STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF KUPFFER CELLS DURING FORMATION OF RESPONSE OF THE MOUSE LIVER TO INJURY

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Kupffer cells are the organ-specific representative of the mononuclear phagocyte system (MPS) and have marked capacity for phagocytosis and degradation of material of heterogenous and endogenous origin [7]. Besides these properties, the study of the number and subcellular organization of these cells in connection with processes taking place in the organ during changes of structural homeostasis is essential in order to understand the function of the Kupffer cells in the liver and as a component part of the MPS of the organism.

Several of the above-mentioned parameters of Kupffer cells were studied in the investigation described below during periods of predominant development of dystrophy, necrosis, and reparative regeneration following toxic injury to the liver.

EXPERIMENTAL METHOD

Male C57BL/6 mice aged 2 months and weighing 19-21 g were given CCl_4 by inhalation. The concentration of the poison was 0.025 ml/liter air and the duration of exposure in the chamber was 10 min. The animals were decapitated 6, 24, 48, 72, and 96 h later. Animals of the second group (same line, sex, and weight), 32 h after inhalation of CCl_4 under similar conditions, were given an injection of latex particles (Dow Latex) 1.1 μ in diameter, in a dose of 0.05 ml/100 g body weight via the caudal vein [6]. Samples of liver from mice of this group were obtained 16 h after injection of latex or 48 h after inhalation of CCl_4. Intact animals served as the control. Each group consisted of five mice. Samples of liver for electron microscopy were fixed in 1% OsO_4 solution in phosphate buffer, and embedded in Epon. Semithin sections were stained with toluidine blue and used to determine the volume of the hepatocytes and the total volume and number of sinusoidal cells. In parallel experiments the liver was fixed in 10% formalin solution and embedded in paraffin wax; sections were stained with Mayer's hematoxylin and eosin and the volume of necrotic areas in them was determined.

The following sinusoidal cells were photographed in the JEM 100S/SEGZ/ASID electron microscope: Kupffer's, Ito's, endothelial, and also monocyte-like, having tight junctions with the sinusoidal surface of the plasmalemma of the endothelium. On the basis of these data and the results of morphometry of semithin "Epon" sections the number of cells of each type was determined. Differentiation of the different types of cells and their morphometry were carried out in agreement with data published previously [3]. Morphometric investigations of Kupffer cells were undertaken outside zones of necrosis. Differences between the mean values compared were considered significant at the $P < 0.05$ level (by Student's test).

EXPERIMENTAL RESULTS

Mainly fatty degeneration and solitary necrotically changed cells were observed in the center of the hepatic lobules 6 h after the end of CCl_4 inhalation in the hepatocytes. After 48 h processes of necrosis of the hepatocytes were predominant there (after 24 h both processes were taking place), but by 72 and 96 h repair processes of the regeneration hypertrophy type were found in the hepatocytes, accompanied by proliferation of

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TABLE 1. Results of Morphometry of Mouse Liver at Different Periods after Inhalation of CCl_4 (M ± m)

Test parameter	Control	Time after inhalation of CCl_4				
		6 h	24 h	48 h	72 h	96 h
Volume of sinusoidal cells, %	6,4±0,79	10,9±1,11	10,1±0,88	10,5±0,91	10,8±0,69	12,9±0,86
Volume of necrotic areas, %	—	—	5,4±0,75	15,0±0,50	7,2±0,41	—
Volume of hepatocytes, μ^3	3883,2±345	2467,0±353	5231,4±450	5203,4±264	5619,0±451	5738,1±517
Number of phagocytic Kupffer cells, %	27,3	28,5	26,3	7,1	5,4	3,6

Legend. Volume of sinusoidal cells corresponds to total relative fraction by volume of endothelial cells, Kupffer cells, Ito's cells, and monocyte-like cells, with tight junction with endothelium (in % of area of test liver section). Volume of necrotic areas given in % of area of test section. Number of phagocytic Kupffer cells shown as a percentage of the total number of Kupffer cells studied.

subcellular structures [2]. A measure of the intensity of these processes was the volume of the hepatocytes (Table 1) and hyperplasia of their ultrastructures.

