Three-dimensional Organization of the Connective Tissue Fibers of the Human Pancreas: A Scanning Electron Microscopic Study of NaOH Treated-Tissues

Osamu OHTANI

Department of Anatomy (Prof. T. MURAKAMI), Okayama University Medical School, Okayama, Japan

Received September 10, 1987

Summary. A method for scanning electron microscope (SEM) study of reticular fibers in their original shapes and locations is described. This technique was employed to demonstrate the three-dimensional organization of the reticular fibers of the human pancreas. The cellular elements were effectively removed by treatment of the tissue pieces with a 10% aqueous solution of NaOH for 3-4 days at room temperature. Thin layers of the reticular fibers surrounding the acini and ducts formed a three-dimensional interstitial compartment. The reticular fiber sheaths for the blood vessels coursed through the compartment. In the lobule, there were scattered round or oval capsules for the islets of Langerhans. The capsule also consisted of reticular fibers. Within the capsule, reticular fiber sheaths for accommodating islet capillaries, representing the pericapillary spaces, formed a three-dimensionally anastomosing network. The channels for the capillaries ensheathed by the reticular fibers in the islet were continuous with those in the surrounding exocrine pancreas; thus, the insulo-acinar portal system was confirmed to exist in the human pancreas. This study also maintains that the present method is useful for examining the microvascular organization of the islet.

A number of early authors gave descriptions of the lattice network made up of argyrophil fibers around the acini, and the islet of Langerhans of the pancreas (OTANI, 1927; PLENK, 1927; FAZZARI, 1935; CLARA, 1936; reviewed by BARGMANN, 1936). However, as these authors used the light microscope, the three-dimensional organization of the connective tissue fibers, skeletal elements of the interstitium, of the human pancreas were not well demonstrated. The author has devised a method which extracts the connective tissue fibers in their original shapes and locations, and demonstrates the three-dimensional organization of the connective tissue fibers of the human pancreas by SEM of the tissues treated by this new technique.

MATERIALS AND METHODS

The pancreases were obtained by necropsy from two Japanese individuals of 45 and 75 years of age. Routine light microscopy of hematoxylin-eosin stained tissue showed no pathological changes of the pancreas.

The pancreas was fixed in 10% formalin and cut into small pieces measuring 2 ×
The pieces were immersed in a 10% aqueous solution of NaOH for 3 to 4 days at room temperature. After rinsing in distilled water for several hours to one day, the pieces were put in a 1-2% aqueous solution of tannic acid overnight, rinsed in distilled water for several hours, and postfixed in an 1% aqueous solution of OsO₄ for 2 to 3 hrs (the so-called conductive staining by Murakami, 1974). The specimens were dehydrated in a series of graded concentrations of ethanol, critical-point-dried in a HCP-2 critical point dryer (Hitachi, Ibaraki) using liquid CO₂. The dried specimens were mounted on brass stubs with double sticky tape and coated with gold in a Polaron SEM coating system (Polaron Equipment, Watford, England).

Observations were made under a JSM U3 SEM (JEOL, Tokyo) with an accelerating voltage of 15 kV. Stereo pairs of scanning micrographs were frequently taken with a tilt separation of six to seven degrees to examine the spatial relationship of the structures.

In some cases, the tissue piece was cut into two, and one was processed for paraffin sections to examine light microscopically the tissue structures of the plane facing the surface of the other piece, which was subsequently observed under the SEM.

RESULTS

Effects of the NaOH treatment

Treatment of the human pancreata with NaOH at room temperature was able to effectively remove the cellular elements, exposing the connective tissue fibers of the organ under the SEM. Stereo observation of scanning electron micrographs showed that, in spite of the treatment with NaOH, the connective tissue fibers preserved their original locations. Thus, the spatial extensions of the parenchymal constituents of the pancreas could be studied by the spaces that were demarcated by connective tissue fibers.

