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Localization and ultrastructure of the Kupffer cells in orthotopically transplanted liver grafts in the rat

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Abstract. Kupffer cells play an important role in the acceptance or rejection of liver grafts. We examined the ultrastructure of the Kupffer cells in transplanted rat livers, from an early to a late stage where the graft is accepted, using a DA-to-PVG combination. Two days after surgery, endocytic activity of the Kupffer cells had increased, as evidenced by worm-like structures and many endocytic vacuoles. There was often close apposition to the monocytes or lymphocytes. By day 4, infiltration of mononuclear cells into the sinusoids was readily noticeable. By day 7, several Kupffer cells had migrated into the space of Disse through the openings in endothelial linings. The number of Kupffer cells reached a maximum at 14 days. They were located mostly outside the sinusoid, adhering to the hepatocytes. At this point in time, however, the Kuppfer cells contained few endocytic vacuoles and phagolysosomes, in contrast to those at 2 days. The number and location of Kupffer cells became almost normal at 2 months. The present results indicate that Kupffer cells are highly activated before mononuclear cell infiltration becomes manifest in the sinusoid, and that when a rejecting reaction reaches a peak, they are usually located extrasinusoidally and show a morphologically immature profile.

Key words: Liver transplantation, in the rat – Kupffer cells, ultrastructure, in liver transplantation

In certain donor-recipient combinations of rats, such as DA-to-PVG, liver allografts are accepted permanently without immunosuppressive drug treatment [6]. However, a mild, rejecting reaction also occurs in such cases, with mononuclear cell infiltration of portal tracts and sinusoids that reaches a peak at 2 weeks after grafting [6]. It is also reported that in surviving liver grafts, donor Kupffer cells are completely replaced by recipient ones 15–30 days after grafting [5, 11].

In this study, we examine the ultrastructural changes and localization of Kupffer cells in the liver grafts, using a DA-to-PVG combination, until the grafts are finally accepted at 2 months. Since Kupffer cells are defined as the resident-typed macrophages of the liver [14] and need to be differentiated from exudate-typed ones, we have used peroxidase cytochemistry here.

Materials and methods

Animals

Inbred DA ($RT1^a$) and PVG ($RT1^c$) rats, 8–12 weeks of age, were purchased from Harlan Olac (Bicester, Oxfordshire, UK).

Liver transplantation

Orthotopic liver transplantation was performed using the cuff technique for the portal vein, inferior vena cava, and biliary anastomoses [7], with DA rats as donors and PVG rats as recipients. No immunosuppressants were used. Two to three animals were sacrificed at 2, 4, 7, and 14 days and 2 months after transplantation. Two cases of hepatic artery-reconstructing liver grafts were also examined.

Peroxidase cytochemistry and electron microscopy

The peroxidase staining reaction was carried out using a method similar to that described by Wisse [14]. Under ether anesthesia, the abdomen was opened and the liver graft was perfused via a portal vein with 1.5% glutaraldehyde in 0.062 M cacodylate buffer, pH 7.4, for 40 s. The liver was taken out and cut into blocks measuring about 1 × 1 × 0.5 cm. After being washed in TRIS-HCl buffer, 50-μm thick sections were cut on a vibratome and incubated in medium containing 0.05% diaminobenzidine, 7% sucrose, and 0.01% hydrogen peroxide for 60 min at 35 °C in the dark. For the routine electron microscopy, the liver graft was perfusion-fixed with the same glutaraldehyde solution as described above for 2 min and cut into small blocks (1 × 1 × 1 mm). The sections and small blocks were further fixed in 1% OsO_4, dehydrated in ethanol series, and embedded in

