Morphological Characteristics of Schwann Cells in the Islets of Langerhans of the Murine Pancreas

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Summary. The present study demonstrated the three-dimensional architecture of peri-insular nerve plexuses in the murine pancreas by the combined use of light microscopy of S-100 immunostained sections, transmission electron microscopy (TEM) of thin sections, and scanning electron microscopy (SEM) of KOH digested tissues. By light microscopy of thin sections immunostained with anti-S-100 antibody, Schwann cells were often found on the margin of the islets as if delimiting the islet and exocrine parenchyma. In thick sections, Schwann cells of the islet connected their thin and slender processes with each other to form a delicate network on the surface of the islet. By TEM, Schwann cells were observed as an attenuated sheet that invested the surface of the islet. Axon terminals were usually found on the outer surface of these membranous Schwann cells. SEM of KOH digested tissues revealed that nerves reaching the islet spread on the insular surface. Schwann cells in this portion extended their thin membranous processes, which directly covered the basal part of several endocrine cells as a whole. Numerous axons with varicosities were usually found on the surface of these membranous Schwann cells, but sometimes crept beneath them.

These findings indicate that “the interstitial cells” described by light microscopists are peculiar-shaped Schwann cells present in the islets. The functional significance of the rich innervation of the islets is also briefly discussed in the present study.

The innervation of the pancreas, i.e., its acinar, insular and vascular compartments, has been studied classically by many light microscopists who used silver impregnation and/or methylene blue-vital staining methods (CAJAL, 1894; CAJAL and SALA, 1891; PENSA, 1905; CASTRO, 1923, PINES and TROPOWA, 1930). Among them, HONJIN (1956) paid special attention to the presence of a very dense nerve network in and around

the islet of Langerhans in the mouse pancreas, and made an impressive schematic drawing of the peri-insular nerve plexus consisting of nerve fibers and specialized interstitial cells of CAJAL (Fig. 1).

On the other hand, transmission electron microscopy (TEM) has revealed that peripheral nerves are usually composed of axons ensheathed by glial elements or Schwann cells. In their TEM study on the dog pancreas, FUJITA and KOBAYASHI (1979) demonstrated that axons incompletely invested by Schwann sheaths concentratedly terminated in the islet, especially around blood capillaries. They further noticed a close spatial relationship of islet endocrine cells to Schwann cells; this was also demonstrated by several other TEM investigators in certain mammals (dog: SMITH 1975; rat: DONEV 1982; sand rat: DONEV 1984). Recent immunohistochemical studies using the anti-S-100 protein antibody have confirmed the existence of Schwann cells in the pancreatic islets (rat: FUJITA et al. 1983; monkey: GIROD et al. 1987, guinea-pig: UCHIDA and ENDO 1989, human: LASZIK et al 1989).

By scanning electron microscopy (SEM), we previously demonstrated three-dimensionally the distribution and ultrastructure of nerves in the mouse and rat exocrine pancreas (USHIKI and IDE, 1988; USHIKI and WATANABE, 1997), and indicated that the “interstitial cells” described by light microscopists correspond to Schwann cells associated with axons. However, the shape of Schwann cells in the islet of Langerhans in connection with “interstitial cells” has not been fully elucidated three-dimensionally, since we mainly focused our attention in those studies to the exocrine innervation.

Thus, the present study was performed to demonstrate precisely the three-dimensional architecture of peri-insular nerve plexuses in the murine pancreas. For this purpose, we used light microscopy of S-100

Fig. 1. Innervation of the islet of Langerhans of the mouse pancreas illustrated by HONJIN (1956). The "neural terminal net" is stated to be composed of nerve fibers and interstitial cells of Cajal (black cells with nuclei). A part of the islet is cut off, wedge-wise, to show the interior of the islet. *p.i.g.* Peri-insular ganglion, *c.* capillary. (From *J. Comp. Neurol.* 104: 331-371, 1956; courtesy of John Wiley & Sons, Inc.)

immunostained sections, TEM of thin sections, and SEM of KOH digested tissues. Special attention was given to the morphological characteristics of Schwann cells on the surface of the islets of Langerhans in the murine pancreas.

