An apparent deficiency of lymphatic capillaries in the islet of Langerhans in the human pancreas

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Abstract

The lymphatic system is crucial for efficient immune surveillance and for maintenance of a physiological pressure in the interstitial space. Even so, almost no information is available concerning the lymph drainage of the islets of Langerhans in the human pancreas. Immunohistochemical staining allowed us to distinguish lymphatic capillaries from blood capillaries. Almost no lymphatic capillaries were found within the islets in pancreatic biopsies from non-diabetic subjects, or from subjects with either T1D or T2D. Lymphatic capillaries were, however, found at the islet-exocrine interface, frequently located along blood capillaries and other fibrotic structures within or close to the islet capsule. Lymphatic capillaries were regularly found in the exocrine pancreas, with small lymphatic vessels located close to and around acini. Larger collecting lymphatic vessels were located in fibrotic septa between the exocrine lobules and adjacent to the ductal system of pancreas. In summary, we report a pronounced deficiency of lymphatic capillaries in human islets, a finding with implications for both immune surveillance and the regulation of interstitial fluid transport in the endocrine pancreas, as well as for the pathophysiology of both type 1 and type 2 diabetes.

Keywords: Islet of Langerhans, Pancreas, Lymphatic capillaries, Blood capillaries, podoplanin
Introduction

Type 1 diabetes (T1D) is caused by a continuing destruction of the insulin-producing cells occurring over a period of several years after diagnosis (1), and autoantibodies with affinity for beta cells as well as exocrine antigens usually appear several years prior to diagnosis (2; 3). The incidence of insulitis, defined as the presence of >2 or 5 T cells infiltrating at least three islets in children (≤14 years of age) dying within one month after diagnosis, is 73% (4). These observations have led to the conclusion that T1D is an immune-mediated disease.

Immune cells are constantly circulating in the body to detect and evoke an immune response against invading organisms and cells recognized as non-self. Antigen-presenting cells leave the extracellular space of the affected organ via the lymphatic capillaries and accumulate in regional lymph nodes, where the encountered antigens are presented to stimulate clonal expansion of T cells with affinity for the foreign peptides presented on the individual’s own HLA.

The beta cell is one of the most metabolically active cells in the body and is critically dependent on a high supply of oxygen and nutrients from the blood. Almost every beta cell is in direct contact with a capillary that has a fenestrated endothelial cell lining to allow optimal transport through the capillary wall and in rodents islet blood perfusion is about 10 times higher than in the exocrine pancreas (5). In all organs, there is a net surplus in fluid transport over the wall of the blood capillary that is correlated with the level of blood perfusion and the permeability of the capillary. This extracellular interstitial fluid (EIF) is transported from the interstitial space via the lymphatic capillaries and disturbances in this system can lead to the formation of edema(6).
A well-organized lymphatic system is crucial for efficient immune surveillance and induction of T cell-mediated immune responses as well as for the maintenance of physiological pressure in the interstitial space (6). An absence of lymphatic capillaries within the islets in rodents have previously been reported (7; 8) and in the fetal human pancreas (9), however, to the best of our knowledge no information is available concerning the lymphatic drainage of the islets of Langerhans in the human pancreas.

Lymph capillaries can be distinguished from blood capillaries by the expression of specific antigens, e.g. podoplanin and LYVE-1, on the endothelial cells (10; 11). The present morphological study was conducted on human pancreases from non-diabetic subjects as well as from subjects with either type 1 or type 2 diabetes (T1D or T2D) in order to describe the extent of the intra-islet lymph capillaries and their relation to the insulin-producing cells and intra-islet blood capillaries.
Research design and Methods

**Ethics** All work involving human tissue was conducted according to the principles expressed in the Declaration of Helsinki and in the European Council’s Convention on Human Rights and Biomedicine. Consent for organ donation (for clinical transplantation and for use in research) was obtained from the relatives of the deceased donors by the donor’s physicians and documented in the medical records of the deceased patient. The study was approved by the Regional Ethics Committee in Uppsala, Sweden, according to the Act Concerning the Ethical Review of Research Involving Humans (2003:460; permit number Dnr 2009/043, 2009/371).

