

Microvascular development: learning from pancreatic islets

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Summary

Microvascular development is determined by the interplay between tissue cells and microvascular endothelial cells. Because the pancreatic islet is an organ composed mainly of endothelial and endocrine cells, it represents a good model tissue for studying microvascular development in the context of a tissue. In this review, we will describe the special morphology of islet capillaries and its role in the physiologic function of islets: secretion of insulin in response to blood glucose levels. We will speculate on how islet-secreted VEGF-A generates a permeable endothelium that allows insulin to pass quickly into the blood stream. In addition, we speculate on how endothelial cells might form a capillary lumen within the islets. At the end, we look at the islet microvasculature from a medical point of view, thus describing its critical role during type I diabetes and islet transplantation. *BioEssays* 26:1069–1075, 2004.

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Introduction

Pancreatic islets consist of four endocrine cell types: the beta cells that secrete insulin and form the islet core, and the non-beta cells (alpha, delta and PP cells) that form the islet mantle (Fig. 1A).⁽¹⁾ The size of islets can range from less than 100 cells or 50 μm in diameter to more than 5000 cells or 500 μm in diameter. Studies using vascular corrosion casts have shown that large islets are supplied by 1–3 arterioles.⁽¹⁾ These arterioles penetrate the islet core through discontinuities of the non-beta cell mantle and branch into the capillary network, which empties into venules in the islet periphery (Fig. 1A).

The islet capillary network is about five times denser than the capillary network of the exocrine tissue.⁽²⁾ Likewise, relative to the tissue mass, five times more blood is flowing through the pancreatic islets than exocrine pancreatic tissue.⁽³⁾ The capillary network in islets is branched and has about ten times more fenestrae than capillaries of the exocrine tissue (Fig. 1B,C). The diameter of the capillary or vascular lumen ranges from 4 to 5 μm .⁽²⁾

On average, only one tight junction is visible on a section through an islet capillary.⁽²⁾ However, sections through capillaries are often found with no cellular junctions or two junctions that connect microvascular endothelial cells with one another.⁽⁴⁾ Most of the endothelial cell body that surrounds the vascular lumen is extremely thin; about 100 nm in width. The basement membrane or basal lamina is located between the pancreatic beta cell and endothelial cell and is less than 500 nm in width.⁽⁴⁾

Insulin is stored in secretory granules (approximately 300 nm in diameter) (Fig. 1C). The granules closest to the basal membrane of pancreatic beta cells are therefore separated from the arterial blood stream by no more than 500 nm. When the arterial blood enters the pancreatic islet, it distributes into a dense network of sinusoidal capillaries, with a morphological resemblance to the renal glomerulus.⁽³⁾ There are different views on how the blood flow is directed through the islet. According to one view, the blood flow is not random, but organized so that arterial blood first reaches the beta cells and then the non-beta cells. This is in line with the observation that arterioles enter the islet at discontinuities in the non-beta cell mantle and then branch from the islet core towards the islet periphery (Fig. 1A). According to other views, however, the arterial blood flow already branches at the islet periphery or is entirely random. The discrepancies might, in part, be explained by the large heterogeneity of islets.

The principal morphology of capillaries in other tissues is roughly the same: the vascular lumen is always around 5 μm in diameter, and the endothelial cell body is always relatively thin (100 to few hundred μm). However, there are some major differences with regard to the vascular density, the morphology of the endothelial cell body as well as the basement membrane.⁽⁴⁾ For example, the exocrine pancreatic tissue has five times less capillaries than islet tissue. Moreover, brain capillaries have no fenestrae at all, and liver capillaries are not separated from the tissue cells by a basement membrane.

