Ultrastructural Study on the Contact between Lipid-Storing Cells and Macrophages

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Summary：The lipid-storing cells (LSC) proposed by Watari, containing a small number of lipid droplets in their cytoplasms, can be found within the interstitial connective tissue of various organs. These cells are considered to dissolve, store and detoxify various toxic materials within their lipid droplets. That the LSC exchange information with macrophages (MPs) is also assumed.

The present study was performed to elucidate the morphological mechanism for the exchange of information between LSC and MP by means of electron-microscopy in the KK-A^y mouse. Two types of cellular contact between LSC and MP were submicroscopically demonstrated. One was a membranous contact, the other a connection of cytoplasmic protuberances. For the latter type, route structures around 1 μm in width were verified electron-microscopically. Their functional roles were also discussed.

Introduction

KK-A^y mice, which are genetically diabetic, are well known for having a condition similar to non insulin-dependent diabetes mellitus (NIDDM)[1-3]. Using these mice, the protective effect of glycyrrhizin (an extract of kanzo, a plant used in Chinese herbal medicine) against pancreatic injuries by genetic diabetes was electron-microscopically studied[4,5]. The existence of lipid-storing cells (LSC), proposed by Watari[6], was confirmed in the interstitial connective tissue of the pancreas of KK-A^y mice, using electron micrographs. Also, the connection between LSC and macrophages (MPs) have been described. As for the connection between LSC and MP, much can be obtained from the work of Watari et al[7-12]. But electron-microscopic evidence for information exchange between these two cells has yet to be obtained.

The present study was conducted in order to determine whether a path-way (protuberance) for facilitating information exchange exists between these cells or not.

Materials and Methods

Twelve KK-A^y male mice (ca.36g, 8 weeks old) were purchased from Japan Clear Co., Osaka. Six mice served as the control group, and received no treatment. The other six mice comprised the experimental group, and were injected twice a week intramuscularly with 20 mg/kg of glycyrrhizin (an extract from the Chinese herb, glycyrrhiza uralensis) supplied by Minophagen Pharmaceutical Co., Tokyo, for a month. All twelve mice (ca.42g,12 weeks old) were sacrificed by decapitation after the experiment. From each, the pancreatic tissue was dissected, and small pieces fixed at 4 ℃ for 2 hours in a mixture of glutaraldehyde (final concentration of 2.5 %) and osmium tetraoxide (final concentration of 1 %) in 0.14M veronal acetate buffer (pH 7.4). Sucrose (0.045 g/ml) was added to adjust the osmotic pressure of the fixative. In the course of the electron-microscopic observations, the author randomly chose 5 pancreatic tissue blocks (size : about 1mm^3) from each mouse in each group. The fixed tissue blocks were embedded in Epon 812 after dehydra-

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tion in ethanol and immersion in propylen oxide. Ultra·thin sections with a thickness of about 70 nm were obtaind by Ultracut (Reichert-Jung). These sections were stained with an uranyl acetate and lead citrate mixture, and then examined under a transmission electron-microscope (JEM 100-S). When a connection between LSC and MP was detected electron-microscopically, this part of the tissue block was sliced into successive ultra·thin sections, and observed more closely.

Results

LSC are generally shaped long and thin, and have many cytoplasmic protuberances. Their nuclei are ellipses, and they have a relatively well-developed rough endoplasmic reticulum and one or more small lipid droplets. Other cell organelles of LSC include small Golgi apparatus, a few mitochondria, and vesicles. On the other hand , fixed MPs were rarely observed in the pancreatic interstitial connective tissues of the control group. Their shapes are irregular and they contained some cell organelles, especially a variety of lysosomal inclusion bodies. Activated MPs contain also a number of heterogeneous phagosomes.

LSC were observed in the pancreatic interstitial connective tissue of the KK-A^y mouse in the control group (Fig. 1). One lipid droplet was confirmed within each cell. Most of the LSC found in the control group were of similar ultrastructure to that shown in Fig. 1. Figure 2 shows a connection between LSC and MP in the pancreatic interstitial connective tissue of the control group. Connections were found in only two of six control animals. In the pancreatic interstitial connective tissues, LSC having contact with MP are observed in the experimental group (Fig. 3). One lipid droplet was found within each of the LSC, which was connected to three MPs. It is quite unusual to find a cell connected to three MPs, as shown in Fig. 3. A higher power picture of a neighboring section is shown in Fig. 4. Electron densities in the connection between both cell membranes were somewhat higher than those without such a

