RESTORATION OF THE KUPFFER CELL POPULATION AFTER LOADING WITH INERT COLLOID

D. N. Mayanskii and V. I. Shcherbakov

UDC 612.354.2:612.112.3]:612.6:03.014.46

KEY WORDS: liver; Kupffer cells; system of mononulear phagocytes.

The system of mononuclear phagocytes (SMP) includes all classes of organ- and tissue-specific macrophages [11]. The resistance of the organism to bacterial and virus infections [7, 8], the character of inflammation [4], and the course of reparative regeneration [1, 2] depend on its functioning. Considering the key role of the tissue macrophages in all these processes, it is important to understand the basic mechanisms of regulation of the SMP. To begin with, we must know what informs the bone marrow of the need to increase or reduce the production of precursor monocytes of the tissue macrophages. The possibility cannot be ruled out that the signal for a decrease or increase in monocytopoiesis reaches the bone marrow from peripheral activated macrophages themselves. In fact, 18 h after the initiation of acute inflammation, a high concentration of a factor disinhibiting monocytopoiesis can be detected in the blood stream [13]. The times of its accumulation in the blood coincide with those of accumulation of macrophages in an inflammatory focus. Activated macrophages are the source of colony stimulating factor (CSF) which induces proliferation of a single semistem cell — for cells of the granulocytic and monocytic series [9]. When peripheral macrophages are loaded with inert colloidal particles some of the macrophages, having ingested the foreign material, undergo "functional elimination," for these cells can no longer participate in clearance of the blood. This phenomenon has been known for a long time as blocking of the macrophages or blocking of the reticuloendothelial system. Because of the general biological principles governing overloading and inactivation of some of the peripheral macrophages by inert colloidal particles there must be some stimulus for disinhibition of monocytopoiesis and for functioning of the SMP as a whole. Accordingly the inert colloid loading test can be recommended as a model with which to study self-regulation of the SMP.

In the investigation described below the response of the Kupffer cells of the liver to "functional elimination" of part of the liver macrophage pool was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. An intravenous injection of 1 ml of 10% colloidal iron carbonyl (R-100F brand) with a particle diameter of 0.5-1 μ, suspended in 5% starch solution, was given into the femoral vein of the experimental animals and the control animals received 1 ml of 5% starch solution. The animals were killed 0.5, 2, 4, 8, 16, and 24 h after the injection. Sections cut from the liver, spleen, thymus, and lung tissues, 5-8 μ thick, were stained with hematoxylin—eosin. The percentage of cells loaded with iron granules was determined in blood films and squash preparations of bone marrow stained with azure-II—eosin. The 11-hydroxycorticosteroid (11-HCS) level was determined in the blood serum [3]. The total number of Kupffer cells per 3000 hepatocytes was counted in the liver sections and the percentage of hepatic macrophages loaded with colloidal particles was determined. The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

After injection the colloidal iron was concentrated in Kupffer cells, macrophages of the red pulp and marginal zones of the splenic follicles, in blood polynuclear cells, in mature

Laboratory of Pathophysiology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 3, pp. 374-376, March, 1981. Original article submitted June 5, 1980.

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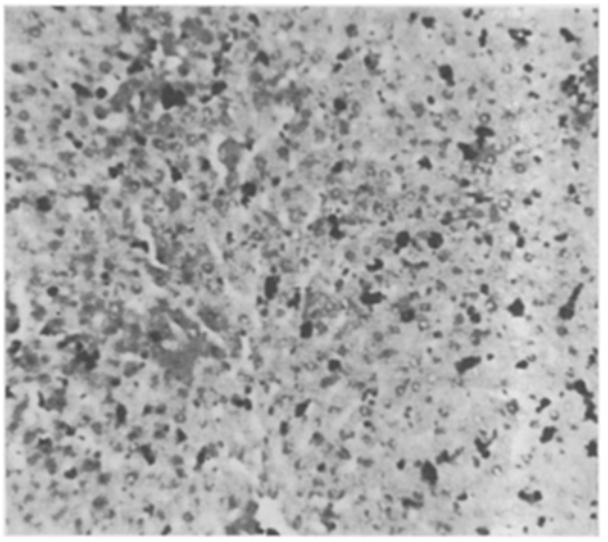

Fig. 1

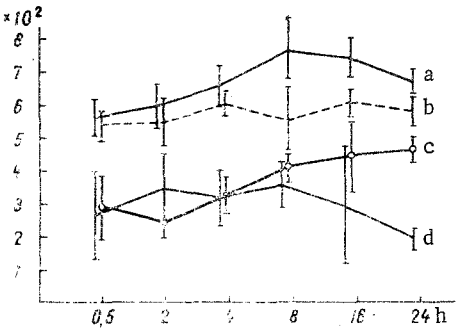

Fig. 2

Fig. 1. Concentration of main mass of colloid in middle and peripheral zones of hepatic lobule 2 h after loading. Hematoxylin–eosin, 160 ×.