General organization

Around the interlobular duct and accompanied by the interlobular arteries and veins were condensations of connective tissue fibers from which layers of connective tissue fibers extended radially as septa that subdivided the gland into lobules. The septa terminated in extremely thin layers that surrounded the parenchymal structures of the pancreas; thus, the lobule was not completely demarcated by its own connective tissue layer. A thin layer of connective tissue fibers or reticular fibers divided the lobule into numerous round or oval spaces which, in the natural state, housed the pancreatic acini, and ductules (Fig. 1-3). Thus, the exposed surface of the lobule displayed a honeycombed structure at low magnification (Fig. 1). Within the lobule were also scattered round or elliptical capsules of the islets of Langerhans of about 60-220 µm in diameter (Fig. 1, 4, 5).

Exocrine pancreas

Stereo observations of scanning electron micrographs clearly showed the three-dimensional organization of the extremely thin layers of the reticular fibers; the spaces demarcated by them accommodated the intralobular ducts and acini in the natural
The sheaths of reticular fiber layer housing those ducts measuring more than 15 \( \mu m \) in diameter possessed many holes (about 6 \( \mu m \) in diameter) that were continuous with the round or oval spaces representing the shapes of the acini (Fig. 2). The terminal portion of the reticular fiber channels of the duct also continued to the reticular fiber layers surrounding the acini (Fig. 2).

The most frequently found spaces for the acini were round or oval ones ap-
Fig. 2. Stereo-pair SEM view of the human exocrine pancreas treated with NaOH. The reticular fiber sheath surrounding the terminal portion of the duct (D) continues to those surrounding the acini (A). The reticular fiber sheath of the duct possesses a fenestration (f) through which the space for the duct is continuous with that for the acinus. A small tubular space (arrows) for the capillary is seen running in the reticular fiber layer associated with the acini. ×800

Fig. 3. Stereo-pair SEM view of the human exocrine pancreas treated with NaOH. The spaces for the acini (A) are demarcated by meshworks of reticular fibers that run in various directions. The reticular fiber meshwork surrounding the acini possesses round fenestrations (f) through which the acinar spaces are continuous with those for the adjacent ones. Between the reticular fiber meshworks associated with the acini are narrow spaces (i) with few reticular fibers in them, representing the spaces for the blood vessels and the constituents of the interstitial stroma. ×1,700
proximately 30 \mu m in diameter (Fig. 2). Most of them were continuous with adjacent ones through holes of about 6 \mu m in diameter (Fig. 2, 3). The thin reticular fiber layers were frequently shared by two adjacent acini, but when three or more acini met, narrow spaces of irregular shapes with few reticular fibers formed between the layers associated with the acini (Fig. 3). In or between the layers of reticular fibers were small
sheaths for blood vessels (Fig. 2, 4). Since these sheaths were formed by extremely thin layers of reticular fibers, only stereo observations of the scanning micrographs were useful to identify the structures as such (Fig. 2, 4).

**Islets of Langerhans**

Each islet of Langerhans was surrounded by a capsule (Fig. 1, 5, 6) which consisted of a meshwork of reticular fibers (50–100 nm in diameter) that ran in various directions (Fig. 7). The reticular fibers in the surrounding exocrine portion also joined the capsule (Fig. 5). The capsule was continuous along almost the entire islet.

From the capsule extended extremely thin layers of reticular fibers along the capillaries in the islet. Thus, there was a three-dimensional network of anastomosing sheaths in the islet which were composed of the meshwork of reticular fibers and, in the natural state, accommodated capillaries (Fig. 5, 6, 8). Almost all of the sheaths in

![Fig. 6. SEM view of a small islet and its surrounding exocrine portion of the human pancreas treated with NaOH. Within the reticular fiber capsule (c) of the islet is a thicker sheath of reticular fibers (a) probably for the afferent vessel that runs centrally and issues smaller sheaths for capillaries to the islet cortex. Note that the reticular fiber sheath for the islet capillary is continuous with that running in the interstitial compartment of the exocrine portion (indicated by arrowheads). ×1,100](image-url)
the islet measured 4 to 8 μm in diameter, but there were occasional string-like structures (Fig. 5). In larger islets, generally uniform sheaths repeatedly branched and anastomosed to form the network; there was no sheath that was significantly thick and reached the islet core (Fig. 5). In smaller islets, however, a slightly thicker sheath (about 15 μm in diameter) entered the islet core where the sheath then divided into thinner sheaths running radially to the islet cortex (Fig. 6). The reticular fiber sheaths for the insular capillaries were continuous with those running in the interstitial compartment of the exocrine pancreas (Fig. 6).

