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Effect of Activation of the Mononuclear Phagocyte System on Binding of Low- and High-Density Lipoproteins to Rat Hepatocytes, Kupffer and Endothelial Cells

I. F. Usynin, L. E. Panin, O. M. Trubitsyna, A. V. Khar'kovskii, and T. A. Tret'yakova

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The liver is the main organ regulating the lipoprotein (LP) and cholesterol (CH) blood levels. Low-density lipoproteins (LDL) are believed to transport CH to the peripheral tissues, whereas high-density lipoproteins (HDL) are responsible for reverse CH transport to the liver, where it is converted to bile acids [6]. Specific receptors to LDL and HDL are found on both hepatocytes and nonparenchymal elements, namely, endothelial and Kupffer cells [15]. Kupffer cells are a component of the mononuclear phagocyte system (MPS) and account for more then 20% of the total pool of macrophages in the organism [10]. Macrophages have been recently established to secrete a broad spectrum of cytokines, which induce various functional changes in different types of cells. Their involvement in the regulation of erythropoiesis, the immune response, and the proliferation of fibroblasts and vascular endothelium has also been shown [9]. At the same time, the role of macrophages in the regulation of lipid metabolism is poorly understood [4]. Previously we showed that stimulation of the MPS with lipopolysaccharides (LPS) leads to marked induction of protein synthesis [1] and activation of key enzymes of carbihydrate metabolism in hepatocytes [3]. The present study aimed to investigate the role of the MPS in regulating the LP receptors in different types of liver cells.

MATERIALS AND METHODS

Experiments were carried out on female Wistar rats weighing 180-200 g. Stimulation of the MPS was effected by injecting intravenously the bacterial LPS prodigiosane (Moskhimfarmpreparat, Russia) in a dose of 0.25 mg/kg. The animals were decapitated under light ether anesthesia after 24 and 72 hours. The liver cells were isolated by enzymatic digestion [2,15] using recirculatory perfusion of the liver with 0.03% collagenase (Boehringer Mannheim, Germany). Hepatocytes and nonparen-

Institute of Biochemistry, Siberian Branch of the Russian Academy of Medical Sciences, Novosibirsk

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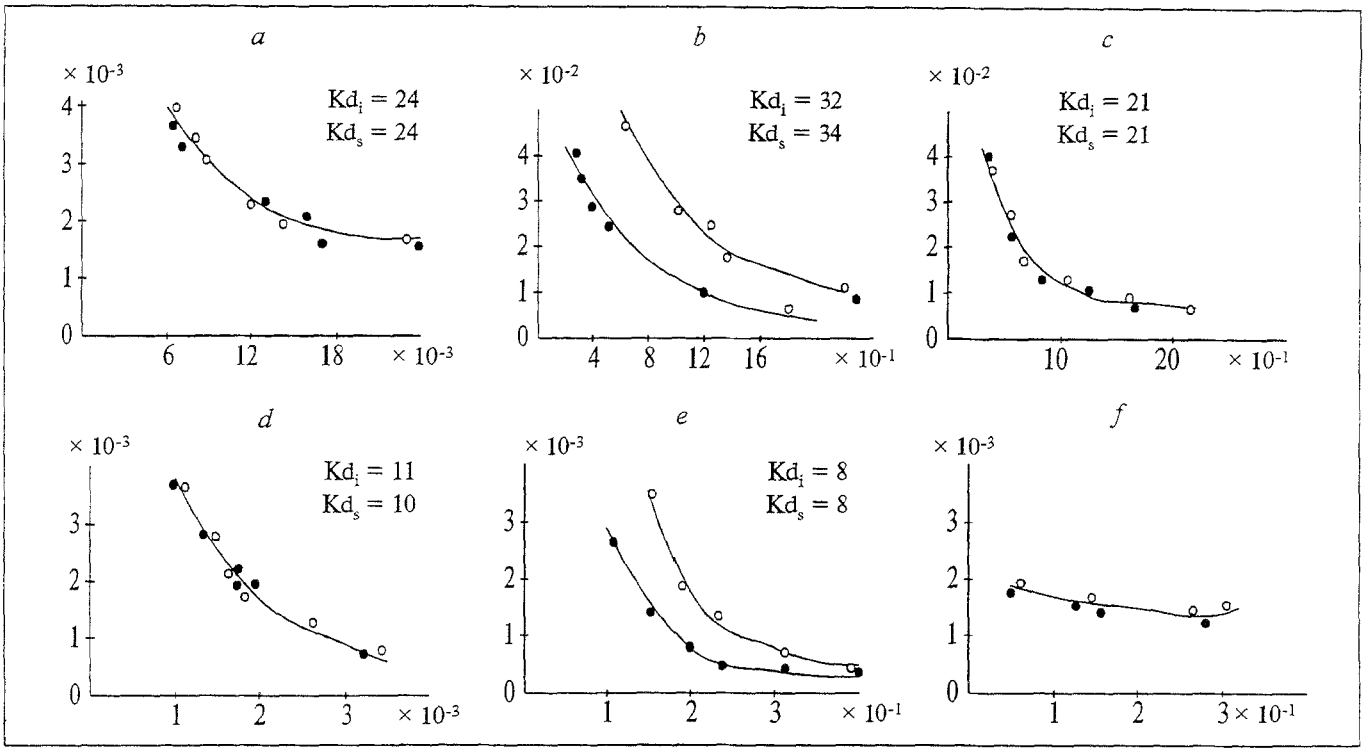