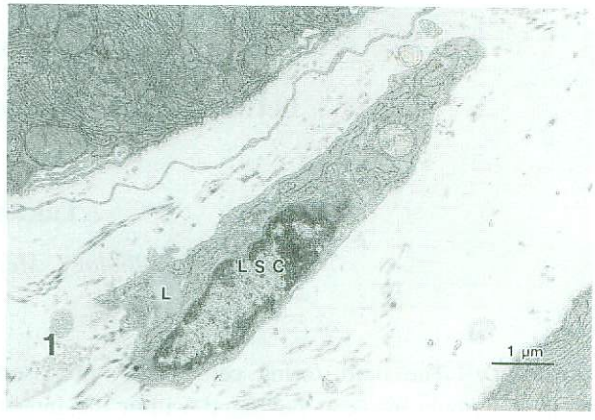

Fig. 1 LSC containing one L are observed in non-treated KK-A^y mouse. LSC are usually observed alone in the control group (male rats, 12 weeks old)
L : lipid droplet, LSC : lipid-storing cells

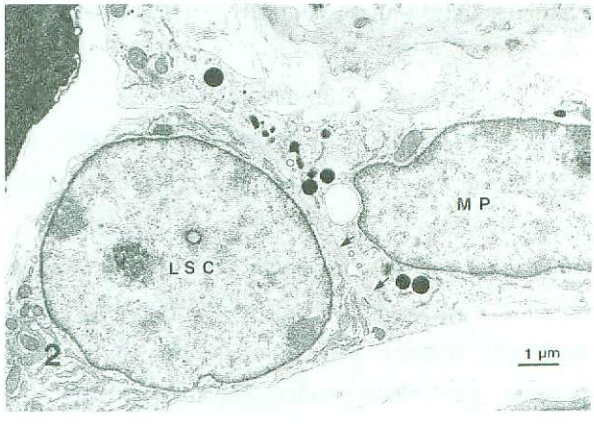

Fig. 2 LSC in contact with MP are observed in non-treated KK-A^y mouse (control group).
LSC : lipid-storing cells, MP : macrophage, arrows : contact area between LSC and MP

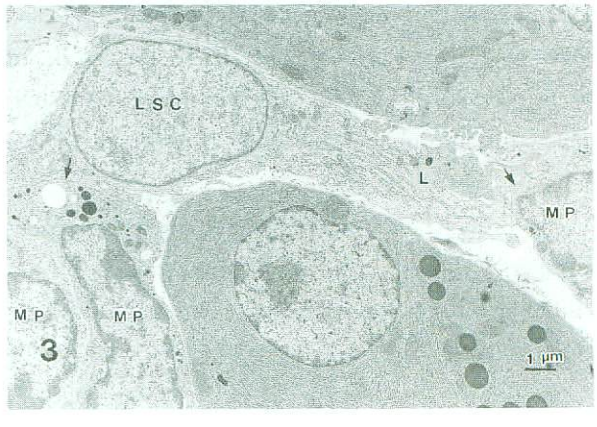

Fig. 3 This electron-micrograph shows one of the connections between LSC and MP in the pancreatic interstitial connective tissue of the experimental group (male rats, 12 weeks old). LSC contain one L
L : lipid droplet, LSC : lipid-storing cells, MP : macrophage, arrows : contact area between LSC and MP

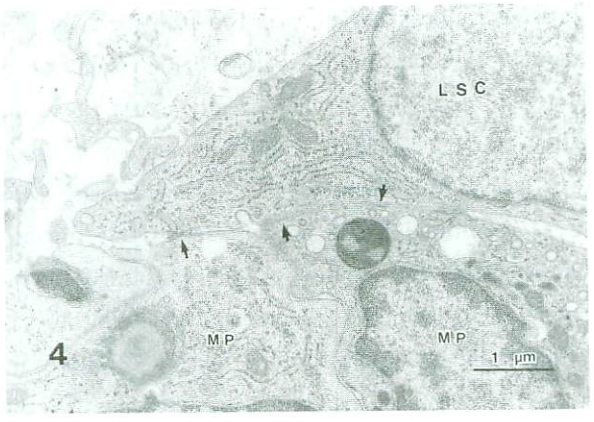

Fig. 4　A higher power view of a neighboring section to that in Fig. 3. LSC contact with MPs.
LSC : lipid-storing cells,　MP : macrophage,　arrows : contact area between LSC and MP

