (pUC18 as negative control; β-actin and glyceraldehyde 3 phosphate dehydrogenase (GAPDH) for loading). We compared expression of 23 genes in EC from endogenous and transplanted pancreatic islets. The microarray assays were conducted according to the manufacturers instructions. In brief, cDNA was prepared from mRNA by reverse transcription with MMLV reverse transcriptase, radiolabeled using (¹³²P) dCTP (3,000 Ci/mM), and hybridized on a positively charged nylon membrane containing the arrayed DNA. After washing, the arrays were visualized by autoradiography. Loading was adjusted based on intensity of hybridization signals to the house-keeping genes beta-actin and GAPDH, followed by scanning densitometry to quantify gene expression.
3.23 Real Time-PCR (V)
Polymerase chain reaction (PCR) was carried out with the Lightcycler real time-PCR instrument using the Faststart DNA Master CYBR Green I kit (Roche Molecular Biochemical, Mannheim, Germany). Primers for the following genes were used; angiopoietin-2 (Ang-2), basic fibroblast growth factor (bFGF), endostatin, pigment epithelium-derived factor (PEDF), pleiotrophin (PTN), Smad7, tyrosine kinase receptor 1 (Tie1), vascular endothelial growth factor (VEGF), all purchased from CyberGene (Stockholm, Sweden).

3.24 Statistical analysis (II-IV)
All values are given as means±standard error of the mean. Multiple comparisons between data were performed by analysis of variance (ANOVA; Statview; Abacus Concepts, Berkeley, CA, USA) with correction of P-values using the Bonferroni method (41) or Fisher´s protected least significant difference test. 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.
4. RESULTS AND DISCUSSION

4.1 Staining of EC in formalin-fixed and paraffin-embedded samples (I)

We found that BS-1, which binds to α-gal epitopes, stained microvascular EC in all tissues examined, with the exception of the kidney where only some of the EC, especially in the medulla, were positive. Of special interest was the finding that BS-1, in contrast to the other examined markers, consistently stained pancreatic islet endothelium. To evaluate this, we searched for unstained capillaries, i.e. blood vessels with a diameter <20 µm and preferably containing erythrocytes, without noticing any such structures. The other investigated markers [Ulex europaeus, CD34, PECAM-1 (CD31), Ox2, Ox43 and vWF (F8/86)] showed no consistency in their staining of EC in sections from either mouse or rat. Thus, antibodies against CD31 stained EC only in white adipose tissue and small intestine. It should be noted in this context that many of the tested antibodies or lectins referred to above perform well in cryosectioned tissue samples (42-45).

Isolectin B4, an iso-form of BS-1, has previously been used as a marker for microvascular EC in mouse tissues; including the islets of Langerhans (46). This lectin also binds to α-D-galactosyl residues (46), which are present on EC in many species. This may explain the ability of this lectin to stain microvascular EC in rodent tissues. We have also evaluated the ability of this isolectin to stain endothelium in endogenous and transplanted mouse pancreatic islets in formalin-fixed and paraffin-embedded material (unpublished observations). We observed no increased specificity in the staining pattern when compared with BS-1, but the background staining was slightly reduced.
4.2 Identification of endothelium in endogenous and transplanted islets (I-III)

Of particular interest was the finding that BS-1 consistently stained endothelium, not only in endogenous pancreatic islets, but also in isolated islets and syngeneically transplanted rat and mouse pancreatic islets irrespective of implantation site (I-III). This means that also newly formed EC in recently revascularized islets and the connective tissue stroma can be identified with this lectin. Moreover, these results imply that the staining properties of endothelium in islet grafts are independent of the origin of the newly formed microvessels, i.e. whether they derive from renal, hepatic or splenic blood vessels. However, the endothelium within the intrasplenic islet grafts, although they were positive, stained less intensely.

Recently, expression of \(\alpha\)-gal epitopes, i.e. those to which BS-1 binds, was demonstrated in neonatal porcine islet cells (47), whilst no expression was seen in adult endocrine cells (48). Therefore, we further evaluated the staining specificity of BS-1 by simultaneous staining with BS-1 and antibodies for insulin, glucagon or somatostatin in the same pancreatic sections (II). Neither insulin-, glucagon-, nor somatostatin-positive cells in endogenous islets were stained with BS-1. Thus, our results are consistent with the notion that \(\alpha\)-gal epitopes are not expressed in adult rodent islet endocrine cells.

