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Perspectives submission

The beta cell/EC axis: how do islet cells talk to each other?

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Abbreviations: Cx Connexin; EC Endothelial cell; ECM Extracellular matrix; eNOS Endothelial nitric oxide synthase; EPC Endothelial progenitor cell; HGF Hepatocyte growth factor; JNK c-Jun N-terminal kinase; NF-kB Nuclear factor kappa-B; Pdx-1 Pancreatic and duodenal homeobox 1; TGF-β Transforming growth factor β; VEGF-A Vascular endothelial growth factor A;

Within the pancreatic islet, the beta cell represents the ultimate biosensor. Its central function is to accurately sense glucose levels in the blood, and consequently release appropriate amounts of insulin. As the only cell type capable of insulin production, the beta cell must balance this crucial workload with self-preservation and, when required, regeneration. Evidence suggests that the beta cell has an important ally in intra-islet endothelial cells. As well as providing a conduit for delivery of the primary input stimulus (glucose) and dissemination of its most important effector (insulin), intra-islet blood vessels deliver oxygen to these dense clusters of metabolically active cells. Furthermore, it appears that endothelial cells directly impact insulin gene expression, secretion and beta cell survival.

This review discusses the molecules and pathways involved in the crosstalk between beta cells and intra-islet endothelial cells. The evidence supporting the intra-islet endothelial cell as an important partner for beta cell function is examined to highlight the relevance of this axis in the context of type 1 and type 2 diabetes. Recent work which has established the potential of endothelial cells or their progenitors to enhance the reestablishment of glycaemic control following pancreatic islet transplantation in animal models is discussed.

THE ISLETS OF LANGERHANS

The pancreatic islet - the ultimate biosensor

Distributed throughout the exocrine pancreas, the islets of Langerhans house the central regulator of glucose homeostasis - the beta cell. Beta cells are the major cellular component of islets,

making up approximately 80% of islet cellularity in mice, and 50-60% in humans. The remainder of the islet comprises other endocrine cells (including glucagon-secreting alpha cells, somatostatin-secreting delta cells, pancreatic polypeptide-secreting gamma cells, ghrelin-producing epsilon cells), as well as endothelial cells and supportive cells including pericytes.

The body's response to glycaemic load is extremely rapid – circulating plasma insulin levels increase within 1 minute of ingestion of food as beta cells release preformed insulin from granules. In addition to this rapid first phase response, islets continue to release insulin in a pulsatile fashion over a longer period (second phase response) in the presence of a persisting glucose stimulus. Pulsatile insulin release is coordinated within the islet and throughout the pancreas by intercellular gap junctions and ATP spreading. Pancreatic hormone secretion is additionally regulated by the autonomic nervous system and endocrine cells in murine islets are heavily innervated by parasympathetic and sympathetic neurons. Human islets, on the other hand, are only sparsely innervated, with most neurons contacting intra-islet smooth muscle cells (1). Thus, neuronal control of human beta cells may be indirect and mediated via the vasculature. The exquisite regulation of blood glucose is vital for the proper function of multiple systems – including neuronal, cardiovascular and renal. Microvascular beds throughout the body are sensitive to glucose-mediated toxicity. In diabetes, hyperglycaemia leads progressively to associated macrovascular and microvascular complications including retinopathy, nephropathy, neuropathy and cardiovascular disease (2).

Intra-islet endothelial cells

Pancreatic islets are highly vascularised and receive 10% of pancreatic blood flow despite comprising only 1-2% of tissue mass. Small islets (<100 μm) are incorporated into the microcapillary beds of the exocrine pancreas, while larger islets are supplied by up to three dedicated arterioles. The vasculature is crucial for pancreatic development, largely via VEGF-A mediated signals. Intra-islet endothelial cells are thin and highly fenestrated, allowing for sensitive detection of blood glucose levels and rapid dissemination of secreted insulin (Figure 1), and it has been postulated that every beta cell is in contact with a vascular endothelial cell. Islet cellular arrangement differs among species. While rodent islets classically consist of a beta cell core surrounded by a non-beta cell mantle, the structure of human islets is more irregular and varies with islet size and perfusion. One in-depth study of human islets proposed a trilaminar arrangement comprising a layer of beta cells sandwiched between two alpha cell layers with vessels lining both faces, and this structure folded upon itself to form an islet (3). This model suggests the existence of more heterologous intercellular contacts compared with rodent islets.