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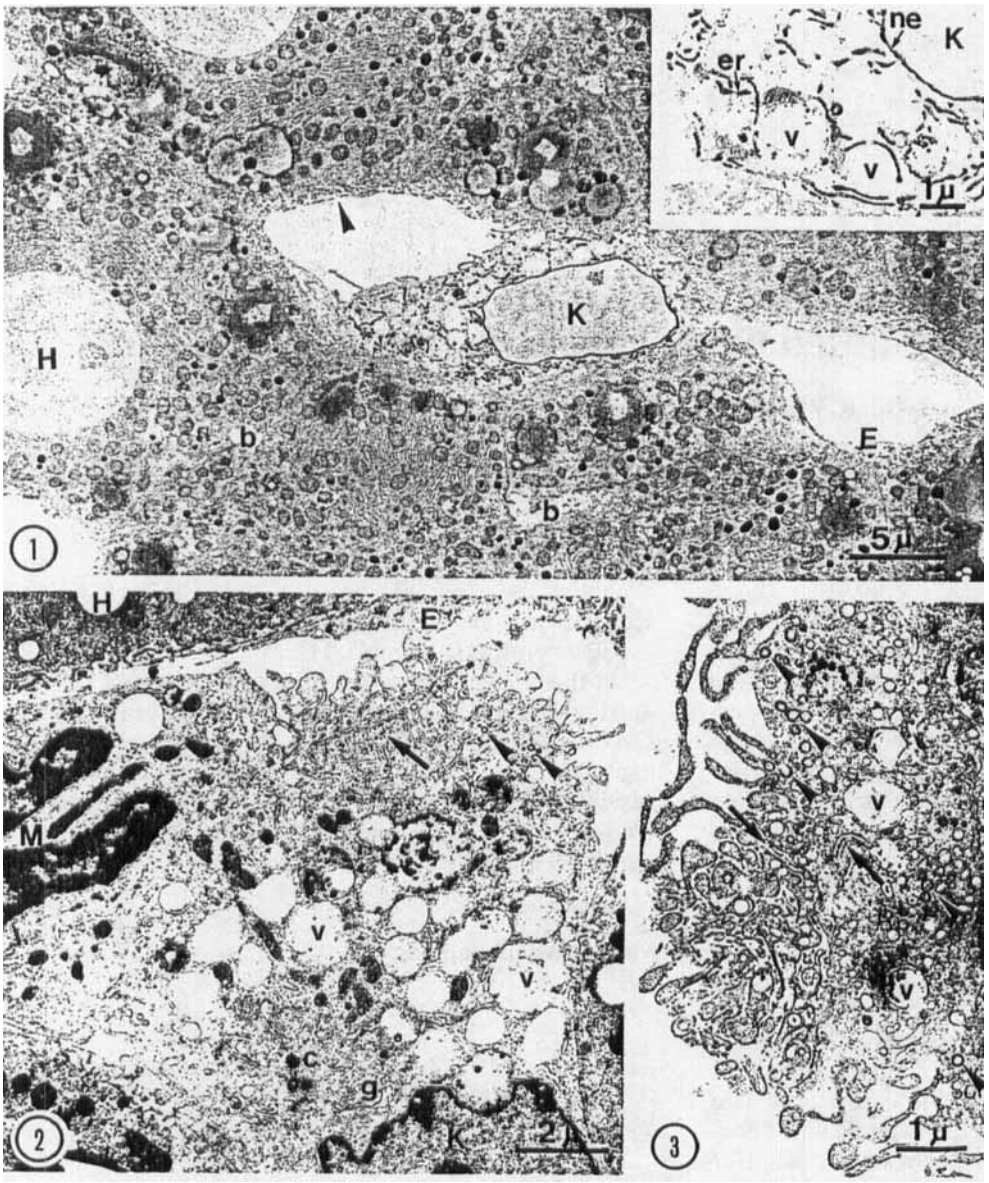

Fig. 1. The liver graft at 2 days post-transplantation. The Kupffer cell *(K)*, whose nuclear envelope *(ne)* and endoplasmic reticulum *(er)* are peroxidase-positive, contains many endocytic vacuoles *(v)*. Endothelial cells *(E)* have well-preserved fenestrations *(arrowhead)*. Bile canaliculi *(b)* are also normal. *H* Hepatocytes

Fig. 2. The liver graft at 2 days post-transplantation. A monocyte *(M)* adheres to the Kupffer cell *(K)* over a wide surface. The Kupffer cell has prominent worm-like structures *(arrows)* with subsurface coated vesicles *(arrowheads)*, well-developed Golgi apparatuses *(g)* and many vacuoles *(v)*. *c* Centrioles; *H* hepatocytes; *E* endothelial cells

Fig. 3. The liver graft at 2 days post-transplantation. A higher magnification of the worm-like structures *(arrows)* and subsurface coated vesicles *(arrowheads)* of the Kupffer cell. *v* Vacuoles

Polybed. Thin sections were stained with saturated lead citrate only (for peroxidase cytochemistry) or saturated uranyl acetate and lead citrate (for routine electron microscopy) and observed under a JEOL 100CX electron microscope at 100 kV.