MATERIALS AND METHODS

Adult mice (dd strain) of both sexes weighing 30–35 g were used for light microscopy, TEM and SEM. Adult male Wistar rats were used for SEM. They were anesthetized either by inhalation of diethyl ether or by intraperitoneal injection of pentobarbital sodium (Nembutal, 50 mg/kg body weight), and prepared as

follows.

S-100 immunohistochemistry for light microscopy

Mice were perfused through the left ventricle with the Gerade's fixative (i.e., Bouin's fluid without acetic acid) for about 3–5 min. After perfusion, the pancreas was removed from the body and immersed in the same fixative for 4–6 h at 4°C. For thin section studies, pieces of the pancreas were embedded in "Tissue-Tek O.C.T. compound" (Sakura Fine Technical Co. Ltd, Tokyo, Japan) and frozen sections, 10 µm thick, were made using a cryostat (Coldtome model CM-41, Sakura, Japan). The sections were mounted on the poly-L-lysine coated glass slide, rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.2), and treat-

Fig. 2. Photomicrograph of a mouse pancreatic islet (*I*) immunostained with anti-S-100 antibody and counterstained with hematoxylin. **a.** S-100 immunoreaction is found on the surface of the islet. **b.** Closer view of a part of Figure 2a. Note a nerve entering the islet (*arrowhead*). An arteriole (*A*) is surrounded by nerves. *V* venules, *EX* exocrine pancreas.

ed in 0.3% H_2O_2 in methanol for 15 min to block endogenous peroxidase activity. The sections were incubated 12 h with rabbit anti-bovine S-100 protein antiserum (Dako Japan, Kyoto) at a 1:1000 dilution. The reaction sites were made visible by the avidin-biotin complex (ABC) method (HSU et al. 1981) using 3,3'-diaminobenzidine as the chromogen. The specimens were counterstained with hematoxylin and observed with a light microscope.

For thick sections, the splenic lobe of the pancreas was embedded in 25–30% agar, and a series of frozen sections, 50 μm thick, were made using a freezing microtome. The sections were immersed again in Gerade's fixative for 12 h at 4°C. After a brief rinsing in the PBS, each section was placed on aminopropylsilane-coated glass slide (Matsunami Co., Ltd, Japan) or poly-L-lysine coated glass slide, and dried for over 24 h at room temperature. The specimens were rinsed again in PBS for 30–60 min, treated with trypsin solution (1:250 DIFCO Lab., Detroit, USA, 100 mg/100ml) in Tris-HCl buffer (pH 7.6) containing 0.1% CaCl_2 for 20–30 min at 37°C, and then incubated for

36–48 h at 4°C with the S-100 antibody as described above. They were colored with a diaminobenzidine solution using the avidin-biotin complex method. The sections were dehydrated in a series of graded ethanol, cleared by immersion in clove oil for 5–10 min and xylene for 30 min., mounted with Eukitt, and observed with a stereo light microscope (High definition 3D microscope, Edge Scientific Instrument, USA).

Transmission electron microscopy (TEM)

Mice were fixed with 2.5% glutaraldehyde in 0.1% phosphate buffer by perfusion through the left ventricle. Immediately after perfusion, the pancreas was removed from the body, cut into small pieces, and immersed again in the same fixative for about 4 h. They were postfixed with 2% OsO_4 , dehydrated in ethanol, and embedded in Epon 812. Ultrathin sections were made with an ultramicrotome, stained with uranyl acetate and lead citrate, and observed in a transmission electron microscope (H-7000, Hitachi, Japan).