DISCUSSION

The present study is the first to demonstrate by SEM the three-dimensional organization of the connective tissue fibers of the human pancreas, previously visualized much less clearly by conventional light microscopy. The study was performed with a new technique using NaOH at room temperature. As the shapes of the spaces demarcated by the reticular fibers of the pancreas treated

Fig. 7. SEM view of a capsule of the islet of Langerhans of the human pancreas treated with NaOH. The capsule consists of fine reticular fibers running in various directions. ×6,700

Fig. 8. Stereo-pair SEM view of the reticular fiber networks ensheathing the capillaries in the islet of Langerhans of the human pancreas treated with NaOH. The reticular fibers run in various directions along the capillaries. ×6,400
with this technique correspond well to the shapes of the other parenchymal constituents of the organ, the reticular fibers appear to preserve their original locations and shapes after the treatment with NaOH. However, the tissues treated with NaOH are so soft and fragile that fixation in a tannic acid aqueous solution is necessary to make the specimens adequately hard and resistant to the following preparation procedures, as well as to give them electric conductivity.

The three-dimensional network of the reticular fibers demonstrated in this paper represents the skeletal structure of the interstitial compartment of the pancreas. As the capillaries run in the interstitial compartment, which occupies the entire space between the parenchymal constituents of the gland, it seems evident that this interstitial compartment serves as a passage for the fluid that comes out of the capillaries. In this sense, the interstitial compartment seems to correspond to what KIHARA (1950) designated as "extravaskuläre Saftbahnen", or extravascular fluid pathways.

Some early authors (PLENK, 1927; FAZZARI, 1935; CLARA, 1936), in their light microscopic studies of silver impregnated human pancreas, reported a reticular fiber capsule surrounding the islet. The capsule is distinct in the human islet but either indistinct or intermittent in some species. The present study confirmed that the human islet is almost entirely surrounded by a capsule made up of reticular fibers. The significance of the islet capsule is not well known at this time, although the capsule is most likely a device for collecting the islet cells together into a functional unit. The capsule may also prevent the insular secretions from spreading too freely to the surrounding exocrine portion.

Within the islet, the reticular fibers occur only around the capillaries, as reported in previous light microscope observations of silver impregnated human pancreas (FAZZARI, 1935; CLARA, 1936). Particularly noteworthy is the finding that the meshwork of the reticular fibers completely surrounds the islet capillaries, and thus forms the three-dimensional network of the sheaths. The network also represents the organization of the pericapillary spaces which facilitate the spread of secretions from one type of islet cell to another.

In the smaller islets, a relatively large sheath for the blood vessel often enters the islet core, from which radiate smaller sheaths to the islet cortex. Such a sheath with a larger diameter probably serves for the so-called "central vessel" or the vas afferens that enters the islet core. GRUBE and BOHN (1983) reported that a heterocellular layer of endocrine cells was observed mainly adjacent to such centrally located capillaries in small islets, whereas the vasa efferentia were surrounded predominantly by B-cells. In this connection, the hypothesis proposed for some mammals (FUJITA, 1973; FUJITA and MURAKAMI, 1973; OHTANI et al., 1986) that the blood flows from the A and D-cell area to the B-cell area also seems applicable to the human islet of Langerhans. In larger islets, however, vessels preferentially entering the islet core have not been observed so far, instead, a rather uniform capillary network was noticed in the islet. Details of the intrainsular vascular organization of the human pancreas will be our next communication.

Previous studies showed that, in mammalian pancreata, there exists an insulo-acinar portal system through which high concentrations of insular secretions are transferred to the exocrine portion, there to exert their actions (HENDERSON, 1969; MCCUSKEY and CHAPMAN, 1969; FUJITA, 1973; FUJITA and MURAKAMI, 1973; FUJITA, YANATORI and MURAKAMI, 1976; OHTANI and FUJITA, 1980; OHTANI, 1983; NISHINO et al., 1985; OHTANI et al., 1986). The present study has shown that the capillaries in the islet are continuous with those running in the interstitial compartment of the exocrine
pancreas. This therefore indicates that the insulo-acinar portal system also exists in the human pancreas, as reported by YAGINUMA et al. (1981) in their reconstruction study.