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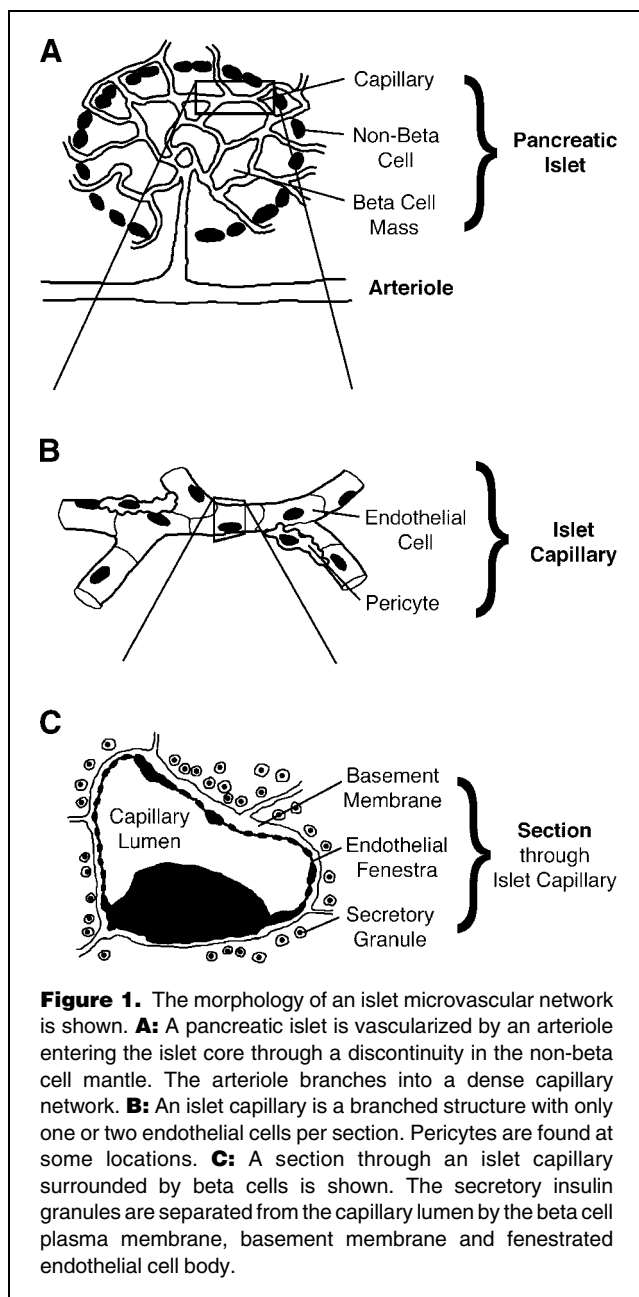
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Abbreviations: NOD, non-obese diabetes; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VVO, vesiculo-vacuolar organelle; T cell, thymus-derived cell



The significance of tissue-specific microvascular morphologies is not yet understood. Using the pancreatic islet as a model for a vascularized tissue, we would like to focus the reader's attention on the function and development of the microvasculature in a healthy and diseased tissue.

VEGF-A and the secretory function of pancreatic islets

High expression levels of vascular endothelial growth factors (VEGFs) observed in endocrine cells have been recently shown to be responsible for the strong islet vascularization and

fenestration.^(5,6) The best-characterized member of the VEGF family, VEGF-A, is expressed more strongly in islets than exocrine cells or pancreatic ducts (Fig. 2). VEGF-A was first identified as vascular permeability factor, VPF, before it was re-discovered as a mitogenic, chemo-attractant and fenestrating factor for vascular endothelial cells.^(7,8)

In order to obtain insights into the role of islet VEGF-A, two studies were performed in which *VEGF-A* was deleted in either most pancreatic beta cells or the entire pancreatic epithelium of mice.^(5,6) Both studies showed that lack of VEGF-A results in less islet capillaries. When VEGF-A was missing, the number of endothelial cells in islets was approximately the same as in the exocrine tissue, thus demonstrating that the high VEGF-A levels were responsible for the high numbers of endothelial cells found in islets.⁽⁶⁾ In addition, ten times less endothelial fenestrae were observed in VEGF-A-deficient islets, while the number of caveolae was significantly increased.⁽⁶⁾

In order to understand how the VEGF-A-induced islet microvasculature contributes to the physiologic function of islets, glucose tolerance tests were performed. These tests demonstrated that VEGF-A was needed for proper blood glucose regulation by pancreatic islets due to defective insulin secretion.⁽⁶⁾ Steady-state blood glucose levels were elevated in mice with VEGF-A-deficient islets. After intra-peritoneal glucose injections, blood glucose levels in these mice went up to 600 mg/dl (compared with 300 mg/dl in normal mice). However, blood glucose levels returned to almost normal levels after three to four hours, showing that insulin secretion is not completely impaired. These data demonstrate that the fenestrated capillary network is required for the full secretory function of islets. More experiments need to be done to analyze whether insulin levels are reduced as a result of missing endothelial signals, which could explain the higher steady-state insulin levels. In addition, pancreas perfusion experiments could provide definitive evidence whether insulin is secreted more slowly, as suggested by the impaired glucose tolerance.