4.3 Connective tissue stroma of transplanted islets (II-III)

Endogenous islets of Langerhans have a connective tissue capsule, which is a minor part of the islet. Thus, the connective tissue within the grafts, which constitutes approximately 30% of the transplant (II, III), is likely to derive, at least partially, from a foreign body reaction. Connective tissue was also formed in association with microspheres implanted into the renal subcapsular space, and constituted 57% of these grafts (II). Intraportally transplanted islets were also surrounded by connective tissue. Since most of these islets were entrapped in periportal areas, it was impossible to separate between graft or liver stroma connective tissue.
4.4 Vascular density of endogenous and transplanted islets (II-III)

The vascular density, i.e. the number of blood vessels per area, of the transplanted islets was decreased compared to that of endogenous islets, irrespective of the chosen implantation organ (II). Furthermore, islets transplanted into the spleen had an even lower vascular density than islets transplanted beneath the renal capsule or into the liver. The capillary density was markedly higher in the connective tissue stroma of grafts implanted into kidney or spleen than in the endocrine parts of these grafts. The density of stromal capillaries appeared to be higher in the immediate vicinity of the islets at all three implantation sites. (II). A markedly lower number of capillaries was found in the connective tissue surrounding microspheres implanted into the renal subcapsular space when compared to connective tissue surrounding implanted islets. Thus, an angiogenic response initiated by the cells within the transplanted islets seems to be of importance to increase the vascular density in the surrounding connective tissue. This preferential distribution of graft blood vessels to the connective tissue stroma rather than among the endocrine cells has not been previously described, and its functional importance awaits further studies. However, when adding the capillaries in the stromal and endocrine compartments, the vascular density in the total graft is almost as high as in the endogenous islets. It should be noted that a lower oxygen tension in grafted islets, as well as other microvascular disturbances (26), which may be related to the location of graft capillaries, have been previously observed. This suggests that the changed capillary distribution may be of functional importance.

The influence of the hormone production from intact endogenous islets on the function and revascularization of ectopically implanted islet grafts is largely unknown. To investigate this we used both normoglycemic and hyperglycemic recipients, i.e. animals with or without functioning endogenous islets (III). The number of islets was chosen to either reverse (500 islets) or not reverse (200 islets) hyperglycemia. The recipients were injected with alloxan prior to transplantation and they all had blood glucose concentrations >14 mM at the time of implantation. Transplantation of 500 islets fully reversed the hyperglycemia (<8 mM), while the animals transplanted with 200 islets remained slightly hyperglycemic (~11.1 mM) throughout the course of the study. The vascular density in transplanted islets was significantly decreased to the same extent, when compared to that of native islets, in both groups of recipients (III). Thus, the present study showed that a remaining normal number of native pancreatic islets does not affect the formation of new blood vessels in an islet graft. Furthermore, the possibility to cure diabetic recipients demonstrates that the function of the
islets can be adequate, even though the vascular density is decreased. We also found that the vascular density in the transplanted islets did not improve up to six months post-transplantation (III). Thus, no redistribution of graft blood vessels from the connective tissue stroma into the endocrine tissue occurred with time.

4.5 Identification of retrieved transplanted islets (IV)

Since islets implanted into the liver are injected directly into the portal vein they distribute in the portal tributaries throughout the hepatic parenchyma. Thus, they are difficult to localize, and few techniques are available for their study post-transplantation. To circumvent this problem we developed a method to retrieve transplanted islets from the liver. On an average 50-100 islets, i.e. 25-50% of those implanted, could be retrieved from each processed mouse liver. The identity of retrieved transplanted islets was confirmed by several different methods. Retrieved islets stained pink/red after intravital staining with neutral red, which allegedly selectively stains pancreatic islets (49, 50). Sections from the re-isolated islets also stained positive with an insulin antibody, and beta and alpha cell secretory granules were observed with TEM. As a final control pancreatic islets expressing enhanced yellow fluorescent protein, obtained from YC3 mice (51), were transplanted to athymic nude mice. These spontaneously fluorescent islets could be recovered to the same extent as those from other mice. Furthermore, such fluorescent islets were stained with neutral red in vivo and with antibodies against insulin in sections.