Fig. 3. Stereo pair of 50 μm thick sections immunostained with the anti-S-100 antibody. Schwann cells with a nuclear swelling connect with each other by their slender processes to form a dense network (*arrowheads*) on the surface of the islet of Langerhans. Note also Schwann cells extending in the islet (*arrows*).

Scanning electron microscopy (SEM)

Mice and rats were fixed by perfusion with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After perfusion, the pancreas was removed from the body and immersed in the same fixative for more than 1 day. The pancreas was cut into small blocks and treated by the following KOH digestion method (USHIKI and MURAKUMO 1991; USHIKI and WATANABE, 1997). The specimens were placed into 30% KOH solution for 8 min at 63°C. They were rinsed in the buffer, treated with 1% tannic acid for 2 h, rinsed in distilled water for 1 h, and conductive stained with 1% OsO_4 solution for 2 h at room temperature (MURAKAMI, 1973).

After dehydration in a series of graded ethanol, they were transferred to isoamyl acetate, critical point-dried using liquid CO_2 , mounted on metal stubs with adhesive tape, coated with platinum-palladium in an ion coater (Eiko IB-5, Eiko Engineering Co., Japan), and observed in a scanning electron microscope (S-2380N, Hitachi).

RESULTS

Light microscopy

Thin sections of the mouse pancreas showed that S-100 immunoreactivity was detected in the fat cells and the peripheral nerves. S-100 immunopositive nerves were present around arterioles, venules, pancreatic ducts, and in the islets of Langerhans. These nerves were usually observed as a slender cord, but revealed nuclear swellings at places, indicating the Schwann cells of peripheral nerves to be immunostained with the S-100 antibody. Ganglion cells, which were often associated with the islets of Langerhans, were also surrounded by Schwann cells (or satellite cells). The sheet-like Schwann cells were often found on the margin of the islets as if to delimit the islet from the exocrine parenchyma (Fig. 2). Schwann cells were also present inside the islets, although their number was small.

Observations of S-100 immunostained thick sections with a stereo light microscope clarified the

Fig. 4. Transmission electron micrographs of the islet of Langerhans in the mouse. **a.** A Schwann cell (*arrows*) is present in the periphery of the islet as if investing the islet cells. *C* blood capillary within the islet. $\times 2,500$. **b.** Closer view of the membranous Schwann cell (*S*) in the periphery of the islet. Note an axon terminal (*arrowhead*) enwrapped by Schwann cell cytoplasm. A basal lamina is found outside the Schwann cell sheet but not present between the Schwann cell and islet cells. $\times 10,000$

three-dimensional distribution of peripheral nerves in the mouse pancreas (Fig. 3). Nerves entering the islets usually accompanied afferent arterioles. These islets were often in contact with a mass of ganglion cells. Some islets received nerves running through the

exocrine parenchyma (Figs. 2, 3). Schwann cells of the islet connected with each other by their thin and slender processes to form a delicate network on the surface of the islet (Fig. 3). These Schwann cells also extended their processes to the inside of the islet and

Fig. 5. High magnification of a nerve found on the surface of the islet. Several axon terminals are either completely or incompletely invested by the Schwann cell cytoplasm which is in close contact with the islet cell. Synaptic vesicles are found in some of the axon terminals. *EX* exocrine acinus. $\times 20,000$

anastomosed with Schwann cells located within it.

Transmission electron microscopy

Unmyelinated nerves were often recognized near the islets of Langerhans. They were composed of axons ensheathed by the cytoplasm of Schwann cells. Schwann cells were often located on the periphery of the islet; they possessed an ovoid nucleus and extended thin sheet-like processes along the basal surface of the islet endocrine cells (Fig. 4). The basal laminae were present outside the membranous Schwann cells but were not found between the Schwann cells and endocrine cells. Thus, the Schwann cells directly invested the surface of the islet, separating it from both the connective tissue capsule and the acinar tissue.