Until now, diabetes research has focused its attention mainly on the production of insulin, the insulin-producing beta cells and the target cells of insulin. Research on the microvascular endothelial cells of pancreatic islets, however, has been largely neglected because of the instant appearance of insulin in the blood upon secretion by the pancreatic beta cells. Given that insulin injected by subcutaneous injections does not instantly appear in the blood, the quick *trans*-endothelial passage of insulin in the pancreas seems remarkable. Subcutaneous insulin injections result in plasma peaks after 100 minutes, which is 50 times slower than the physiological situation.⁽⁹⁾ Because of this problem, the pharmaceutical industry has put a huge effort in improving insulin uptake by the circulatory system. This includes the production of new forms of insulin that are more easily absorbed as well as research on the development of inhaled forms of insulin.

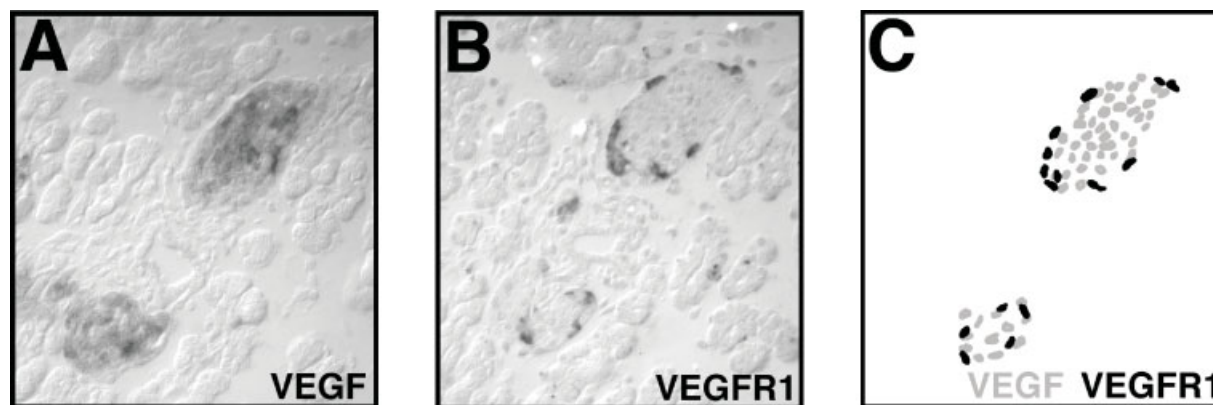


Figure 2. Adjacent sections through a mouse pancreas showing two islets in which VEGFR1-expressing cells surround VEGF-A-expressing islet cells. **A:** VEGF-A staining. **B:** VEGFR1 staining. **C:** Schematic image.

The secretion of insulin and VEGF-A by the same cell, the pancreatic beta cell, represents part of the mechanism of how the healthy organism has solved the problem of insulin delivery into the circulatory system. Interestingly, insulin inhibits endothelial cell growth. This is suggested by the fact that insulin-deficient mice have more islet capillaries as well as bigger islets.⁽¹⁰⁾ The different effects of VEGF-A and insulin might enable enough islet capillaries to form so that insulin can be secreted, but prevent capillary overgrowth.

Development of the islet microvasculature

The experiments described above show that islet VEGF-A is required for the proper physiological function of islets. Vascular endothelial cells migrate to the source of VEGF-A, proliferate and form blood vessels in response to VEGF-A. This leads to the question of where the vascular endothelial cells targeted by islet VEGF-A are coming from.