4.6 Insulin release and insulin content of retrieved intraportally transplanted islets (IV)

Insulin release from retrieved islets incubated with 1.67, 16.7 or 16.7 mM glucose + 5 mM theophylline was investigated either immediately after retrieval or after 3-4 days of culture and compared to non-transplanted freshly isolated or cultured islets. Immediately after retrieval, the insulin release was lower compared to non-transplanted control islets during all three incubations. After culture, the insulin response to 16.7 mM glucose and 16.7 mM glucose + theophylline remained markedly impaired in retrieved islets, whereas basal insulin release was similar to that from similarly cultured control islets. Retrieved transplanted islets contained less insulin, both immediately after retrieval and after culture, compared to control islets.
However, whereas the insulin content of control islets decreased markedly after culture, the insulin content of retrieved islets did not change.

4.7 Glucose oxidation rate and DNA content of retrieved intraportally transplanted islets (IV)

Glucose oxidation rates of retrieved islets were markedly lower than those of control islets when exposed to 16.7 mM glucose, both immediately after retrieval and after culture. The glucose oxidation rates were not affected by culture in any of the groups. There were no differences in DNA content between freshly retrieved transplanted islets and freshly isolated control islets, thereby arguing against the notion that the decreased glucose-stimulated insulin release, insulin content and glucose oxidation rate of retrieved islets compared to control islets merely reflect a lower amount of islet tissue in the former preparations. However, it cannot be excluded that some hepatocytes adhered to the transplanted islets during experiments on freshly obtained retrieved islets, thereby affecting the DNA content and/or function of the islets, but this is likely to be of minor importance.

Another possible explanation for the poor function of the retrieved islets is that the re-isolation procedure in itself damages the islet cells. However, we used the same amount of collagenase as for isolation of islets from the pancreas (32, 52). In separate experiments we also decreased this concentration, or even omitted it completely, and nevertheless obtained similar results. However, the number of retrieved islets was markedly reduced. The mechanical dispersion of the liver used for retrieval is similar to that used during normal islet isolations and is therefore unlikely to inflict any further functional impairment, as suggested by the similar survival of the retrieved islets. An issue of importance is to what extent the observed impaired function is dependent on the implantation organ, that is the liver, or if it mainly reflects the transplantation and engraftment trauma in itself. The present functional impairment seen in intraportally implanted islets is much more pronounced than that seen after transplantation under the kidney capsule, suggesting that the implantation organ is indeed of major importance. In confirmation of this notion, experimental studies in rodents have also indicated a lower long-term rate of function of intraportally transplanted islets (53). It might also be a difference if the islets are implanted as single islets or as a cluster of islets.
4.8 EC from endogenous or transplanted islets (V)

We were able to isolate EC from intact and dispersed freshly isolated islets, islets pre-cultured for 7 days as well as transplanted islets retrieved from both the kidney and liver. By the use of BS-1 coated Dynabeads we could separate EC from contaminating cells and achieve a purity >90%. The identity of the EC was confirmed by uptake of Dil-Ac-LDL (data not shown), and staining with the lectin BS-1, as well as antibodies against ACE and eNOS.

4.9 Angiogenesis GEArray assays (V)

In order to obtain reliable and reproducible results with the GEArray method several considerations have to be made. The main possible errors with the technique are cross hybridization, due to sequence homology of the tested genes, impurities of the examined cellular preparations or the extracted RNA. Also a correct sample labeling and performance of the hybridization procedure are of importance (54).

We obtained expression responses in all investigated groups, i.e. EC from dispersed islets, EC outgrown from freshly isolated islets or 7 days pre-cultured islets, as well as EC outgrown from retrieved transplanted islets from both the kidney and liver, when applying the angiogenesis GEArrays. We found that the expressions changed with time during culture. Thus, immediately after islet isolation, the EC only expressed endostatin, a major angiogenesis inhibitor (55). The lack of expression of other angiogenic factors should be interpreted with some caution, since it may be that the enzymatic treatment during islet isolation may have affected the possibility to obtain adequate RNA samples. However, the culture step necessary to produce the EC is likely to allow such effects to wear off.