In common with other microvascular cells, intra-islet endothelial cells express CD31 and von Willebrand factor, internalise acetylated LDL, upregulate endothelial markers upon activation including E-selectin and L-selectin, contain Weibel-Palade bodies in the cytoplasm and form tight junctions (4). In humans, intra-islet endothelial cells are the only microvascular cell type found to express high levels of alpha-1 proteinase inhibitor at cell junctions (4), which appears to maintain them in a non-proliferative state.

In addition to providing a conduit for blood flow, intra-islet endothelial cells directly enhance insulin transcription, secretion and stimulate beta cell proliferation (5). This may be through the

secretion of humoral factors, the production of basement membrane components or via cell-contact dependent mechanisms.

THE BETA CELL/EC AXIS

Within the pancreatic islet, the intimate association between beta cells and endothelial cells has implications for both cell types, and it is more appropriate to consider the components within the multicellular islet as a mosaic unit, rather than distinct cellular entities. Multiple mechanisms for driving bidirectional communication between beta cells and endothelial cells exist – including those mediated by soluble, extracellular matrix and cell-bound molecules (Figure 2).

Soluble factors

Within the pancreatic islet, soluble factors are an important component of the crosstalk between beta cells and endothelial cells. While multiple soluble islet-derived factors have been implicated in beta cell survival and insulin secretion, it is only recently that their cellular origin has been more accurately investigated.

As the major secreted beta cell product, insulin acts in an autocrine and paracrine manner to promote beta cell survival. Additionally, insulin causes the upregulation of eNOS in endothelial cells (6) which is likely to promote intra-islet blood flow and enhance its own dissemination. Beta cells secrete VEGF-A in large amounts early in development and throughout adult life (7). VEGF-A expression is further upregulated in islets by hypoxia and glucose (8), and is important for the establishment of native intra-islet vasculature (9), maintenance of beta cell mass (10) and the revascularisation of islets following transplantation (11). In endothelial cells, VEGF-A

induces cell migration, proliferation and maintains fenestrations. The potential of VEGF-A to enhance the transplantation of pancreatic islets has been thoroughly investigated in animal models (12; 13). However, additional VEGF-A is not necessarily beneficial - while VEGF-A overexpression may increase the number of intra-islet vascular endothelial cells, the resultant vasculature is dysfunctional and islet function is impaired (14). Within islets the abundant production of proangiogenic VEGF-A is balanced by angiostatic and antiangiogenic factors, including thrombospondin-1 (15). Thus it appears that in the steady state VEGF-A production is strictly controlled to maintain the intra-islet vasculature at an appropriate density and with functional architecture.

Vascular endothelial cells produce multiple factors that modulate gene expression, proliferation and cell survival in beta cells. In islets, thrombospondin-1 is almost exclusively expressed by the intra-islet endothelium. Thromobspondin-1 is upregulated in human islets by high glucose (16) and while knockout mice have increased beta cell mass and improved revascularisation of transplanted islets (17), they are glucose intolerant and display impaired islet function (15). In addition to its anti-angiogenic properties, thrombospondin-1 activates latent TGF-β (a potent regulator of adult beta cell insulin gene transcription and islet function (18)). Endothelin-1, a vasoconstrictive protein, is produced by endothelial cells and may directly stimulate insulin secretion in beta cells (19). HGF is a proangiogenic factor expressed by intra-islet endothelial cells (20). HGF signals by high affinity binding to its receptor c-Met, which is expressed by beta cells. The overexpression of HGF in islets reduces beta cell death in islet transplantation models (21), and may be responsible for beta cell proliferation during pregnancy (20). Most recently, conditional knockout of HGF signalling in the pancreas increased the susceptibility of mice to

streptozotocin-induced hyperglycaemia (22). While these mice had normal glucose homeostasis and beta cell mass, they were more vulnerable to beta cell damage mediated via the NF-kB pathway. Thus, HGF/c-Met signalling is likely to be of importance for islet survival, particularly during cytokine-mediated damage, as occurs following islet transplantation and during the development of diabetes.