Experiments on early pancreatic development have demonstrated that vascular endothelial cells provide inductive signals for islet cell development.⁽¹¹⁾ As a result, islet cells develop adjacent to major blood vessels, which provide a rich source of endothelial cells. A different experimental approach has recently supported the conclusion that endothelial cells induce islet development.⁽¹²⁾ Given that vascularized islets are always found adjacent to an artery and pancreatic duct epithelium, we proposed a two-step model for islet development:⁽⁶⁾ in the first step, vascular endothelium induces islet development from pancreatic epithelium. As a result, an islet develops adjacent to a blood vessel. In the second step, the developing islet induces endothelial cells from the vessel to form a capillary network inside of the islet. As a result the islet is now intimately connected with the vascular system. This two-step model explains how pancreatic beta cells interact with the vascular system from the earliest stages of their development

onwards, thereby ensuring their proper secretory function after birth.

It is interesting to speculate why arterioles enter the islet through discontinuities within the non-beta cell mantle, thus bringing the blood stream right to the beta cell core (Fig. 1A). We have found that the VEGF-A receptor, VEGFR2, is highly expressed by the vascular endothelial cells in the islet, while the other VEGF-A receptor, VEGFR1, is mainly expressed by the cells of the islet periphery (Fig. 2). VEGFR2 is the main signaling receptor for VEGF-A, while VEGFR1 is thought to be an antagonist of VEGF-A signalling. This has been shown by deletion of these VEGF-A receptors in mouse embryos: while VEGFR2 deletion leads to loss of blood vessel formation, deletion of VEGFR1 results in excessive blood vessel formation.⁽¹³⁾ The antagonistic effect of VEGFR1 is explained by its stronger VEGF-A binding affinity and weaker signaling activity.⁽¹⁴⁾

Because beta cells express the soluble, more diffusible splice-forms of *VEGF-A* (*VEGF-A120* and *164*), it is possible that the VEGFR1-expressing islet cell mantle reduces the spreading of these factors into the exocrine tissue. The regions where VEGFR1 is not expressed could be the regions where vascular endothelial cells enter the islet core. According to this scenario, high VEGF-A levels would be locally active and attract vessels to the islet core where they branch into a fine capillary network. Protecting ducts and exocrine tissue from VEGF-A might be a way to limit the vascular permeability of their vessels. Indeed, mice expressing VEGF-A in the entire pancreatic epithelium often reveal pancreatic cysts, possibly resulting from the extended vascular permeability.⁽¹¹⁾

Vascular lumen formation in islets

It is still an open question how VEGF-A attracted vascular endothelial cells form capillary structures within a tissue such

as an islet. As mentioned above, the principal morphology of capillaries in tissues is the same: all capillaries have a vascular lumen, which is few micrometers in diameter and surrounded by one or two thin endothelial cell bodies with few or no cellular junctions. How do these structures develop, when endothelial cells penetrate a tissue?

The landmark study by Folkman and Haudenschild using cloned endothelial cells derived from capillaries provided the first evidence that endothelial cells have all information needed to form a vascular lumen.⁽¹⁵⁾ Even though the ability to form a lumen is a key feature of endothelial cells, little is known about the process of lumen formation.⁽¹⁶⁾ Based on the observation that islet capillaries are composed of thin endothelial cell bodies and few cellular junctions, we suggest three possible ways of islet capillary formation (Fig. 3).

During the process of cell hollowing, single cells form an intracellular vascular lumen with no cellular junctions (Fig. 3). The resulting capillaries are so-called "seamless".⁽¹⁷⁾ The only junctions that exist in these capillaries are those connecting the lumen-forming cells with each other. Experiments with human umbilical vein endothelial cells (HUVEC) have revealed that vascular endothelial cells can form vacuoles under certain culture conditions.⁽¹⁸⁾ Moreover, vacuoles of adjacent cells can coalesce to form a continuous lumen.⁽¹⁸⁾

Alternatively, vascular endothelial cells surround a lumen by wrapping (Fig. 3). In this scenario, endothelial cells digest the surrounding extracellular matrix and thereby create a loose space, in which they form a lumen. Cell body extensions (filopodia and lamellipodia) have been described in endothelial cells during tissue invasion.^(19,20) They are dynamic and particularly abundant in the tip cells of endothelial cords, which

have not yet formed a lumen.⁽²¹⁾ Filopodia of tumor cells are sometimes called invadopodia, because they secrete digestive enzymes to invade the tissue.⁽²²⁾ Intracellular cell wrapping would result in one cellular junction.