Axons were usually found on the outer surface of these Schwann cells, but were often enclosed by the Schwann cell cytoplasm. Most of these axons contained small Synaptic and large dense-cored vesicles (Fig. 5).

Scanning electron microscopy

Simple KOH digestion method (USHIKI and MURAKUMO, 1991) used in the present study effectively removed the connective tissue matrices without any severe damage to cell components, thus enabling direct visualization of the surface of the islet in relation to the structures of nervous elements (Fig. 6).

In the mouse pancreas, both nerve bundles and single unmyelinated nerves were recognizable near

Fig. 6. Scanning electron micrographs of the mouse islet of Langerhans. **a.** Overview of an islet of Langerhans (*L*) which appears as a spherical body. $\times 350$. **b.** Closer view of a part of Figure 6a. Numerous axons with varicosities are found on the surface of the islet. *C* blood capillary. $\times 2,200$. **c.** Closer view of a part of Figure 6b. The Schwann cell cytoplasm is colored in yellow, and axons in green. *Asterisk* indicates the basal part of the islet endocrine cell not covered by the Schwann cell sheet. $\times 4,000$. **d.** Higher magnification of the lower part of Figure 6b. Axons (green) are present on the surface of the Schwann cell sheet (green). Note a swelling (*arrow-head*) of the axon terminal. $\times 10,000$

Fig. 6. Legend on the opposite page.

Fig. 7. Legend on the opposite page.

Fig. 8. Scanning electron micrograph of the rat islet of Langerhans. Schwann cells and their cytoplasm are colored in yellow and axons in green. Asterisks indicate the basal parts not covered by the Schwann cell cytoplasm. *S* nuclear portion of the Schwann cell, *C* blood capillary. $\times 3,300$

the islet (Fig. 7a). The single unmyelinated nerves were observed as slender cords which sometimes branched and anastomosed with each other. At higher magnification, these nerves were composed of axons with Schwann cell investments (USHIKI and IDE, 1988; USHIKI and WATANABE, 1997). After reaching the islet, the nerves spread on the surface of the islet. Schwann cells in this portion extended their thin membranous processes, thus investing the islet as a whole (Fig. 6c). Schwann cell bodies were sometimes observed as an ovoid elevation with varying degrees of height (Fig. 7). Axons with varicosities were usually found on the surface of these membranous

Schwann cells. The axons, in most of their courses, ran in corresponding furrows on the Schwann cells, exposing their upper aspect. Some of the axons crept beneath the Schwann cell membranous processes (Figs. 6d, 7c). In the area where endocrine cells were not covered with the membranous Schwann cells, axons were seen also directly attaching to the basal part of the endocrine cells (Fig. 7).

In the rat pancreas, the membranous Schwann cells associated with axons were also observed on the surface of islet, although the area covered by them was smaller than in the mouse islet (Fig. 8).

Fig. 7. Scanning electron micrographs of the mouse islet of Langerhans. **a.** Schwann cell body with a nuclear swelling (*S*) is found on the surface of the islet. *N* nerve and nerve bundle, *C* blood capillary. $\times 1,800$. **b.** Schwann cell in this micrograph has a spindle-shaped cell body (*S*). Note axons on the surface of the islet. *C* capillaries, *P* pericyte. $\times 2,400$. **c.** Closer view of a part of Figure 7b. Schwann cell and its cytoplasm is colored in yellow and axons in green. *P* pericyte. $\times 6,000$

DISCUSSION

The present study has demonstrated precisely the three-dimensional architecture of peri-insular nerve plexuses in the murine pancreas by the combined use of light microscopy of S-100 immunostained sections, TEM of thin sections, and SEM of KOH digested tissue. A dense network of unmyelinated nerves has been shown on the surface of the individual islet, which supplements our previous studies (USHIKI and IDE, 1988; USHIKI and WATANABE, 1997).