Extracellular matrix proteins

ECM proteins form depots for growth factors to support cellular proliferation and function. The majority of ECM proteins exist within basement membranes (underlying vessels and other cellular structures) or in the interstitial matrix. Pancreatic islets are encased within a peri-islet basement membrane and associated interstitial matrix, containing multiple ECM components including laminins, perlecan, collagen type IV and nidogens (23). In addition, intra-islet blood vessels have their own basement membrane (5). In the mouse islet, endocrine cells are either associated with peri-islet or vascular basement membranes while in human islets intra-islet blood vessels contain a double basement membrane (24).

Within pancreatic islets, the ECM plays a multifaceted role and is implicated in islet development (25), function (5) and survival (26). The peri-islet membrane is an important physical barrier for immune cell infiltration during diabetes development (23; 27). Beta cells do not contribute to the islet ECM directly and instead depend on intra-islet endothelial cells to synthesise their basement membrane (5). ECM components increase beta cell survival and proliferation within pancreatic islets (26), and promote insulin gene expression (5), mainly via β1-integrins on the surface of beta cells. This has been demonstrated in experiments where the

improved insulin secretion found in beta cells cultured with intra-islet endothelial cell-derived factors (28) or attached to ECM (29) is prevented by anti β1-integrin blocking antibodies.

Cell surface proteins

There are multiple cell surface proteins that are likely to be involved with beta cell/EC crosstalk. Cellular contacts within pancreatic islets are crucial for calcium flux and insulin secretion following glucose sensing, and glucose-stimulated insulin release is enhanced in whole islets compared to isolated beta cells (30). These contacts allow the islet to respond to stimuli as an entire unit via coordinated calcium oscillations that amplify insulin secretion, and quickly return to the resting state when appropriate. Connexins, ephrins and cadherins are expressed in pancreatic islets and have all been implicated in beta cell function. Here we will concentrate on the connexin molecules - a family of proteins well known for their gap junction properties that may play a newly appreciated role in paracrine signalling (31).

Connexins. Connexins cluster on the cell surface at gap junctions, oligermizing to form hemi-channels which dock with identical or different hemi-channels on neighbouring cells. The major beta cell connexin is Cx36 (this nomenclature indicates a connexin with subunits of 36 kDa), which was initially thought to be restricted to neurons. Cx36 junctions allow the passage of ions (in particular Ca^{2+} and cAMP (32)) between beta cells during synchronized glucose-induced Ca^{2+} oscillations (30) and work to dampen Ca^{2+} elevations following membrane depolarizations (33). Thus Cx36 within islets allow less excitable beta cells to act as a buffer, suppressing electrical activity in their neighbours. Islets deficient in Cx36 have increased basal insulin release with diminished glucose-stimulated insulin responses (30) and, in humans, Cx36 is expressed on a

genomic region that mediates increased risk of type 2 diabetes (34), further suggesting an important role for this molecule in islet function.

Cx43 and Cx45 have also been identified in pancreatic islets, and are specifically expressed on intra-islet endothelium rather than on beta cells (35). Interestingly, islet-specific overexpression of Cx43 increases islet size and insulin content (36). A new islet-associated connexin, Cx30.2 has been very recently described, and is expressed at cell-cell contacts in both endothelial and beta cells (37). Thus, it is tantalizing to suggest that beta cells and endothelial cells may communicate directly via heterotypic connexin junctions, as has been postulated to occur in the retina (38) and kidney (39). Alternatively, recent evidence that uncoupled hemi-channels sample small molecules (eg. ATP) from the extracellular environment (31) suggests that the role of connexins within the islet may not be limited to physical cellular contact as they may also participate in paracrine signalling.

