Mast Cell-Ito Cell Pairings Found in the Disse’s Spaces in the Liver of the Beagle Dog

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Summary. Ito cells (fat-storing cells), containing a few lipid droplets, appeared in the Disse’s spaces of the Beagle dog liver. They ranged in diameter from 0.5 to 5 μm. The present study revealed that mast cells were also distributed in the Disse’s spaces, while they were encountered only in the interlobular connective tissue in other animals. Although mast cells as well as Ito cells were distributed solitarily in the Disse’s spaces, pairing of both types of cells could be frequently observed. Direct contact between both cells was verified using both light and electron microscopy. Also encountered were some peculiar figures of certain Kupffer cells engulfing the cytoplasmic processes and secretory granules of mast cells.

These findings indicate a previously unknown intimate relationship between the mast cell and other perisinusoidal cells in the dog liver: the Ito cell, Kupffer cell and endothelial cell.

Many kinds of cells are known to exist in the Disse’s spaces in the liver. Among these, Ito’s fat-storing cells (Ito, 1951) are most common and are widely distributed among animals ranging from mammals down to fishes. Plasma cells, macrophages, mononuclear lymphoid cells, nerve fibers and Schwann cells are less common constituents of the Disse’s spaces. Mast cells in the liver have been occasionally found in the Glisson’s sheath of some mammalian species, but have not yet been observed in the Disse’s spaces of any other vertebrate species except the dog. The dog has been long known to be a special animal that contains numerous mast cells in its liver (Nakajima, 1928; Nagayo, 1928; Arvy et al., 1955; Mota et al., 1956). Fujita (1964) clearly showed the distribution of numerous mast cells in the walls of hepatic vein branches as well as in the hepatic lobules of the dog. In the liver of the present beagle, figures were frequently encountered which indicated that the mast cell and Ito cell were in direct contact with each other in the Disse’s space. We therefore focussed on the fine structure of these mast cell-Ito cell pairings that appeared in this particular animal, and discussed the possible functional relationships between these paired cells with other types of sinusoidal liver cells.
MATERIALS AND METHODS

Eight adult beagles of both sexes were used in the present study. Under Nembutal anesthesia, liver tissues were picked out and were cut into moderate sizes, and then were fixed in either Bouin's solution or 10% formalin for light microscopic observation. After embedding in Paraffin, sections were stained by hematoxylin and eosin, azocarmin, Nile blue and PAS reaction. For electron microscopy small pieces of the tissue were fixed in cold Karnovsky's solution (KARNOVSKY, 1965) for 15–20 hr; after washing

Fig. 1. Light micrograph showing a part from a thick section of the liver of a beagle dog stained by toluidine blue. Metachromatic mast cells (arrows) are distributed throughout the lobule.  ×350

Fig. 2. A higher magnification showing a metachromatic mast cell (m), Ito cells with fat droplets (f), a Kupffer cell (k) and endothelial linings (e). Thick section from epon embedded material. Toluidine blue staining.  h Hepatocytes, s sinusoids.  ×1,500
in cacodylate buffer they were post-fixed in 1% OsO₄ (MILLONIG's methods, 1962) for 1.5 hr, and then embedded in Araldite-Epon. Sections, 1 μm thick, were stained by toluidine blue for light microscopy and thin sections were stained by both uranyl acetate and lead, and observed with H-500 and JEM-1200EX type electron microscopes.

RESULTS

In the liver tissue of the beagle, the connective tissue of Glisson's sheath is not generally well developed and consequently the contour of each lobule appears not so distinct. Mast cells that show metachromasia by toluidine blue (Fig. 1), positive to Nile blue were distributed just beneath the endothelial cells of large interlobular blood vessels and also among the smooth muscle cells of these vessels. Many more mast cells were encountered in the adventitia of these blood vessels and in the Glisson's sheath around them. Furthermore, mast cells were numerous in the Disse's spaces within the hepatic lobules. Along the Disse's spaces, mast cells were distributed as if they might have invaded from the interlobular connective tissue. Thus, there was a tendency for the mast cells to become denser in population in the peripheral zone and become sparser towards the central part of each lobule. There were often found central veins that contained mast cells just beneath the endothelial cells.