The total volume of sinusoidal cells began to increase 6 h after injection of CCl_4 (Table 1), evidently partly as a result of an increase in the number of Kupffer cells in the liver, their hypertrophy, and an increase in the number of monocyte-like cells (Fig. 1), having a tight junction with the plasmalemma of the endothelial cells, which is characteristic of Kupffer cells. By the shape of their nucleus and the subcellular organization of their cytoplasm, these cells had a definite resemblance to monocytes. However, they had more extensive cytoplasm with a larger number of large lysosomes, more characteristic of macrophages with outgrowths of cytoplasm. It can accordingly be postulated that in this case mononuclear cells in the course of differentiation into Kupffer cells were observed. These cells had evident differences from other types of sinusoidal cells. The increase in the number of Kupffer cells preceded the development of both necrotic and repair processes (Table 1, Fig. 1). The number of Kupffer cells outside the zones of destruction of the parenchyma reached a maximum 24 h after the end of CCl_4 inhalation, and a minimum after 48 h (Table 1, Fig. 1). Both at these periods and during the previous period, corresponding changes were observed in the number of monocyte-like cells, "fixed" to the endothelium. Incidentally, at the time of maximum of the necrotic areas (48 h after inhalation of the poison, Table 1) the number of these cells was 0, and the number of Kupffer cells was 58% less than in the control, and 4.3 times less than during the previous period (Fig. 1). The problem of the causes of the decrease in the number of Kupffer cells in uninjured zones of the hepatic lobules arises. Mass destruction of Kupffer cells can be postulated in this connection, but this was not observed at any of the periods of investigation, or migration of Kupffer cells from the liver or from uninjured zones into zones of necrosis, which cannot be ruled out in view of data in [4], when colloidal carbon was used as marker of the Kupffer cells for light-microscopic investigations. The use of colloidal carbon as marker is less suitable than the use of latex particles, for because of the small size of the carbon particles, they are taken up by endothelial cells and by macrophages of nonhepatic origin.

During investigation of zones of necrosis mainly Kupffer cells and single monocytes were observed during this period. However, at this same time (48 h after injection of CCl_4), in animals receiving an injection of latex, this substance was not found in the Kupffer cells in the foci of necrosis. It can accordingly be postulated that Kupffer cells, capable of migration, migrated into the zones of necrosis up to 32 h after inhalation of CCl_4. In the zones of necrosis the Kupffer cells largely lost their lysosomes and did not contain heterophagosomes or heterophagolysosomes.

Their ability to ingest and degrade material of heterogenous and endogenous origin may serve as a measure of functional activity and "maturity" of macrophages. According to the results in Table 1, the number of Kupffer cells outside zones of necrosis, containing heterophagosomes and heterophagolysosomes, starting from 48 h after inhalation of CCl_4, was reduced (outside zones of necrosis) by 80% or more. However, this does not unequivocally indicate reduction of the phagocytic capacity of the Kupffer cells. It may be that during these periods the volume of circulating material, which is usually contained by heterophagosomes of Kupffer cells, in the blood was reduced as a result of some disturbance of the microcirculation and degradation of remnants

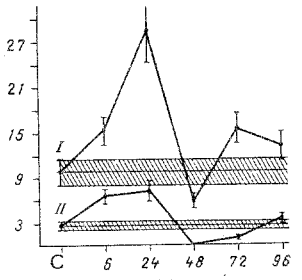

Fig. 1

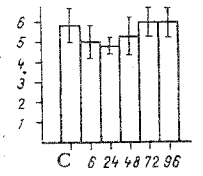

Fig. 2

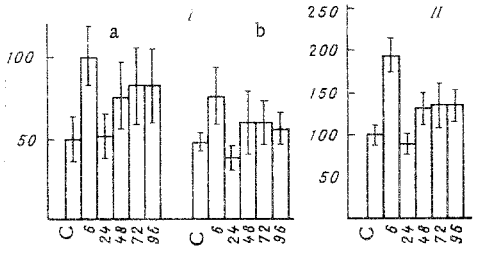

Fig. 3

Fig. 1. Results of investigation of number of Kupffer cells (I) and of monocyte-like cells (II) in $10^5\ \mu^2$ of a section of the liver. Abscissa, time after end of CCl_4 inhalation (in h); ordinate, number of cells. C) Control.