Results

Two days after grafting, the frequency of the Kupffer cells was almost the same as that in a normal liver (Fig. 1). In this study, we identified Kupffer cells as the resident macrophages that showed positive reactivity of peroxidase in the endoplasmic reticulum and nuclear envelope (Fig. 1). They usually existed inside the sinusoid. The cytoplasm became significantly enlarged due to many endocytic and phagocytic vacuoles that contained aqueous substances or debris (Figs. 1, 2). They had well-developed cytoplasmic projections, prominent worm-like structures, and many subsurface coated vesicles (Figs. 2, 3). Golgi apparatuses and lysosomal granules were also well developed. Kupffer cells often adhered to the monocytes or lymphocytes over a wide surface area and, in the contact area, coated vesicles were numerous (Fig. 2). Endothelial linings were continuous, without tears, and showed well-preserved fenestration (Fig. 1). Hepatocytes had normally arranged cell organelles, except for the existence of lipid droplets at the luminal side.

After 4 days, infiltration of mononuclear cells into the sinusoid became obvious, but their extravascular migration was rare (data not shown).

After 7 days, Kupffer cells had increased in number significantly, and some of them began to migrate extrasinusoidally through endothelial openings. Fourteen days after grafting, we saw many Kupffer cells becoming localized in the space of Disse and adhering widely to hepatocytes (Fig. 4). Hepatocytes and bile canaliculi were almost normal (Fig. 4). There was no cytochemical or ultrastructural difference between intrasinusoidal and extrasinusoidal Kupffer cells. Their mitotic figures were often seen (data not shown). In contrast to the Kupffer