REFERENCES

Bargmann, W.: Die Schilddrüse. In: (ed. by) W. v. Möllendorff: Handbuch der mikroskopischen Anatomie des Menschen, VI/2. Springer, Berlin, 1936 (p. 2-136).

Clara, M.: Über das argyrophile Gewebe ("Gitterfasern") in der menschlichen Bauchspeicheldrüse. Z. mikrosk.-anat. Forsch. 39: 231-242 (1936).

Fazzari, I.: Comportamento del tessuto reticolare del pancreas. Anat. Anz. 80: 355-361 (1935).

Fujita, T.: Insulo-acinar portal system in the horse pancreas. Arch. histol. jap. 35: 161-171 (1973).

Fujita, T. and T. Murakami: Microcirculation of monkey pancreas with special reference to the insulo-acinar portal system. A scanning electron microscope study of vascular casts. Arch. histol. jap. 35: 225-263 (1973).

Fujita, T., Y. Yanatori and T. Murakami: Insulo-acinar axis, its vascular basis and its functional and morphological changes caused by CCK-PZ and caerulein. In: (ed. by) T. Fujita: Endocrine gut and pancreas. Elsevier, Amsterdam, 1976 (p. 347-357).

Grube, D. and R. Bohn: The microanatomy of human islets of Langerhans, with special reference to somatostatin (D-) cells. Arch. histol. jap. 46: 327-353 (1983).

Henderson, J. R.: Why are the islets of Langerhans? Lancet ii: 469-470 (1969).

Kihara, J.: Extravasculare Saftbahnsystem. In: (ed. by) Japanese Association for Hematology: Symposium on hematology. (In Japanese), Vol. 3. Nagai-shoten, Tokyo, 1950 (p. 118-159).

McCuskey, R. S. and T. M. Chapman: Microscopy of the living pancreas in situ. Amer. J. Anat. 126: 395-407 (1969).

Murakami, T.: A revised tannin-osmium method for non-coated scanning electron microscope specimens. Arch. histol. jap. 36: 189-193 (1974).

Nishino, H., K. Ozawa, M. Takeishi, K. Nagata and Y. Watanabe: Pancreatic microcirculation in rats (particularly the islet of Langerhans). In: (ed. by) M. Tsuchiya, M. Asano, M. Oda and I. Okazaki: Microcirculation annual 1985. Excerpta Medica, Amsterdam, 1985 (p. 325-337).

Ohtani, O.: Microcirculation of the pancreas: A correlative study of intravital microscopy with scanning electron microscopy of vascular corrosion casts. Arch. histol. jap. 46: 315-325 (1983).

Ohtani, O. and T. Fujita: Microcirculation of the pancreas. A scanning electron microscope study of vascular casts. Biomed. Res. 1: 130-140 (1980).

Ohtani, O., T. Ushiki, H. Kanazawa and T. Fujita: Microcirculation of the pancreas in the rat and rabbit with special reference to the insulo-acinar portal system and emissary vein of the islet. Arch. histol. jap. 49: 45-60 (1986).

Otani, S.: Studies on the islands of Langerhans in the human pancreas. II. Significance of variations in structure. Amer. J. Pathol. 3: 123-134 (1927).

Plenk, H.: Über argyrophile Fasern (Gitterfasern) und ihre Bildungszellen. Erg. Anat. Entw.-Gesch. 27: 302-412 (1927).

Yaginuma, N., T. Takahashi, K. Saito and M. Kyogoku: Reconstruction study of the structural plan of the human pancreas. (Japanese text with English abstract). Jap. J. Gastroenterol. 78: 1282-1292 (1981).
Dr. Osamu OHTANI
Department of Anatomy
Okayama University Medical School
Shikatacho 2-5-1, Okayama
700 Japan