Fig. 2. Total concentration (in μ^2/μ^3 of cytoplasm) of membranes of Kupffer cell organoids: outer and inner mitochondrial membranes, endoplasmic reticulum, Golgi complex, lysosomal structures. Remainder of legend as to Fig. 1.

Fig. 3. Number of ribosomes in Kupffer cells (N_a – number in 10 μ^2 of area of section through cytoplasm). I: a) Free ribosomes, b) fixed ribosomes, II) total fixed and free ribosomes. Remainder of legend as to Fig. 1.

of destroyed hepatocytes in the center of the hepatic lobules after inhalation of CCl_4. The results of the experiments in which latex was injected showed that this was a likely cause, because outside the zones of necrosis all Kupffer cells contained latex.

The total concentration of membranes of the cytoplasmic organoids (surface area of membranes in 1 μ^3 of cytoplasm) was used as a sufficiently adequate integrative morphological criterion of the "maturity" and homogeneity of the Kupffer cell population with respect to this feature. Not until 24 h after inhalation of CCl_4 did the value of this parameter (Fig. 2) fall significantly, possibly in connection with the inflow of a considerable number of "young" Kupffer cells (Fig. 1). The decrease in the concentration of ribosomes during the same period (Fig. 3) could be due to similar causes. However, 6, 48, 72, and 96 h after inhalation of CCl_4 the number of ribosomes was significantly increased. This indicates intensification of synthetic processes in the cells, which was not reflected in the relative values of concentration of ultrastructural membranes. However, in connection with the considerable increase in volume of the cells, the absolute content of ultrastructures also evidently increased, as was observed during the study of Kupffer cells. Partly on this account the total volume of sinusoidal cells evidently increased even in periods when the number of Kupffer cells in the sinusoids was minimal (Table 1, Fig. 1). The increase in the number of ribosomes and "mature" Kupffer cells 6 h after inhalation of CCl_4 was evidently significant, because the value of this parameter did not reflect an increase in the number of "young" Kupffer cells, evidently because it was less than 24 h after administration of the poison (Fig. 3).

During the formation of the response of the liver to toxic injury two peaks of increase in the number of Kupffer cells outside zones of injury to the liver parenchyma were observed: the first, at a time of dystrophic changes in the hepatocytes, before the necrotic changes developed, the second in the period of predominant development of regeneration. This pattern had a definite similarity with that described in [1], based on the results of a study of the rat liver during regeneration after partial hepatectomy. Starting from the period of dystrophic changes in the hepatocytes, and during subsequent periods of formation of the response of the liver to injury, morphological features of an intensification of synthetic processes in the Kupffer cells were observed.

The decrease in the number of Kupffer cells in the undamaged areas of the hepatic lobules and the increase in their number in zones of necrosis could be evidence in support of their ability to migrate into foci of necrosis – a property characteristic of macrophages, but which has received little study in relation to Kupffer cells [4]. Comparison of the ultrastructure of these cells in areas of necrosis and in the sinusoids of the intact liver, and also the results of experiments with injection of latex suggest that in zones of necrosis they evidently secrete lysosomal enzymes into the surrounding medium, as was stated in [5], thus preserving heterophagal function outside the zones of necrosis. On the assumption that the monocyte-like cells fixed to the sinusoidal surface of the endothelium are precursor cells of Kupffer cells in the stage of differentiation, the question of to what degree fixation is an essential condition for their differentiation into organ-specific macrophages is an interesting one.

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