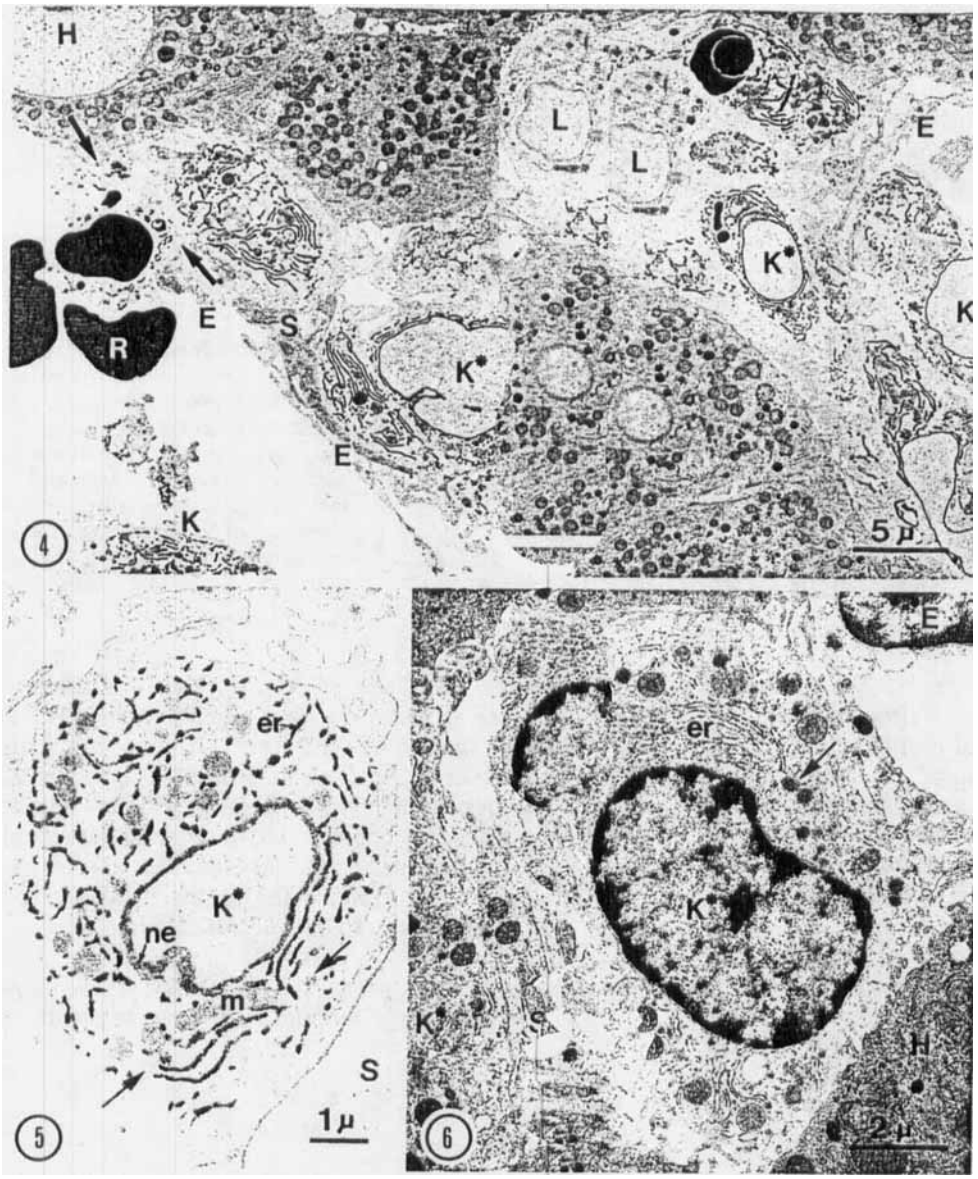

Fig. 4. The liver at 14 days post-transplantation. There are many Kupffer cells *(K, K*)* inside and outside the sinusoid. One Kupffer cell is situated transluminally at a wide opening (between two *arrows*) in endothelial linings *(E)*. *H* Hepatocytes; *S* stellate cells; *L* lymphocytes: *R* red blood cells; *K* Kupffer cells inside the sinusoid; *K** Kupffer cells in the space of Disse

Fig. 5. The liver at 14 days post-transplantation. A Kupffer cell *(K*)* seen in the space of Disse on day 14. It has a peroxidase-positive nuclear envelope *(ne)* and endoplasmic reticulum *(er)*, negatively stained lysosomal granules *(arrows)*, and few phagosomes or endosomes. *S* Stellate cells; *m* mitochondria

Fig. 6. The liver at 14 days post-transplantation. Kupffer cells *(K*)* in an arterialized liver graft on day 14, located outside the sinusoid. They have several lysosomal granules *(arrows)* and few phagosomes or endosomes. *H* Hepatocytes; *S* stellate cells; *E* endothelial cells

cells at 2 days, those at 14 days contained few endocytic vacuoles and lysosomes (Fig. 5). In arterialized liver grafts, although the degree of mononculear cell infiltration was weaker than in nonarterialized ones, Kupffer cells existed predominantly in the space of Disse and had immature features as well (Fig. 6).

Two months after grafting, the frequency and localization of Kupffer cells returned to almost normal. Some of them, however, still remained outside the sinusoid (Fig. 7). They had many phagolysosomes that often contained the debris of peroxidase-positive erythrocytes (Fig. 8). The architecture of the endothelial cells and hepatocytes was well preserved.

Discussion

In this study, we have revealed ultrastructurally that the endocytic activity of the Kupffer cells in liver allografts becomes highly enhanced 2 days after surgery. Since cellular infiltration into the sinusoid was not yet prominent at this point in time, these cells are considered to be of donor origin. It is reported that rat livers stored in a UW cold storage solution for longer than 16 h show a selective endothelial killing, i.e., cellular rounding or disruption, when reperfused with warm, oxygenated buffer for 15 min [2]. During such reperfusion injury of liver grafts, endothelial damage-induced Kupffer cell activation occurs [10]. In this study, however, we observed no damage of endothelial cell linings, probably due to a shorter (3–4 h) and non-cold ischemia. We, therefore, assume that the Kupffer cell activation seen here was caused not by the injured endothelial cells but probably by the stimuli of incoming recipient serum and blood cells, as demonstrated by the presence of many serous material-containing endocytic vacuoles in the Kupffer cells. Furthermore, we often recognized the close apposition of activated Kupffer cells to leukocytes in the sinusoid. This phenomenon of cellular association is also reported in OK-432-stimulated [3] or regenerating [12]