IMPLICATIONS OF THE BETA CELL/EC AXIS IN DIABETES

Type 1 diabetes

Type 1 diabetes is caused by the specific autoimmune destruction of beta cells within pancreatic islets. Cells infiltrating pancreatic islets during the early stages of type 1 diabetes include macrophages and cytotoxic T cells. The intra-islet vasculature represents a barrier for infiltrating autoreactive cells in type 1 diabetes and thus, endothelial cells have been implicated as an important target in the diabetogenic process. During islet damage, such as after streptozotocin treatment in mice, lymphocytes adhere to the intra-islet endothelium more readily (40). In

addition to permitting the entry of infiltrating immune cells, there is evidence that cytokine-activated intra-islet endothelial cells mediate beta cell apoptosis via the release of nitric oxide, which activates apoptotic caspase and DNA damage pathways (41; 42). Human intra-islet endothelial cells become activated and upregulate their expression of adhesion molecules and proinflammatory cytokines following infection with coxsackie B virus (43) – a postulated initiator of type 1 diabetes.

Type 2 diabetes

The pathogenesis of type 2 diabetes includes genetic and environmental factors that result in elevated circulatory free fatty acid levels and insulin resistance, followed by beta cell dysfunction. Mechanistically, glucotoxicity, lipotoxicity, pro-insulin biosynthesis and advanced glycation end products act in concert to inhibit beta cell function and increase apoptosis (44). In the hyperglycaemic, hyperlipidemic type 2 diabetes patient, vascular endothelial cells are in constant contact with these potentially cytotoxic substances. While hyperglycaemia may directly induce apoptosis in intra-islet endothelial cells by upregulating the expression of reactive nitric oxygen species via a JNK-mediated pathway (45), an earlier vascular-associated pathogenic mechanism has been postulated to exist. In models of type 2 diabetes, early microvasculature changes are detectable within islets and precede the onset of hyperglycaemia, overt endothelial cell destruction and beta cell degeneration. In the Zucker fatty rat, endothelial thickening and loss of fenestrations were accompanied by *increases* in VEGF-A mRNA and protein levels (46) – contrary to what might be predicted by VEGF-A knockouts that have a similar islet vascular phenotype (47). However, VEGF-A causes the overproduction of ECM proteins and in this way may contribute to inflammation and fibrosis in islets via the recruitment of macrophages and

other leukocytes (48; 49). Thus, onset of type 2 diabetes may be preceded by VEGF-A-driven endothelial dysfunction, overproduction of ECM and inflammation, which combine to bring about the ultimate destruction of the beta cell.

IMPLICATIONS OF THE BETA CELL/EC AXIS IN PANCREATIC ISLET TRANSPLANTATION

Islet transplantation

Despite improved insulin delivery techniques and drug design, around 90% of diabetic patients develop severe cardiovascular complications over time. Pancreatic islet transplantation represents a promising therapy for type 1 diabetes that is effective in reducing HbA1c and hypoglycaemia (50; 51) and is beginning to yield improved protection from diabetes-associated complications (52). Upon organ procurement, islets are separated from their dense capillary network and rich blood supply and are dependent upon the diffusion of oxygen and nutrients from the islet periphery until revasularisation can occur. During enzymatic isolation from the donor pancreas, islets come under a myriad of cellular assaults including ischemia, physical stress, and loss of contact with key basement membrane proteins and signalling molecules (53), resulting in a substantial loss of viability before transplantation. Clinically, islets are cultured prior to transplantation, and intra-islet endothelial cells reduce rapidly in this period (54 and *CFJ unpublished*). Therefore, not only do islet transplants face multiple cytotoxic challenges, but they are depleted of the building blocks required for rapid revascularisation. In the first few days post-transplantation islets undergo a dramatic remodeling process accompanied by changes in insulin content and hypoxic and inflammatory insults have a detrimental effect on islet survival and

function. A major limitation for islet transplantation is the substantial amount of cell death seen in this early post-transplant period which accounts for the loss of up to 60-80% of transplanted islet mass (55). This necessitates the use of multiple donors for each recipient – a major limitation for the roll out of this therapeutic option into the wider type 1 diabetes community.