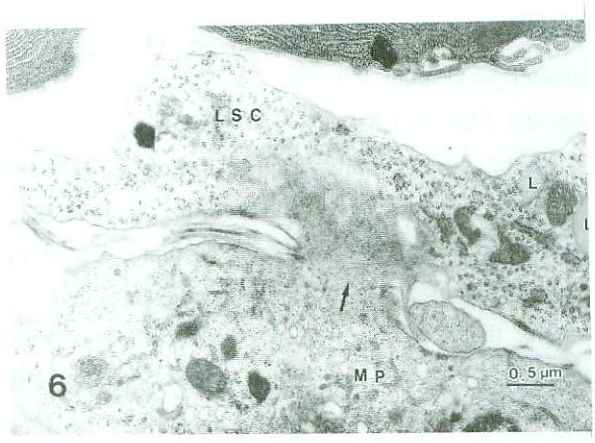

Fig. 6　A high magnification view of the area marked by the arrow in Fig. 5. LSC contact with MP.
L : lipid droplet,　LSC : lipid-storing cells,　MP : macrophage,　arrow : protuberance

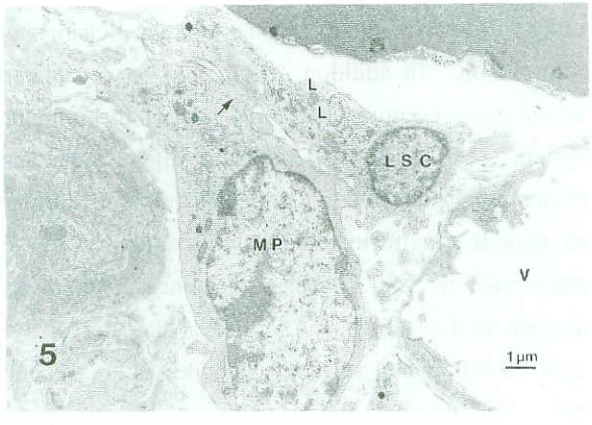

Fig. 5　This electron-micrograph shows one of the connections between LSC and MP in the pancreatic interstitial connective tissue of the experimental group. A protuberance (path-way)-like structure is seen between the two cell types.
L : lipid droplet,　LSC : lipid-storing cells,　MP : macrophage,　V : blood capillary,　arrow : protuberance

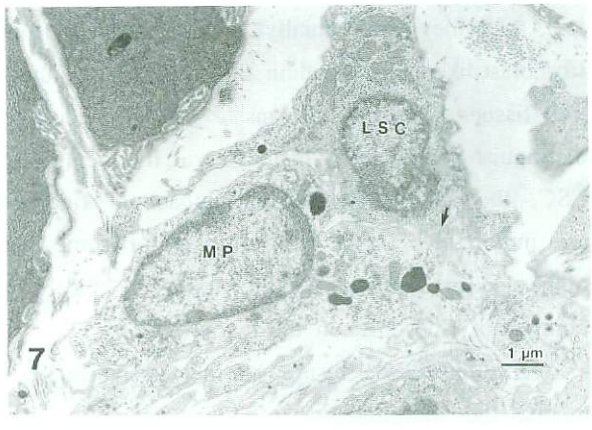

Fig. 7　LSC are in close contct with MP (experimental group).
LSC : lipid-storing cells,　MP : macrophage,　arrow : protuberance

connection, but the membranes of the boundaries of both cells were indistinct (Fig. 4). Fig. 5 shows an example of the connections between LSC and MP in the pancreatic interstitial connective tissue of the experimental group. Two lipid droplets were confirmed within the cell, and rough endoplasmic reticulum and a lot of free ribosomes were observed within the cytoplasm. Between LSC and MP there was a formation that could be a path-way : protuberance. The protuberance (path-way) of the MP was 1.4 μm in width, and was thought to be extending toward the LSC. Higher power revealed a protuberance (path-way) (Fig. 6). The tip of the 1.4 μm protuberance is considered to connect with the LSC. It is not certain, however, whether the materials of both cells mix together through this connection. The protuberance of MP might touch closely the LSC or cover it. To make this more clear, the author obtained a morphological image by tilting the axis of the sections at 15° intervals when taking electron-microscopic pictures. Though these pictures were compared with pictures not taken at an angle, no clear differences in morphology could be detected. The reason for this was that the thickness of the sections was only around 70 nm. Then, the author obtained evidence for the morphology of this protuberance (path-way), though it was less clear than those in

Figs. 5 and 6. In the other areas (Fig. 7), its diameter was about 0.9 μm. It seemed to be connecting LSC and MP as in Figs. 5 and 6.

In the present study, eight connections between LSC and MP were observed in four of six experimental mice injected with glycyrrhizin, but only two membraneous type connections were detected among the six control mice. This difference suggests that in the experimental group injected with glycyrrhizin there were more connections between LSC and MP.