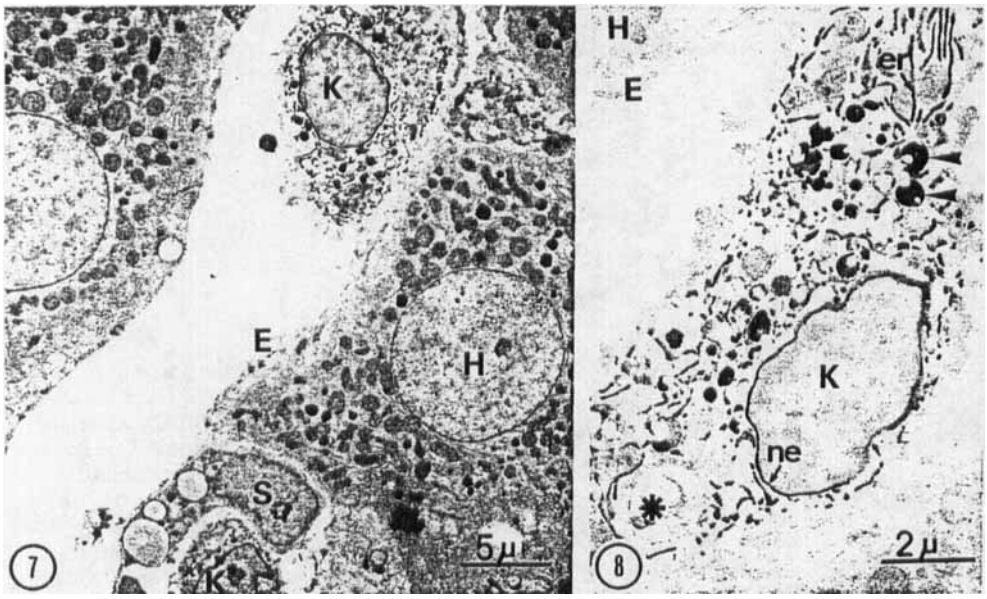

Fig. 7. The liver at 2 months post-transplantation. Kupffer cells *(K, K*)* exist inside and outside the sinusoid. *E* Endothelial cells; *S* stellate cells; *H* hepatocytes; *K* Kupffer cells inside the sinusoid; *K** Kupffer cells in Disse's spaces

Fig. 8. A Kupffer cell *(K)* in the liver graft 2 months post-transplantation. It has peroxidase-positive endoplasmic reticulum *(er)* and nuclear envelope *(ne)*. It also contains a large phagosome *(asterisk)* and many phagolysosomes that include fragments of peroxidase-positive erythrocytes *(arrowheads)*. *H* Hepatocytes; *E* endothelial cells

livers where Kupffer cells are activated. We think that the Kupffer cell activation seen soon after grafting leads to the subsequent accumulation of mononuclear cells in the liver, either by direct trapping of circulating leukocytes or by the release of certain chemotactic factors into the circulation. We have further demonstrated that the Kupffer cells gathering in the space of Disse after 14 days had an immature profile in contrast to those after 2 days. Previous immunohistochemical studies [5, 11] using models similar to those used here have reported that donor Kupffer cells are replaced completely by recipient ones between 15 and 30 days after grafting. We, therefore, conclude that the increased number of Kupffer cells seen here at 14 days mostly consist of recipient ones. The immaturity of the cells indicates that they are newly formed in the liver graft. Kupffer cells are resident-typed macrophages and considered to be generated either by the differentiation of monocytes via exudate-typed macrophages and a transition form [4] or through local proliferation [1]. After 14 days, neither an exudate-typed macrophage nor a transition form was very frequent, but the mitotic figure of Kupffer cells was often seen, indicating that the increase in Kupffer cells at this point in time could have been attributed to both local proliferation and differentiation from incoming recipient monocytes.

In a DA-to-PVG model, a rejecting reaction is spontaneously self-limiting, as we have reported previously [8]. In contrast to this, in a DA-to-BN combination, an acute rejection occurs and animals die between 10 and 30 days after surgery. The reaction is more vigorous and mononuclear cells in the sinusoids, which often come into contact with hepatocytes, are more numerous than in a DA-to-PVG model [13]. We have also observed ultrastructurally many Kupffer cells in the space of Disse in a DA-to-BN combination (unpublished data). The phenomenon of extrasinusoidal localization of macrophages is thus a common feature of both nonrejecting and rejecting combinations. An artery-reconstructing model has shown the extrasinusoidal migration of Kupffer cells in a nonarterialized model as well. We have already demonstrated that, basically, there is no significant difference in the histology of the liver in these two models [9]. The accumulation of Kupffer cells in the space of Disse and their contact with hepatocytes may be related to the process of presenting class I major histocompatibility complex antigens on hepatocytes by the Kupffer cells, but the detailed, functional significance needs to be elucidated further.

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