**Human pancreatic specimens** Biopsies from 33 human pancreases were included in the study. Before islet isolation, a clamp was used to compress the main pancreatic duct at the head of the pancreas, and the tissue proximal to the clamp was taken as a biopsy and stored in formalin. Donors were chosen based on factors such as weight, age, and health (Table 1). Two pancreases were obtained at the onset of T1D (previously described in detail in (12)), seven were obtained from patients with longstanding T1D, seven were obtained from patients with longstanding T2D, and the remaining pancreases were collected from multi-organ donors without any known pancreatic disease, divided according to age and body mass index (BMI) (Table 1).

**Immunohistochemical staining** Formalin-fixed and paraffin-embedded tissues were cut into 5-µm sections. Consecutive sections were processed and labeled using a standard immunoperoxidase technique, as previously described in detail (12). With the exception of insulin, all other antigens were unmasked by heat-induced epitope retrieval. Antibodies against chromogranin A (1:150, clone LK2H10; NeoMarkers,
Thermo Fisher Scientific Inc, Fremont, CA), insulin (1:200, clone A564; Dako, Glostrup, Denmark) and synaptophysin (1:50, clone DAK-SYNAP; Dako) were used to identify islets. A monoclonal antibody against CD34 (1:50, clone EP373Y; Dako) was used to detect blood endothelial cells and antibodies against podoplanin (1:50, clone D2-40; Dako) or LYVE-1 (1:200, clone ab 36993, Abcam, Cambridge, UK) were used to detect lymphatic endothelial cells. Bound antibodies were visualized using Dako EnVision or an EnVision DuoFLEX Doublestain System (both Dako) and diaminobenzidine-based substrate or 3-amino, 9-ethyl carbazole (Dako). Sections were counterstained with hematoxylin and scanned and analyzed by Aperio Imagescope and by light microscopy by two investigators, who were blinded to their origin.
Results

**Immunohistochemical staining** Optimization of the staining for lymph and blood capillaries was performed on sections of the intestinal wall and on human pancreases. Double staining of lymph and blood capillaries revealed that the two types of capillaries were distinctly and specifically stained, without overlap or background.

**Blood and lymph capillaries in the pancreas** Larger collecting lymphatic vessels (Figure 1 A, B, D, and M) were often located in fibrotic septa between the exocrine lobules and adjacent to the ductal system of the pancreas. Lymphatic capillaries were frequently found in the exocrine pancreas (Figure 1 E, F, K, and L) located close to and around acini.

A total of 4365 islets from 35 subjects were examined (Table 1). Lymphatic capillaries were found in only 24 islets from 9 different subjects. In one subject with longstanding T2D, a total of 15 islets with lymphatic capillaries were found. The pancreas of this subject showed significant fibrosis in both the islets and the exocrine parenchyma. Also in the other subjects, one in the high BMI group, three in the high age group, one in the group with long-standing T1D and one with recent onset T1D, the islets with lymphatic capillaries showed signs of fibrosis. The lymphatic capillaries were located in fibrotic strands within the islet and were often close to blood capillaries. Fibrotic strands in islets frequently occur and these strands are usually devoid of lymphatic capillaries (figure 1 F), i.e. less than 5% of these islets contain lymph capillaries. However, the rare islets with extensive fibrosis or hyalinized islets consistently contained lymphatic capillaries within these strands of non-endocrine tissue (Figure 1 I).
In the remaining 4341 islets examined, no lymphatic capillaries were found, but an extensive network of blood capillaries was seen (Figure 1N). Lymphatic capillaries were, however, found at the islet-exocrine interface (Figure 1 C, F, G, H and J), frequently located along blood capillaries and other fibrotic structures within or close to the islet capsule.
Discussion

There are few tissues in the human body lacking lymphatic capillaries, and hence lymph drainage. The observations reported herein show that in addition to the brain, the islets of Langerhans also show a pronounced deficiency of lymphatic capillaries. This finding has implications for both immune surveillance and regulation of interstitial fluid transport in the endocrine pancreas.