Revascularisation of transplanted islets

Many groups have examined revascularisation events following islet transplantation. One difficulty has been correlating results from the renal subcapsular mouse model in which an aggregate of pristine rodent islets are transplanted into a syngeneic avascular site, to the human situation, where islets of variable purity and quality are dispersed throughout the portal circulation of an often hyperglycaemic, immunosuppressed patient. Nevertheless, the field has gained important insight into the mechanisms underlying islet revascularisation. This has been further enabled by powerful models such as the transplantation of islets into the rodent anterior chamber of the eye, to allow real-time microscopic analysis of cellular events (56).

Unlike solid organs, transplanted islets are not directly reconnected to the blood supply. Following intraportal transfusion, around half of surviving transplanted islets remain within the portal vein tributaries, while the remainder migrate further into the vessel wall (57). Vascular sprouting, angiogenesis and revascularisation occur within the first few days post-transplantation. These processes involve recruited bone marrow-derived cells, recipient local vascular cells and donor ‘passenger’ endothelial cells derived from the islet transplant itself (54; 58; 59). The contribution from donor cells may explain the superior function of freshly isolated islets (60), which are endowed with additional donor intra-islet endothelial cells, and small islets

(61), which are less dependent on revascularisation. Multiple bone marrow lineages, including hematopoietic, mesenchymal and endothelial, all participate in the early stages of islet engraftment, via the production of soluble factors, recruitment of accessory cells and/or incorporation into newly formed vasculature. Ultimately, the functional intra-islet vasculature in a transplanted islet is a mosaic of donor and recipient-derived cells, the majority of these being generated from existing local vessels, with bone marrow-derived cells comprising less than 10% of long term intra-islet vasculature (57). Islet engraftment is a slow process, and while islet blood flow is reestablished in 7-14 days (62), the maturation of these vessels is likely to take several months. In addition, immunosuppressive drugs, including mTOR inhibitors (eg. rapamycin), potentially compound the problem as they have been found to inhibit angiogenesis in human intra-islet endothelial cells (63). Although some studies, including post-mortem analysis of an islet transplant recipient (64), show little difference in the ultimate vascular density of transplanted islets, most studies suggest that revascularised islets display a decreased vascular density and lower oxygen tension compared to native islets, regardless of the transplantation site (65). Recent work using an accurate microsphere technique showed that the perfusion of intraportally transplanted islets was 5% that of native islets at 1 month post-transplantation, with 24% the vascular density (57).

FUTURE DIRECTIONS TO IMPROVE ISLET TRANSPLANTATION

Considering the importance of the vasculature for islet function, it is of little surprise that research has been directed at enhancing the revascularisation response post-transplantation. While a number of molecules and pathways have been targeted, the approach showing most

potential to date is the overexpression of VEGF-A by transplanted islets. However, as discussed above, the question of optimal VEGF-A dose for islet vasculature is not a simple one. While VEGF-A is crucial for the vascularization of islets during development and following transplantation (7; 47), continued beta cell overexpression of VEGF-A can impair islet morphology and result in poorly functioning islets with an inflammatory phenotype (14; 49).