Ito cells appeared in the Disse's spaces, usually containing a few lipid droplets of

Fig. 3. Electron micrograph of an Ito cell (ITO) located in the Disse's space found between hepatocytes (H) and an endothelial lining (EN). Three lipid droplets (L), a Golgi apparatus (G) and granular endoplasmic reticulum (ER) are found on one side of the nucleus (N) of the Ito cell. The cytoplasmic membrane of the Ito cell partly contacts the hepatocyte where inconspicuous patches of a junctional complex (arrows) are found. Ly lysosome, M mitochondria, S sinusoidal lumen, V microvilli of hepatocyte. ×13,300
moderate sizes, 0.5–5 μm in diameter (Fig. 2), which caused variable indentations on the surface of the nuclear envelope. In many cases the lipid droplets were gathered on one side of the cell, pushing the nucleus on the other side, while in other cases the droplets were distributed evenly around a centrally located nucleus. Mast cells scattering in the Disse’s space also showed differing contours, varying from large oval to slender according to the amount of the secretory granules contained in their cytoplasm.

Kupffer cells covered with irregular cytoplasmic processes and containing phagosomes, or lysosomes of various sizes were observed in the sinusoidal lumen in addition to red blood corpuscles and leukocytes.

Electron microscopy clarified that both Ito cells and mast cells were usually located in the Disse’s space, being separated from the sinusoidal lumen by a thin endothelial cell lining. As shown in Figure 3, one Ito cell appeared with three lipid droplets on one side of the nucleus, containing a granular endoplasmic reticulum, Golgi apparatus, lysosomes and smaller sized mitochondria compared with those of the adjacent hepatocyte. Sometimes, long cytoplasmic processes were extended from Ito cells underneath the endothelial cells of the blood sinusoids. Though a part of Ito cells was located close to the endothelial cell lining, a narrow gap remained between the two (Fig. 3). Two kinds of cell-to-cell contact were noticed between Ito cells and the hepatocytes. On the left hand side of Figure 3 can be seen a hepatocyte projecting many microvilli as well as an Ito cell with a somewhat rugged contour with some microvilli facing each other, while on the right hand side the surfaces of both cells are smooth and closely confronting each other, leaving a narrow space between them, though at some parts they closely front (arrows in Fig. 3).

Mast cells are characterized by numerous secretory granules ranging from round

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**Fig. 4.** A mast cell (MC) containing secretory granules of irregular shape found in the Disse’s space (SD) among an endothelial lining (EN) and hepatocytes (H). G Golgi apparatus of mast cell, S sinusoidal lumen. ×15,800
Fig. 5. A cytoplasmic bleb (arrow) with a few secretory granules of a mast cell (MC) whose perikaryon is located in the Disse's space. The cytoplasmic bleb is protruded into the sinusoidal lumen (S) passing through the endothelial lining (EN). E erythrocyte, H hepatocyte. ×13,000

Fig. 6. A mast cell (MC) containing numerous secretory granules and an Ito cell (ITO) containing a lipid droplet (L) contact closely at the two points of their juxtaposed cytoplasmic membranes (straight arrows) in the Disse's space, while the facing surface of Ito cell and hepatocyte show a button-like attachment at several points (curved arrows). E erythrocyte, EN endothelial cell, H hepatocyte, S sinusoidal lumen. ×7,900
to ellipsoid in shape; the contents of the granules vary from fine granular structures of moderate electron density to dense compact granular ones (Fig. 4). Among these secretory granules, granular endoplasmic reticulum, Golgi apparatus and small mitochondria are found. Sometimes a mast cell shoots cytoplasmic processes with some secretory granules beneath the endothelial lining. Bulbous cytoplasmic processes with secretory granules, protruding into the sinusoidal lumen through the endothelial cell sheath can also be often encountered (Fig. 5).