Fig. 2. Changes in macrophage population of the liver after loading with inert colloid. a, b) Total number of macrophages in experiment and control, respectively, c, d) number of unloaded and loaded macrophages respectively in experiment. Abscissa, time after injection of colloid (in h); ordinate, number of macrophages.

granulocytes and monocytes of bone marrow, in macrophages of the interstitial tissues of the lung, and in free alveolar macrophages. No particles of iron could be detected in macrophages in the thymus. Attention was concentrated on reorganization of the hepatic macrophage population after loading of some of the Kupffer cells with inert colloid. The main object of interest was how quickly the functionally active pool of Kupffer cells was restored through the inflow of fresh precursor cells from bone marrow [10], for as a rule mature tissue macrophages do not proliferate [12]. Colloid was distributed 30 min after its injection mainly in Kupffer cells from the subcapsular zone. In addition, an irregular distribution of colloid was observed among individual lobules of the liver. After 2 h colloidal iron was distributed more uniformly among individual hepatic lobules, although inside the lobule it was ingested more actively by Kupffer cells of the peripheral zones (Fig. 1). On average granules of colloidal iron were found in 57% of Kupffer cells. Iron particles did not penetrate into the endothelial cells because of their comparatively large sides [14]. The total number of Kupffer cells began to increase as early as 2 h after loading, and it was significantly higher than the control after 4 h. After 8 h their number reached a maximum, and then began to fall until 16 h or, even more, until 24 h after loading, although it still remained above normal at that time. The increase in the number of cells took place on account of macrophages free from colloid (Fig. 2). The inflow of fresh macrophages into the liver was most demonstrative 4 h after injection of the colloid and it continued until 24 h (period of observation). A more intensive inflow of fresh cells took place after 4 and 8 h than after 16 and 24 h (Fig. 3). The number of Kupffer cells ingesting colloid showed little change between 2 and 8 h after injection, but then it began to decrease, as could be observed after 16 h and, in particular, after 24 h ($P < 0.01$). Loading of some Kupffer cells with inert colloid thus served as the stimulus for the supply to the liver of new portions of macrophage precursors, as a result of which the population of functionally active Kupffer cells was fairly quickly restored. The subsequent fate of the loaded cells was studied in separate experiments for 1 month. After 2 days accumulations of loaded macrophages began to form in the liver and were concentrated in different parts of the hepatic lobules. In later stages, after 2 weeks and 1 month, the individual loaded macrophages began to fuse together at the points of their concentration, with the formation of giant colloid-containing cells (Fig. 3).

In the control slight changes were observed in the 11-HCS level, whereas in the experiment it fell by almost half 24 h after loading of the SMP with colloidal iron (Fig. 4). This

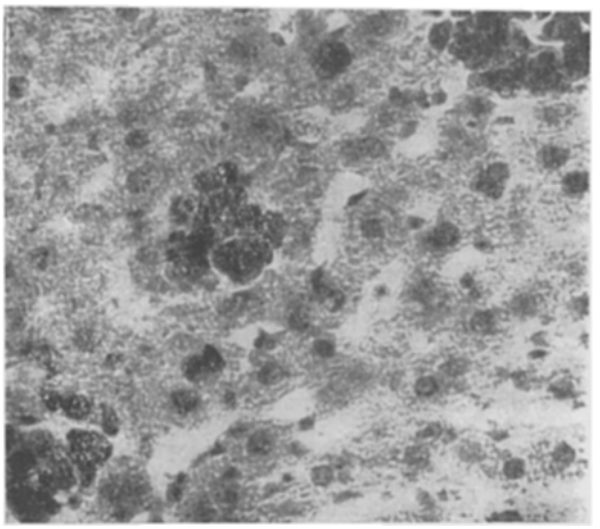

Fig. 3

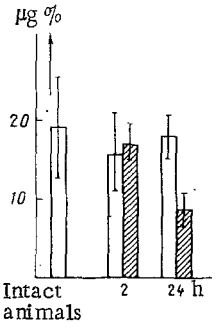

Fig. 4

Fig. 3. Accumulation of giant colloid-containing cells along course of hepatic sinusoids. Hematoxylin–eosin, 400 ×.

Fig. 4. Serum 11-HCS concentration after loading of SMP with colloidal iron. Shaded columns – experiment, unshaded – control. Abscissa, time after injection of colloid (in h); ordinate, 11-HCS concentration (in μg%).

was possibly an adaptive reaction, providing favorable conditions for stimulation of monocyte migration from the bone marrow into the liver and other organs with cells of the SMP, restoring the number of functionally active peripheral macrophages [5, 6].

The authors are grateful to Candidate of Biological Sciences A. L. Markel' for help with the biochemical analyses.

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