Another approach to improve outcomes for islet transplantation aims to promote beta cell survival (66; 67). While multiple studies have shown potential for maintaining viable beta cells in the early post-transplant period, it is known that even long term-surviving islets have impaired function and this may be due to the paucity of functional intra-islet vasculature. Human embryonic stem cells may now be differentiated into insulin-producing glucose responsive cells that express markers of islet cell development such as Pdx-1 (68) and cure diabetes in rodent models (69). While stem cell-derived beta cells hold enormous promise for alleviating the problem of islet donor shortage, it is likely that these cells will need to be co-transplanted with cells or factors capable of directing their appropriate differentiation and supporting beta cell survival. Thus, assuming we are able to overcome the acute phase of immunological and cytotoxic attack of the islet mass, a parallel challenge is to establish appropriate vasculature, sufficient to support an optimally functioning pancreatic islet in the longer term.

Restoration of the beta cell/EC axis with endothelial progenitor cell therapy

A cellular co-therapy at the time of transplantation is one strategy to improve islet revascularisation and/or enhance beta cell function. The ideal candidate should be able to migrate to the required site, deliver supportive factors, and persist for the appropriate time. Mesenchymal

stem cells stimulate vessel ingrowth into islets *in vitro* (70) and their co-transplantation improves islet transplant outcomes in rats (71). While somewhat efficacious, concerns linger regarding the potential invasiveness and pluripotency of mesenchymal stem cells especially in the immune-compromised transplant population.

Endothelial progenitor cells (EPCs) are a circulating bone-marrow derived cell population first described by Asahara and colleagues (72). EPCs are able to home to sites of tissue damage or ischemia and can participate in wound healing, post-natal vasculogenesis and re-endothelialisation of blood vessels (73). Over 200 clinical trials involving EPCs are currently underway and significant progress has been made in the utilisation of these cells as a therapeutic tool. Given their complex interactions with multiple cell types, augmentation of EPC function is as important as increasing gross peripheral numbers. Human autologous cell therapies using EPC-containing products (such as bone marrow or mobilized peripheral blood) are feasible and effective in the treatment of coronary and peripheral ischemic syndromes (74). Most recently, human clinical cell therapy trials have applied bone marrow derived mononuclear cells, CD34+ or CD133+ isolated hematopoietic progenitor cells because of their easy accessibility and safety (reviewed in(75)).

EPCs represent an ideal candidate to provide an engraftment niche for transplanted islets. EPCs may enhance islet engraftment in multiple ways - by providing not only the building blocks for revascularisation, but also angiogenic and cell survival factors to augment the process. EPCs secrete multiple factors including HGF, which enhances the survival of transplanted islets and stimulates beta cell proliferation. While mature endothelial cells lack the survival characteristics

and plasticity to re-establish intra-islet vasculature, EPCs possess these properties. In addition, EPCs isolated from peripheral blood or bone marrow permit the use of autologous EPCs, lessening the risk of sensitisation to multiple alloantigens in the transplant setting. Considering the ability of EPCs to migrate to distant sites, alternate forms of delivery including intravenous infusion of exogenous EPCs or upregulation of endogenous EPCs from the bone marrow, may be considered as an adjunct to pancreatic islet transplantation in the future. Another alternate mode of delivery is the construction of mosaic structures *in vitro* (76), which may comprise combinations of islet cells, beta cell progenitors, EPCs, mature endothelial cells and/or mesenchymal stem cells, prior to transplantation. This approach has the advantage of delivering a minimal number of adjunct cells placed in the optimal anatomical location to exert their supportive roles. Consideration should also be given to additional pro-angiogenic supportive cells such as microvascular pericytes, which encircle capillaries and microvessels and regulate microvascular physiology (77), and pro-angiogenic myeloid cells such as Gr1+CD11b+ cells, which are also mobilised from the bone marrow in response to G-CSF, migrate to sites of neovascularisation and promote angiogenesis (78).