As briefly mentioned in Introduction, HONJIN (1956) reported the presence of the "neural terminal nets" consisting of nerve fibers and the "interstitial cells of Cajal" in and around the islet of the mouse pancreas (Fig. 1). While calling the cells "interstitial cells", HONJIN apparently regarded them as a particular kind of neuron, having recorded a "neurofibrillar network" around their nuclei in his silver-impregnated sections. In fact, we admit that the distribution and morphological features of our S-100 immunoreactive cells are closely similar to those of the interstitial cells of Cajal. Nevertheless, the cells now in question seem to be endowed with every feature characteristic of Schwann cells, especially their possession of S-100 immunoreactivity and their fine structure investing nerve axons. It is thus most probable that the "interstitial cells of Cajal" reported by HONJIN correspond to the terminal Schwann cells, as noted previously in our studies on the exocrine pancreas (USHIKI and IDE, 1988, USHIKI and WATANABE, 1997) and intestine (USHIKI, 1992). In this context, KOBAYASHI (1990, 1996) correctly pointed out the confusion in the usage of the term, "interstitial cells of Cajal", in various tissues from both historical and morphological view points.

The present TEM and SEM findings have further demonstrated that Schwann cells extend their membranous processes on the surface of the islets of the mouse and rat pancreas, although such thin processes were not clearly detected in thick sections immunostained with S-100 antibody. A close relation between Schwann cells and islet endocrine cells has been reported by previous TEM investigators in some mammals (dog: SMITH, 1975; FUJITA and KOBAYASHI, 1979; rat: DONEV, 1982; sand rat: DONEV, 1984). Previous immunohistochemical studies using an anti-S-100 protein antibody have also shown the existence of Schwann cells in the pancreatic islet (rat: FUJITA et al., 1983; monkey: GIROD et al., 1987; guinea-pig: UCHIDA and ENDO, 1989; and human, LASZIK et al., 1989). Although our findings are basically in accordance with these studies, the spatial relation-

ship among Schwann cells, the islet cells, and axons in the murine pancreatic islets were able to be visualized more evidently and three-dimensionally than before. We have also shown that, in the murine pancreas, Schwann cells invest the islet as a whole with their attenuated cytoplasm, on which axons run like a creeping plant.

The reason why Schwann cells extend membranous cytoplasmic processes on the surface of the islets and have a special relationship with the pancreatic endocrine cells remains unknown. One possibility is an affinity between the Schwann cells and endocrine cells. FUJITA (1976) originated the concept of the "paraneuron," in which he stated that peptide producing endocrine cells and sensory cells share structural, functional, and metabolic features with neurons (FUJITA et al., 1988). The pancreatic endocrine cells are one of the typical paraneurons, and this view accounts for their high affinity to neurons and glial elements. In fact, Schwann cells have been reported to invest islet cells and neurons simultaneously, suggesting that both elements might be equally recognized by the Schwann cells (SERIZAWA et al., 1979; FUJITA and KOBAYASHI, 1979). It has even been stated that the islet of Langerhans, which consists of paraneurons and neurons — the population of the latter being variable among animal species, can be regarded as a modified ganglion (FUJITA, 1959, SERIZAWA et al., 1979).

Concerning the innervation pattern in the islets, FUJITA and KOBAYASHI (1979) demonstrated in TEM studies of the dog pancreas that the axons supplying the islets mainly ended in the pericapillary space and only a few axons terminated on the endocrine cells. However, in the mouse pancreas axons terminate mainly on the endocrine cells of the isles, as shown in the present and previous studies (USHIKI and WATANABE, 1997). This provides morphological evidence supporting that axons directly innervate the islet cells in the mouse pancreas. Although its functional significance is still unknown in the present study, the rich innervation of the islet cells may suggest that secretion of endocrine cells are intimately controlled by autonomic nerves in the mouse pancreas. Physiological data showing that stimulation of the autonomic nerves and treatment with neurotransmitters affect islet hormone secretion (e.g., see review by AHREN, 2000) may also support our findings.

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