Despite the fact that mast cells and Ito cells appear to be solely distributed in the Disse’s space, “mast cell-Ito cell pairings” involving direct contact with each other were frequently noticed there. The pairings were recognized with both light (Fig. 2) and electron microscopy (Fig. 6–10). Figure 6 shows a mast cell-Ito cell pairing with cell contact at two points on their surfaces (straight arrows), while they show a button-like attachment at several points against an Ito cell and hepatocyte (curved arrows). A cytoplasmic process of an Ito cell containing three lipid droplets appears in Figure 7. This cytoplasmic process extends between two hepatocytes from underneath and embraces a mast cell from above. Interrupted contact areas (curved arrows) are seen between the cytoplasmic process of the Ito cell and the hepatocytes. Figure 8 shows a similar pairing of the two types of cells contacting with a relatively longer part of both surfaces of the cytoplasm. In none of the cases of Ito cell-mast cell contact was found any specific junctional differentiation such as a tight junction, gap junction or desmosome (Fig. 9); only poor tight junctional structures were found between the Ito cell and hepatocyte in a few cases (Fig. 3).
Occasionally we met with figures suggesting that a mast cell might be migrating from the Disse’s space into the sinusoidal lumen through the endothelium barrier. Figure 10 illustrates one such cases. Just half of the cell body with a constricted nucleus seems to have passed into the sinusoidal lumen. The secretory granules appear in the cytoplasm of both sides of the constricted cell. An Ito cell with few lipid droplets is seen in contact with the mast cell in the Disse’s space.

In Figure 11, a large part of a mast cell is seen escaping from the Disse’s space, leaving only a small cytoplasmic process with several secretory granules in it. In this case the cytoplasm of an elongated Kupffer cell covers the escaping part of the mast cell in the sinusoidal lumen. Figure 12 shows also an invading mast cell from the Disse’s space. It’s remaining cytoplasmic process closely contacts the surface of an Ito cell. The migrating part of this mast cell is covered with a Kupffer cell and its cytoplasmic process is anchored to the endothelium. Figure 13 is an ultra- section slipped several sections from that of Figure 12, showing a figure of Kupffer cell that seems to engulf the granule-containing processes of a mast cell.

Fig. 8. A mast cell (MC) containing irregular-shaped secretory granules contacts an Ito cell (ITO) that contains two large lipid droplets (L). Note that the contact surface is running along the longitudinal cell axis in the Disse’s space. EN endothelial lining, H hepatocyte, N nucleus, S sinusoidal lumen. × 13,300
DISCUSSION

It is well known that the liver of the dog is particularly rich in mast cells (Nakajima, 1928; Nagayo, 1928; Arvy and Quivy, 1955; Mota et al., 1956). The relationship between these mast cells and anaphylactic and peptone shock has been investigated by several authors (Wilander, 1939; Jacques and Waters, 1941; Mota et al., 1954; Kawamoto, 1958; Nishiyama, 1959a, b). Fujita (1964) demonstrated a peculiar distribution of mast cells beneath the endothelium of hepatic vein branches. He discussed these structures in relation to shock derived from peptone and other substances. As Fujita

Fig. 9. An Ito cell (ITO) containing lipid droplets (L) and granular endoplasmic reticulum (ER) in close contact (arrows) with a mast cell (MC). a × 18,000, b × 44,000
(1964) pointed out, mast cells are also numerous in Disse's space in the dog hepatic lobule, and this was confirmed by us in the beagle (KOBAYASHI et al., 1985). Thus the mast cell has come to be referred to as the common resident in Disse's space of the dog liver. On the other hand, the Ito cell has been established to be a constant component of the Disse's space of vertebrate species (ITO, 1969, 1973, 1978), and this was also the case in the present beagle. Therefore, these two types of cells have to be considered as residential cells in the Disse's space of dog liver.

Desmosomal junctions between an Ito cell and hepatocyte were found in some teleostean species (NOPANITAYA et al., 1979) and in the crucian liver (TANUMA and ITO 1980), while junctions between the Ito cell and endothelial cell and between two Ito cells have been found in the goldfish (FUJITA et al., 1980). Thus the appearance of the desmosomal structure between the Ito cell and other types of cells may be a normal figure of the liver of teleostean fishes; no desmosomes have been found between these sinusoidal cells in other vertebrate animals. It is reasonable to consider that the Ito cell is not a wandering cell but a stationary one—at least in fish (FUJITA et al., 1980). In the present study on the dog liver, a few cases of close contact with very poor desmosome-like structures were found only between the Ito cell and the hepatocyte.