In 2012, Kang and colleagues (79) demonstrated the potential of human EPCs for enhancing the engraftment of transplanted porcine islets in an immunodeficient mouse xenograft model. After co-transplanting EPCs with islets under the kidney capsule they saw an increase in vascular cell density within the first two weeks of transplantation. Despite showing no difference in vessel density at 1 month, there was improved glycaemic control in co-transplanted animals due to an increase in engrafted beta cell mass, highlighting the impact of hastening early revascularisation events. More recently, researchers have used syngeneic models to show that co-transplanted

murine EPCs improve the cure rate, function and vascularization of a marginal transplanted islet mass (80 and *CFJ unpublished*). Observations that the EPCs localized within and around the islet mass and that VEGF was produced by these cells, suggest that EPCs may act in a paracrine and autocrine manner to improve islet engraftment. Taken together, these studies support the notion that EPCs, or the factors they produce, may be of benefit in enhancing the survival and engraftment of transplanted pancreatic islets.

THE INTRA-ISLET ENDOTHELIAL CELL – A KEY PLAYER IN DIABETES AND ISLET TRANSPLANTATION?

While the destruction of otherwise healthy beta cells is a hallmark of type 1 diabetes, type 2 diabetes is characterized early by beta cell dysfunction. Regardless of these differences, it is clear that the vasculature, in particular within the pancreatic islet, is likely to play an important role in both diseases. Likewise, the successful reestablishment of intra-islet vasculature following pancreatic islet transplantation will be important both for beta cell survival in the early post-transplant period and optimal islet function in the longer term.

As such the intra-islet endothelial cell may represent an important common breaking point – whereby failure of this multifunctional cell type could result in multiple downstream events with detrimental effects on glycaemic control. Dysfunctional intra-islet endothelium is likely to enhance the recruitment and infiltration of autoreactive cells, and ultimately further stimulate the autoimmune process. Disruption of vascular tone and suboptimal intra-islet blood flow will

result in altered dissemination of insulin, interrupted clearance of toxic metabolites and reduced provision of blood-borne oxygen and nutrients. Considering the vascular endothelial cells themselves, alteration in phenotype during islet isolation, culture and transplantation would perturb the production of ECM proteins, secreted beta cell-supportive factors and direct cellular signals that are so crucial for the proper operation of the pancreatic islet unit.

In short, a dysfunctional intra-islet vascular endothelium may contribute to the progression of type 1 diabetes, worsening of type 2 diabetes, and the failure of transplanted pancreatic islets. Therapies that prevent breakdown of the intricate beta/endothelial cell axis within the pancreatic islet, or restore this crosstalk once it has been interrupted, have the potential to improve outcomes for diabetic patients in the future.

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Figure legends.

Figure 1. Ultrastructure of pancreatic islets. TEM image of a native islet in the mouse pancreas. Thin endothelial cells of intra-islet capillaries are only separated by a basement membrane (arrows) from the islet cells. The characteristic abundant diaphragm-covered fenestrations are observable (arrowheads). Reprinted with permission from Nyqvist et al. (81).

Figure 2. Proposed mechanisms of intercellular communication within the pancreatic islet. *Soluble factors:* Beta cells secrete large amounts of vascular endothelial growth factor-A (VEGF-A) which is mitogenic for endothelial cells and crucial for maintaining the density and specialty phenotype of fenestrated intra-islet endothelial cells. Insulin induces changes in endothelial cells, including the upregulation of eNOS. Endothelium-derived factors, including hepatocyte growth factor (HGF), improve beta cell survival and promote insulin transcription/secretion. Other endothelial cell-derived factors include thrombospondin (TSP-1), fibroblast growth factor (FGF) and vasoconstrictive endothelin-1. *Extracellular matrix proteins:* collagens, laminins and preoteoglycans interact with β1-integrins on beta cells to enhance islet cell survival and function. In the islet the majority of these components are synthesized by the intra-islet endothelial cells. *Cell surface molecules:* Connexin 36 is the major islet connexin and is involved in coordinated pulsatile insulin release. Connexins are capable of forming homo- or hetero-junctions to directly pass molecules between cytoplasms, while uncoupled connexin hemi-channels may sample ATP from the extracellular environment. Other cell surface molecules implicated in intercellular signalling in the islet include ephrin A and E-cadherin.