Notably, there are marked similarities in the morphological structure of the blood capillaries in the CNS and in human pancreatic islets, with a unique paravascular space surrounded by a double basal membrane structure (13; 14). This peculiar anatomical structure, which functions as the prevailing transport system for EIF, has been described in the CNS (13; 15; 16). Disturbances in the glymphatic pathway have been associated with accumulation of metabolic degradation products and the development of neurodegenerative diseases (15; 16). Even if a similar paravascular space surrounded by a double basal membrane is present in human islets (14), there is no report thus far to describe glymphatic transport of interstitial fluids and waste products in human islets.

EIF is continuously formed by filtration from blood capillaries (6), collected by lymphatic capillaries, and finally re-circulated back to the blood via the thoracic duct. The extracellular volume in human islets has been estimated to be about 14% of the total islet volume (17). Ligation of the thoracic duct in rats induces edema and the accumulation of inflammatory cells in the exocrine parenchyma (18), demonstrating the importance of the lymphatic system in maintaining physiological levels of EIF volume and pressure in the exocrine pancreas. Notably, no comment was made concerning a similar edema in the islets (18). Disturbances in a tentative glymphatic
transport system within the islets would have implications for the development of both T1D and T2D. Remarkably, there is a 5-fold increase in the accumulation of hyaluronan and hyaladherins in the pericapillary space in islets from subjects with T1D when compared to non-diabetic controls (19).

In our study, the human exocrine pancreas showed a well-developed lymphatic system, as evidenced by the frequent lymph capillaries close to the acini and the presence of larger lymph vessels in the interstitial septa of the pancreas. Drainage of the thoracic duct has previously been applied to reduce the number of circulating lymphocytes in order to induce systemic immunomodulation in autoimmune disorders and after organ transplantation; the amount of lymph collected per day was in the range of 1.5-2 liters. When patients were examined after a meal or during a secretin test, the levels of the exocrine enzymes in the lymph rose prior to and to higher concentrations than those simultaneously measured in the blood, supporting the notion of a substantial direct delivery of these enzymes into the lymph capillaries adjacent to the acini (20; 21). In line with the morphological observations reported here, corresponding measurements of insulin during an intravenous glucose tolerance test gave no support for a direct delivery of insulin into lymphatic capillaries (22).

The absence of lymphatic capillaries in most subjects examined also makes the islets a “locus minoris resistentiae” (23) for infections and dysregulated immune responses resulting from the lack of normal trafficking of immunocompetent antigen-presenting cells from the parenchyma via the lymphatic capillaries to the regional lymph nodes. The few lymphatic capillaries identified within the islets was in most cases not in direct contact with the endocrine cells; instead, the capillaries detected were found in
conjunction with fibrotic strands entering the islets. Lymphatic capillaries were instead often found in the peri-islet area, close to the blood vessels supporting the islet, i.e., the site into which a tentative glymphatic transport system would empty (14). The importance of this observation for the development of T1D is unknown and beyond the scope of this study. However, the observation of an extensive peri-islet network of lymphatic capillaries is consistent with the frequently reported, predominant accumulation of immune cells in this area in subjects with recent-onset T1D (4).

Even if lymphatic capillaries in the exocrine pancreas were readily detected using antibodies to podoplanin or LYVE-1, the apparent absence of lymphatic capillaries within the islets could be a result of the absence of these proteins specifically on the lymphatic endothelial cells within the islets. It should, however, be noted that these antisera readily detected lymphatic capillaries in pancreatic endocrine tumors, including benign and malign insulinomas (24) and transplanted islets (25).