Cord hollowing is a process by which more than one cellular junction arises (Fig. 3). The number of cellular junctions to be found in the resulting vessel would equal the number of endothelial cells surrounding the vascular lumen. Given that a capillary lumen is only 4–5 μm in diameter, we feel that only very few endothelial cells would be able to form a cord. Cord formation followed by hollowing had been described as a mechanism for the development of large blood vessels such as the dorsal aorta in vertebrate embryos.⁽²³⁾

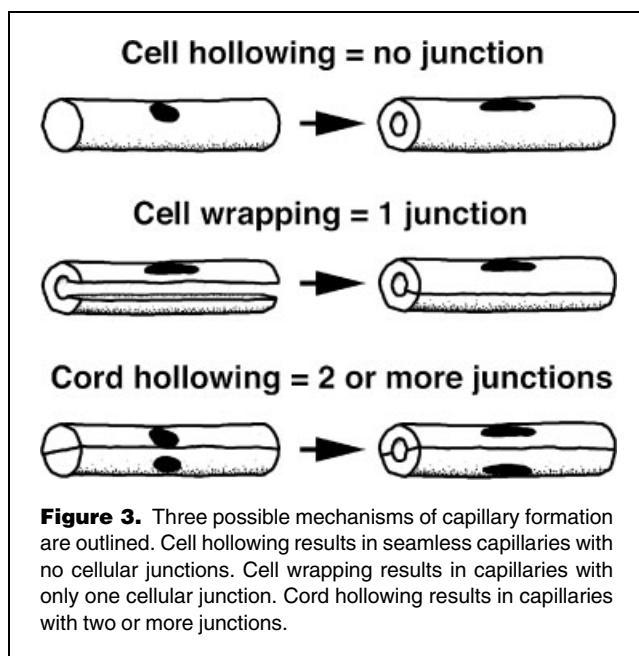
It is not yet known which of the mechanisms lead to the formation of capillaries within islets. However, the observation of islet capillaries with no junction, one junction or two cellular junctions points to more than one mechanism. Whatever mechanism leads to the development of a capillary, there must be a polarization of the endothelial plasma membrane during the process of vascular lumen formation. At the end of this process, the endothelial cell has two distinct plasma membranes: an outer basal membrane facing the basement membrane and an inner apical membrane facing the vascular lumen. In this regard, the cell and cord hollowing models are particularly attractive, because the inner apical plasma membrane would be formed by intracellular vacuoles with properties distinct from the outer basal plasma membrane.

Trans-endothelial passage of insulin

Once endothelial cells have formed capillaries within an islet, the capillaries need to be permeabilized to allow the pancreatic beta cells to secrete insulin through the endothelial cell layer into the vascular lumen. Pancreatic beta cells face one or two fenestrated capillaries, and perfusion experiments with horseradish peroxidase have shown that the endothelial fenestrae are sites through which proteins can quickly permeate.⁽²⁴⁾ Thus, the question was raised whether insulin enters the vascular lumen through the fenestrae of islet capillaries.

Fenestra is the Latin word for window. A fenestra results from fusion of apical and basal plasma membranes, thus producing a pore within the endothelial cell body (Fig. 4). These pores are 50 to 200 nm in diameter and connect the basement membrane on the basal side with the blood stream on the apical side of the endothelial cell. Thus fenestrae represent the fastest way for insulin to enter the vascular lumen. Indeed, mice with non-fenestrated islets display defective glucose tolerance. However, these mice are still capable of lowering blood glucose levels.⁽⁶⁾ This shows that secretion through endothelial fenestrae cannot be the exclusive mode of insulin entry into the circulatory system.