Discussion

LSC, proposed by Watari[6], have been reported to be derived from pericytes (surrounded capillaries), but they are actually mostly derived from fibroblast-like cells found in the interstitial connective tissues of various organs[12]. It is difficult to distinguish LSC from histocytes. In identifying LSC, the following electron-microscopic findings are important : small lipid droplets of somewhat high electron density in the cytoplasma ; a large number of ribosomes in the cytoplasma.

According to the results of the present experiments, the morphologies of the connections between LSC and MPs could be divided into two groups. One is the type of connection seen in Figs. 2, 3 and 4. Compared with the unconnected areas, connected areas have higher electron-densities in border regions of the cell membranes, which are somewhat unclear. The cell membranes of the connected areas show normal thickness, and the unclear membranes may suggest that the electron-densities are increased due to either the concentration of information-transmitting materials or the increase of enzymes that digest these transmitting materials. This type of connection may not involve the adhesion of cells by desmozomes, but it might have some other functional role, such as information exchange. The author supposes that one from of information exchange between LSC and MP is diffusion or active transport.

Another type of connection is seen in Figs. 5 and 6. Here, the connected areas of both cells possess pathway structures. The path-way which is thought to connect LSC and MP is a protuberance, 1.4 μm in diameter. If it really exists, it would allow the exchange of a large amount of information-transmitting materials. As mentioned, MPs were confirmed to be present among LSC materials, but the morphology of the tip of MP membrane is unclear. Moreover, it is impossible to say whether the materials forming in both cells are actually mixed, or merely linked closely to each other. The morphological images were obtained by tilting the axis of the sections (70 nm thick) at 15° intervals when taking electron-microscopic pictures. But, it could not be determined whether the two cell types were connected or adhered to each other. However, there is some sort of exchange of information. In addition, MPs could be considered as antigen-presenting cells or a part of the immune system. MP could take antigens, digest them and present their information on their own cell surface as peptide fragments. This information could be intercepted by LSC. The protuberance (path-way), as seen in Figs. 5, 6 and 7, had a diameter of 1.4 μm$\sim$0.9 μm and seemed to connect the LSC and MP. This finding suggests the existence of a pathway (protuberance), though more evidence should be collected.

Based on electron-microscopic images, it is suggested that there occurs information exchange between LSC and MP through cellular contact or protuberance. The experimental group injected with glycyrrhizin exhibited more connections between LSC and MP, and from an electron-microscopic image, the protuberance (path-way) was estimated to be about 1 μm in diameter, extremely strong evidence supporting Watari et al[7-12] hypothesis.

Conclusion

Ultrastructural connections between LSC and MP were searched in interstitial connective tissues within the pancreases of KK-A^y mice with genetic diabetes, and the following results obtained.

1) Electron-microscopic observations suggest one

possible form of information exchange between LSC and MP is ion transportation through the contact of both cell membranes.

2) Electron-microscopy also suggests another possible form of information exchange is the connection of cytoplasmic protuberance (path-way) structures between the two cell types.
Some protuberances were 1 μm in diameter. There was a path-way (protuberance) where information exchange was conducted smoothly.

3) Such connections were detected more in the experimental group injected with glycyrrhizin than the control group.

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脂質貯蔵細胞とマクロファージの接触像（情報交流）についての超微形態学的研究

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要旨　渡の提唱する脂質貯蔵細胞（lipid-storing cell：LSC）は，いろいろな臓器の間質結合組織内に出現する細胞で、その細胞質には，少数の小脂質滴が認められる．渡らは，LSC は，その脂質滴のなかに，生体にとって毒性のある物質の多くを溶解，貯蔵し，その毒性を弱めるように働くと考えている．従来，LSC とマクロファージ（MP）との接触像が電顕的にしばしば認められ，その所見から，両細胞間での情報交流が予測される．

本研究は，上記の両細胞の接触像を電顕的に検索し，情報交換ないし情報伝達を行いうる，いわゆる連絡路の存在を，超微形態学的に追求したものである．その結果，LSC と MP の接触像には，二つのタイプが認められた．一つは，お互いの一部細胞膜同志が直接接触している所見であり，他は，細胞膜突起を介する連結所見であった．とくに後者については，その巾が１μｍもある太い連絡路の構造として認められた．以上の両細胞接触像の機能についても若干考案した．

キーワード：脂質貯蔵細胞　Lipid-storing cell，マクロファージ　Macrophage，
突起　Protuberance，電子顕微鏡　Electron-microscopy