**Fig. 10.** A mast cell (MC) migrating from the Disse's space (SD) into the sinusoid (S) penetrating through the endothelial lining (EN). A part of the cytoplasmic membrane of the mast cell in the Disse's space contacts an Ito cell (ITO) which contains two lipid droplets (L). E erythrocyte, H hepatocyte, N nucleus. ×13,000
Fig. 11 and 12. Legends on the opposite page.
Though most of the mast cells and Ito cells in the hepatic lobule of the dog were distributed solitarily, some appeared paired. Particularly close contact between a mast cell and Ito cell was frequently encountered and confirmed by both light and electron microscopy. Specific junctions like desmosomes or gap junctions between these cell membranes could not be recognized even when these two cells contacted each other with closely apposed cell membranes running parallel for a long distance. However, an
intimate functional relationship for the two cells may be conceivable from their morphological relation. The reason why these two cells contact each other in the narrow Disse’s space may be found in the possibility of their mutual reactions. There are some reports that administered vitamin A enhances the collagen formation in the liver, suggesting that the Ito cell is responsible for this effect (Ito, 1973; Takahashi et al., 1978). On the other hand, Taylor (1971a, b) found that lysis of collagen fibers in cultured mesenteries with explants of gingival tissue occurred around degranulating mast cells and their dispersed granules, and that this mast cell effect was augmented by vitamin A and heparin, but was inhibited by cortisol and histamine. Heparin and histamine, which may be released locally from mast cells, were found to have opposite effects on collagen lysis (Taylor, 1971a, b). Presumably these antagonistic substances may possibly be released selectively from the cells in response to specific agents (Johnson and Moran, 1969), a condition that would be necessary if the mast cell products are to act as a controlling mechanism of collagenolysis (Taylor, 1971b). The present finding that mast cells and vitamin A storing Ito cells are located close to each other in the Disse’s space leads to the assumption that collagenogenesis and/or collagenolysis might be finely controlled by these two types of cell.

McCusky et al. (1979) pointed out that histamine and serotonin are released by a cholinergic mechanism from mast cells in the portal tract and reach the sieve plate of the endothelial cell, thus affecting the permeability of the sinusoidal wall. Ohata (1984), studying the innervation of guinea pig liver, proposed that the adrenergic effects arrive at the sinusoidal endothelium and Kupffer cells through synaptic contacts or through diffusion from nerve endings. Concerning the release of histamine and serotonin, McCusky et al. (1979) proposed a cholinergic mechanism, while Ohata (1984) opposed this view since no cholinergic terminals have ever been detected. Though the present study of dog liver did not reveal innervation in the Disse’s space it can be reasonably presumed that histamine and serotonin might be liberated by nervous or humoral stimuli from mast cells dispersed in the Disse’s space, and may affect the permeability of the endothelial sieve plates of sinusoids. Mast cells of the dog liver showed a positive reaction by Falck-Hillarp’s method (Kobayashi et al., 1985), but in general it must be said that serotonin of mast cells is demonstrable only in the rat and mouse; the presence of serotonin in mast cells of dog is still to be confirmed.

It has been reported that mast cells are able to mediate phagocytosis (Padawer and Fruhman, 1968; Padawer, 1971; Spicer et al., 1975; Vranian et al., 1981; Ohtani et al., 1982; Czarnetski, 1982; Coble et al., 1984), so it may be speculated upon that mast cells in the Disse’s space may affect the phagocytic action of Kupffer cells in the sinusoidal lumen. The figures of a Kupffer cell that seems to be engulfing cytoplasmic processes of mast cell containing secretory granules encountered in the present study might be due to activation of the Kupffer cell by substances from the mast cell. Whether this figure shows only a temporary interaction between the Kupffer cell and mast cell or results in a complete phagocytosis, can not be determined at present.

The tendency that an Ito cell contacts the phagocyte has been reported by Watari (1980) who found a direct contact between phagocytes and fat-storing cells (Ito cells) leading them to call it a “phagocyte-fat-storing cell complex” and speculate that these cells might cooperate mutually. From the present findings on the intimate relationship among the mast cell, Ito cell, Kupffer cell and endothelial cell, it can be speculated that these four types of cells which intermediate between the sinusoidal blood and parenchymal hepatocytes may act as an effective functional unit in the dog liver.
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