In summary, we report that there is a marked deficiency of lymphatic capillaries in the islets of Langerhans with the human pancreas, a finding that may have important implications for our understanding of the physiology of the endocrine pancreas as well as for the pathophysiology of both T1D and T2D.
Author contribution statement

EK and OK designed the study, analyzed and interpreted the obtained data, and wrote and approved the manuscript. No potential conflict of interest relevant to this article was reported. OK is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Figure legends**

**Figure 1: Blood and lymph capillaries in the human pancreas.**

A) Immunohistochemistry showing distinct staining of several large lymph vessels (LYVE-1, brown) close to a vein and an artery in a subject with longstanding T1D. B) Several large lymph vessels (D2-40, brown) close to a small duct in a subject with T2D. C) A network of lymph capillaries (D2-40, brown) in the islet-exocrine interface in a subject with T2D. The arrow indicates a tiny lymph capillary (D2-40, brown) in a fibrotic strand in the center of the islet (SYN, red). D) Lymph capillaries (D2-40, brown) surrounding a small duct, but not in the islets (SYN, red), in a subject with high age. E) Lymph capillaries (D2-40, brown) in the exocrine parenchyma, but not in the islets (SYN, red), in a subject with high age. F) Lymph capillaries (D2-40, brown) in the exocrine parenchyma, but not in the islet (SYN, red), in a subject with recent onset T1D. G) Lymph capillaries (D2-40, brown) in the islet-exocrine interface in a subject with recent onset T1D. The arrow indicates a lymph capillary in a fibrotic strand in the islet (SYN, red). H) Lymph capillaries (LYVE-1, brown) in the islet-exocrine interface in a subject with T2D. The arrow indicates a tiny lymph capillary in a fibrotic strand in the center of the islet. I) Several tiny lymph capillaries (arrows, D2-40, brown) in a hyalinized islet (SYN, red) in a subject with T2D. J) A tiny lymphatic capillary (LYVE-1, brown) in a fibrotic strand within an islet from a subject with T2D. K) Lymph capillaries (LYVE-1, brown) in the exocrine parenchyma in a subject with T2D. L) Lymph (D2-40, brown) and blood (CD34, red) capillaries in the exocrine parenchyma in a subject with high age. M) A lymph vessel (D2-40, brown) close to an islet (SYN, red) in a subject with longstanding T1D. N) Frequent blood (CD34, red) but no lymph (D2-40, brown) capillaries in an islet.
depicted by a dotted line in a non-diabetic subject. O) Lymph vessels (LYVE-1, brown) close to a ganglion (marked with *) in a subject with longstanding T1D.
Table 1 Characterization of the groups of subjects included and the number of lymphatic capillaries found.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>BMI</th>
<th>Total number of islets examined</th>
<th>Total number of islets with lymph capillaries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic, BMI &lt;18,8 (n=4)</td>
<td>48.5 ± 17.4</td>
<td>18.2 ± 0.5</td>
<td>508</td>
<td>0</td>
</tr>
<tr>
<td>Non-diabetic, BMI &gt;40,7 (n=4)</td>
<td>58.5 ± 5.3</td>
<td>42.6 ± 2.2</td>
<td>187</td>
<td>1 in 1 subjects</td>
</tr>
<tr>
<td>Non-diabetic, age &lt; 24 (n=5)</td>
<td>20.6 ± 2.4</td>
<td>22.7± 1.9</td>
<td>452</td>
<td>0</td>
</tr>
<tr>
<td>Non-diabetic, age &gt; 70 (n=5)</td>
<td>75.6 ± 1.5</td>
<td>26.0 ± 3.2</td>
<td>810</td>
<td>4 in 3 subjects</td>
</tr>
<tr>
<td>T2D (n=8)</td>
<td>52.9 ± 18.1</td>
<td>30.6 ± 8.0</td>
<td>765</td>
<td>17 in 3 subjects</td>
</tr>
<tr>
<td>Longstanding T1D (n=7)</td>
<td>38.4 ± 20.7</td>
<td>23.9 ± 3.7</td>
<td>1121</td>
<td>1 in one subject</td>
</tr>
<tr>
<td>Recent-onset T1D (n=2)</td>
<td>34.5 ± 7.8</td>
<td>25.7 ± 2.1</td>
<td>522</td>
<td>1 in one subject</td>
</tr>
</tbody>
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Values are presented as means ± standard deviation.
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