Endostatin was expressed in EC from all investigated groups except for those obtained from islets implanted beneath the kidney capsule. EC outgrown from freshly isolated islets also expressed PEDF and PTN. PEDF normally decreases EC proliferation (56), and induces apoptosis in EC in sprouting blood vessels, but spares those in quiescent microvessels (57). Indeed, previous studies have shown that the pancreas develops substantial stromal vascularity and epithelial cell hyperplasia in the absence of PEDF (58). We also found expression of PEDF in EC obtained from islets transplanted beneath the kidney capsule. All these findings are of interest in view of our previous reports suggesting the presence of a vascular dysfunction in transplanted islets, manifested by a decreased vascular density, low oxygen pressure and low blood flow (59). It is tempting to
speculate that expression of an angiogenesis inhibitor, such as PEDF, may participate in this response. However, more quantitative studies of both the EC and the grafts as a whole are needed to verify this speculation.

The 7 days pre-cultured islet EC expressed VEGF, which is a potent, EC specific angiogenesis inducer with widespread localization in the body (55). VEGF is known to be produced in isolated islets in culture, and after transplantation (60). This is likely to reflect its upregulation by tissue ischemia (57, 61). Ang-2 was also expressed, this is a substance involved in the remodeling of vasculature. In a normal blood vessel from adults another peptide, Ang-1, is associated with the receptor Tie-2, to keep the endothelium quiescent. Up-regulation of Ang-2, by hypoxia or increased VEGF concentrations (62), disrupts this interaction between Ang-1 and Tie-2, resulting in destabilization of the blood vessels (55). This leads to active vascular remodeling providing the presence of VEGF (63). One interpretation of the expression of Ang-2 is therefore that the islet vasculature is actively reorganized during culture of these islets. Such reorganization is seen in vivo after transplantation (64), but, as discussed further below, expression of Ang-2 was seen only in some groups in the present study (see below).

It would have been expected that transplanted islets should express several angiogenic factors to improve their vascularization after implantation. Surprisingly, EC obtained from syngeneically transplanted mouse islets into the liver only expressed endostatin, i.e. an angiogenesis inhibitor. However, when we performed the same test on intraportally transplanted human islets retrieved from nude mice, they expressed several angiogenic factors or receptors; namely Ang-2, bFGF, endostatin, Gro1, PEDF, PTN, Smad7, Tie1, VEGF-D/FGF and VEGF. The reason for this discrepancy between species is not clear and the accuracy of these experimental results has to be verified by an independent and species-specific method (such as Northern or RT-PCR analyses) after performing such cross-species hybridization experiments. The differences may be due to differences in the degree of engraftment or merely reflect species differences. Thus, it was previously reported that transplanted human islets responded better to glucose stimulation 12 weeks as compared to 4 weeks post-transplantation (65). Another explanation can be that human islet preparations are less pure and contain contaminating tissues with a different EC population than the one found within islets.

In the normal situation of mature tissues, EC quiescence without proliferation prevails. During tissue ischemia an angiogenic switch, where the inhibitors are downregulated and angiogenesis inducers upregulated normally occurs. It can be hypothesized that the angiogenic switch is operating in transplanted islets, which results in a reduced intrasilet vascular
density. This may also explain why most blood vessels are found in the connective tissue stroma of the grafts. The newly developed methods described in this thesis now provide a unique opportunity to further investigate the engraftment and angiogenic processes in transplanted islets.
5. CONCLUSIONS

- The lectin BS-1 stains endothelium in both endogenous and transplanted rodent islets of Langerhans in formalin-fixed, paraffin-embedded sections. Other investigated markers showed no consistency in their staining pattern during these conditions.

- The vascular density of syngeneically transplanted islets of Langerhans is decreased when compared to endogenous islets irrespective of implantation site.

- A great majority of the blood vessels in the islet transplants were located in the graft connective tissue stroma.

- Plastic microspheres implanted beneath the kidney capsule induce a much lower degree of vascular density in stroma when compared to islet grafts. This is likely to reflect the lack of released angiogenic factors from the inert microspheres.

- Neither hyperglycemia nor prolonged transplantation time increased vascular density of syngeneically transplanted islets when compared to endogenous islets.

- Syngeneically transplanted islets retrieved from the liver have decreased insulin release, insulin content and glucose oxidation compared to isolated non-transplanted islets.

- EC can be isolated from freshly isolated, cultured and transplanted pancreatic islets.

- EC obtained from endogenous and transplanted islets differ in their expression of angiogenically active substances.