Transcytosis of proteins through the microvascular endothelium could be an alternative mechanism for trans-endothelial insulin secretion.^(25,26) Most probably transcytosis



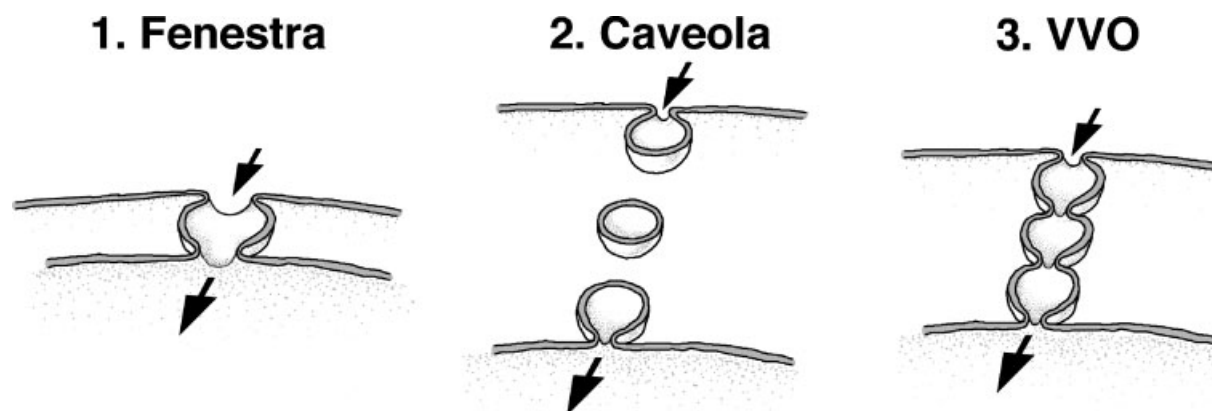


Figure 4. Three possible ways of *trans*-endothelial insulin secretion are shown. Arrows label the way that brings secreted insulin from the basal to the apical side of the endothelial cell. 1. Fenestrae are perforations within the endothelial cell body that result from fusion of apical and basal plasma membranes. 2. Caveolae are cavities, which can pinch off from the endothelial plasma membrane and fuse with the other side of the endothelial cell. 3. VVOs or vesiculo-vacuolar organelles are vesicular channels of venules.

involves an organelle also found in other cell types: the caveola, the Latin word for cavity (Fig. 4). Caveolae appear as smooth, 50- to 100-nm-diameter invaginations of the plasma membrane. When *VEGF-A* is deleted in islets, the non-fenestrated capillaries show an increased number of caveolae. This points to caveolae as a possible backup mechanism for *trans*-endothelial insulin secretion.⁽⁶⁾

In contrast to the view of insulin secretion into the capillary lumen, a study using two-photon microscopy suggested that 70% of insulin is secreted into the interstitial space between the beta cells.⁽²⁷⁾ However, this study was complicated by the fact that islets were isolated with collagenase, an enzyme known to remove capillaries from islets.⁽²⁸⁾ Since some of the empty channels in islets might have been misinterpreted as interstitial spaces, the high percentage of insulin secreted into the interstitial space should be considered with some caution. Whether fenestrated capillaries inside of islets or venules outside of islets take up the interstitial insulin is not known. The vesiculo-vacuolar organelle (VVO) of venules might play a role in interstitial insulin up-take (Fig. 4). These organelles are large channels that connect the extracellular space with the vascular lumen.⁽²⁹⁾ In accordance with a role in *trans*-endothelial traffic, the VVO is induced by factors known to increase vascular permeability including *VEGF-A*, serotonin or histamine.⁽²⁹⁾

More experiments need to be performed to provide definitive evidence for how the diverse endothelial cell organelles (fenestra, caveola or VVO) contribute to the kinetics of *trans*-endothelial insulin secretion.

The islet vasculature in type I diabetes mellitus

The endothelial cell layer allows the tissue to secrete proteins such as insulin into the vascular lumen, but it also allows entrance of blood cells into the tissue during inflammation.

Type I diabetes or juvenile diabetes results from the invasion of thymus-derived lymphocytes, so-called T cells, into the pancreatic islet tissue. Once inside the islet, such T cells destroy the pancreatic beta cells among other islet cells.

The NOD (Non-Obese Diabetic) mouse is the most frequently used animal model for this autoimmune, inflammatory form of diabetes.⁽³⁰⁾ During the course of type I diabetes, the endothelial cell layer, which normally represents a barrier to blood leukocytes, allows T cells to home to, enter and destroy the islets. A recent study investigated the homing of insulin-specific cytotoxic T cells to islets.⁽³¹⁾ The authors showed that insulin-specific cytotoxic T cells destroy both islet endothelial cells and beta cells, which is in line with our unpublished observation that islets lose their capillaries during the early stages of type I diabetes. The destruction of capillaries requires beta cell-secreted insulin or insulin peptides together with the appropriate MHC (Major Histocompatibility Complex) class I molecules to present the insulin peptides to the cytotoxic T cells. Thus, presentation of islet antigens by pancreatic endothelial cells might be a way to home T cells specifically to islets. The authors did not strictly discriminate between homing to vascular versus lymphatic endothelial cells in the *in vivo* situation.⁽³¹⁾ However, they clearly demonstrated that cytotoxic T cells adhere to and destroy vascular endothelial cells in an islet antigen-dependent manner.

Another recent study has shown *in vitro* that cytokine-treated vascular endothelial cells can present islet antigens to helper T cells, so they can adhere to and transmigrate through the vascular endothelium.⁽³²⁾ Pancreatic endothelial cells upregulate a variety of adhesion molecules to which T cells adhere.^(33–35) Blocking antibodies against endothelial cell adhesion molecules and their lymphocyte integrin receptors has been shown to block onset of diabetes in NOD mice.

In summary, the ability to present islet antigens to T cells along with the upregulation of adhesion molecules and cytokines makes endothelial cells critical players in the specific homing of autoimmune cells to the pancreatic islets. Because endothelial cells are accessible through the circulatory system, proteins expressed on pancreatic endothelial cells during type I diabetes could be valuable drug targets for the prevention and treatment of the disease.

The role of vascular endothelial cells during islet transplantation

In recent years, islet transplantation has been suggested as a possible treatment of type I diabetes.⁽³⁶⁾ However, a number of problems would have to be solved to make islet transplantation a feasible treatment. Interestingly, most problems involve endothelial cells.

Firstly, more human islets are needed. This could be achieved by *in vitro* differentiation of islets from human embryonic stem cells, since only human embryonic stem cells would be an unlimited source for human cell production.⁽³⁷⁾ Vascular endothelial cells of the embryonic aorta have been shown to induce the development of endocrine cells from pancreatic epithelium in the mouse.^(11,12) The identification of the inductive signals involved could help to generate protocols for islet differentiation from human embryonic stem cells.

Secondly, the transplanted islets need to survive in the host and secrete insulin in a physiological manner. During islet isolation from donor pancreas tissue, most islets lose their vascular endothelial cells due to the collagenase treatment.⁽²⁸⁾ It takes about one to two weeks until the establishment of an islet blood flow, a circumstance that reduces the survival rate of the transplanted islets.⁽³⁾ Moreover, islets with vascular defects do not regulate blood glucose levels properly.⁽⁶⁾ Attempts to improve islet vascularization are therefore expected to improve the success of islet transplantations. Indeed, VEGF-A overexpression in transplanted mouse islets leads to improved insulin secretion and blood glucose regulation in the recipient mice.⁽³⁸⁾

Thirdly, the autoimmune destruction of transplanted islets must be prevented. To date, this has been achieved by nonspecifically suppressing the immune system.⁽³⁶⁾ It would be desirable to suppress more specifically the homing of cytotoxic T cells to the implanted islets, thereby avoiding side effects that arise from a nonspecific immune suppression. Analyses of the molecular changes in pancreatic endothelial cells during type I diabetes could help to identify new targets for preventing autoimmune islet destruction.

Conclusions

The pancreatic islet is a tissue particularly well studied because of its critical involvement in diabetes mellitus, a disease that affects about 130 million people worldwide. The dense vasculature plays a role in the physiology and disease of

islets. We therefore think that the microvascular endothelium of islets represents a good model for understanding (1) how endothelial cells form capillaries with specific properties within a given organ, (2) how endothelial cells enable the physiological function of an organ, (3) how endothelial cells participate in organ-specific inflammatory diseases. In addition, pancreatic endothelial cells can be used to better understand the mutual signaling between organ-specific cells and endothelial cells, a subject recently covered by a number of reviews.^(4,39,40) Although this topic was not addressed in this article, we would like to mention that islets have also been used to explore the role of vascular endothelial cells during cancer development.⁽⁴¹⁾ In summary, we think that the pancreatic islet is an ideal model tissue to learn more about microvascular development in the context of a tissue.

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