- The results presented above provide a useful platform for future studies of the morphology and function of islet EC, especially with a view for elucidating changes induced by islet transplantation.
6. SWEDISH RÉSUMÉ

odlas i ett odlingskåp i väntan på transplantationen överlever cellerna genom diffusion av syrgas från den omgivande miljön. Syre kan normalt endast transporteras ca 200 µm i tillräcklig mängd för att en cell skall överleva, och en genomsnittsö är just cirka 200-300 µm stor i diameter. Ett annat problem är att det kärlnät som finns i öarna bryts ner och försvinner efter några dagars odling. Det är av yttersta vikt att detta blodkärlsnät återuppbyggs snarast efter transplantationen för att öarna ska kunna överleva i den nya miljön. Detta sker genom att öarna utsöndrar olika ämnen, som stimulerar inväxten av nya blodkärl i den omkringliggande vävnaden och de transplanterade öarna. En av de celler som är involverad i denna revaskulariseringsprocess är endotelcellen. Endotelceller är de celler som finns inuti blodkärlen och skiljer blodet från den omkringliggande vävnaden. Endotelceller har ett flertal uppgifter; bland annat att reglera blodflöden, vara en skyddande barriär (blod och hjärnbarriären), hjälpa till vid angiogenes (kärlnybildning) och mediera upptag av ämnen från blodet. Endotelceller är en mycket heterogen grupp av celler, och de har anpassat sin funktion till de behov som råder i det organ eller vävnad där de återfinns. Denna heterogenitet gör det svårt att hitta markörer som fungerar för alla endotelceller.

I delarbete nr I studerade vi olika endotelcellsmarkörer för att kunna påvisa öarnas endotelceller. Vi kom fram till att ett ämne vid namn Bandeiraeasimplicifolia märker in öendotel, både i endogena och transplanterade öar. Med hjälp av detta kunde vi påvisa endotelceller i öarna samt nybildade kärl i de transplanterade öarna.

I delarbetena nr II och III studerades om kärlnät i öarna återuppbyggdes efter transplantation och om antalet kärl blev detsamma efter transplantationen. Vi kunde konstatera att öarnas kärlantal reducerades kraftigt jämfört med vad som ses i endogena öar. Vi noterade även att transplantatet bestod av ca 30% bindväv och att de flesta blodkärl återfanns i denna bindväv. Anledningen till detta är okänd, men vid transplantation av mikrosfärar (plastmaterial) i samma storlek och antal som öar, noterades att levande celler i öarna inducerade den kärlinväxt som noterats i bindväven kring dessa. Det spelade ingen roll vilket organ (under njurkapseln, i levern eller mjälten) öarna transplanterades till, kärltätheten blev densamma i alla implantationslokaler. Inte heller en längre tid efter transplantationen eller förhöjda blodsockervärden påverkade kärltätheten.

När öar transplanteras till levern sprids de ut i alla delar av leverparenkymet, vilket närmast omöjliggör studier av dem. För att kunna studera dem närmare behövs metoder för att återfå dessa öar efter transplantation. Därför utvecklade vi en metod i delarbete nr IV, som möjliggjorde återisolering av de i levern transplanterade öarna. Det visade sig att öarnas insulininnehåll och deras förmåga till insulinfrisättning
drastiskt sänkts jämfört med isolerade icke-transplanterade öar. Denna sänkning verkade inte bero på vårt sätt att isolera öarna, utan på transplantationen till levern i sig.


Slutsatserna av dessa arbeten är att lektinet Bandeiraea simplicifolia är en väl fungerande markör för endotelceller, i såväl endogena som transplanterade Langerhanska öar. Kärltätheten efter ötransplantation är reducerad jämfört med endogena öar, och kärltätheten påverkas inte av hyperglykemi och tycks ej förbättras med tiden efter transplantation. Vi kan isolera öar transplanterade till levern och sådana öar har sänkt insulinsekretion, insulininnehåll och glukosoxidation. Slutligen har vi visat att vi kan utvinna öendotelceller från endogena och transplanterade öar, och dessa skiljer sig i uttrycket av angiogenesaktiva substanser, vilket kan ha betydelse för öarnas kärlnybildning och överlevnad efter transplantation. Vi har nu tillgång till metoder som möjliggör fortsatta och nödvändiga studier av engraftmentprocessen av transplanterade Langerhanska öar.
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8. References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to October, 1985, the series was published under the title “Abstracts of Uppsala Dissertations from the Faculty of Medicine”.)