Fig. 1. Scatchard plot of ^{125}I–HDL (*a, b, c*) and ^{125}I–LDL (*d, e, f*) binding to hepatocytes (*a, d*), Kupffer cells (*b, e*), and endotheliocytes (*c, f*). Abscissa: ^{125}I–LP bound, μg/mg cell protein; ordinate: bound to free ^{125}I–LP ratio; open circle: intact animals; dark circle: prodigiosane–stimulated animals (24 h). Kd$_i$ and Kd$_s$: dissociation constants for intact and prodigiosane–stimulated animals, μg/ml.

chymal cells were obtained from the suspension by differential centrifugation [15]. Nonparenchymal cells were fractionated in a JE-6 flow-through rotor of a J2-21 centrifuge (Beckman, USA) at 2500 rpm and buffer flow rates of 22 and 42 ml/min for endothelial and Kupffer cells, respectively [8]. The cells were counted in a Goryaev chamber. Viability and purity of the cell fractions were assessed by light and electron microscopy.

LP were isolated from blood plasma using the method of preparative ultracentrifugation in KBr solution [7]. The LDL (d=1.019-1.063 g/ml) and HDL (d=1.063-1.210 g/ml) thus obtained were than labeled with ^{125}I using an IodoGen apparatus (Sigma, USA) [5]. The specific radioactivity of the ^{125}I-LP was 100-300 cpm/ng protein. More than 97% of the total radioactivity was precipitable with 10% trichloroacetic acid. For assessment of LP-binding capacity the cells were incubated at 0°C in medium 199 containing 0.4% albumin and various concentrations of ^{125}I-LP (from 3 to 80 μg/ml). Specific binding was estimated as the difference between ^{125}I-LP binding with and without a 50-fold excess of unlabeled LP per 10^6 cells. The protein content in LP and isolated cells was determined after Lowry [11]. Statistical processing of the results was carried out using the Student t test.

RESULTS

The yield of hepatocytes from one liver was 216±35×10^6 cells. More then 90% of the cells were trypan blue-negative. The protein content in the hepatocytes was 2284±98 μg per 10^6 cells. Elutriation centrifugation of the suspension of nonparenchymal cells yielded 86±7×10^6 Kupffer cells and 190±26×10^6 endothelial cells, with protein content of 185±19 and 76±4 μg per 10^6 cells, respectively. Reciprocal contamination of the cell fractions did not exceed 10%. Similar results were obtained by other workers [8]. Comparative analysis of the cell fractions isolated from either intact or LPS-treated animals revealed no reliable differences in the above-mentioned parameters.

The isolated cells maintained their high LP-binding capacity. In the presence of excess unlabeled LP the ^{125}I-LP binding decreased by 40-60%. It should be noted that we found no saturation in ^{125}I-LP binding to endotheliocytes, which suggests the lack of highly specific binding sites for LDL. This is also indicated by morphological and biochemical data on a low LDL-binding capacity of endothelial cells both *in vivo* and *in vitro* [12]. Scatchard analysis [13] showed that LP binding to cells occurs via both low- and high-affinity mechanisms. The dissociation constants

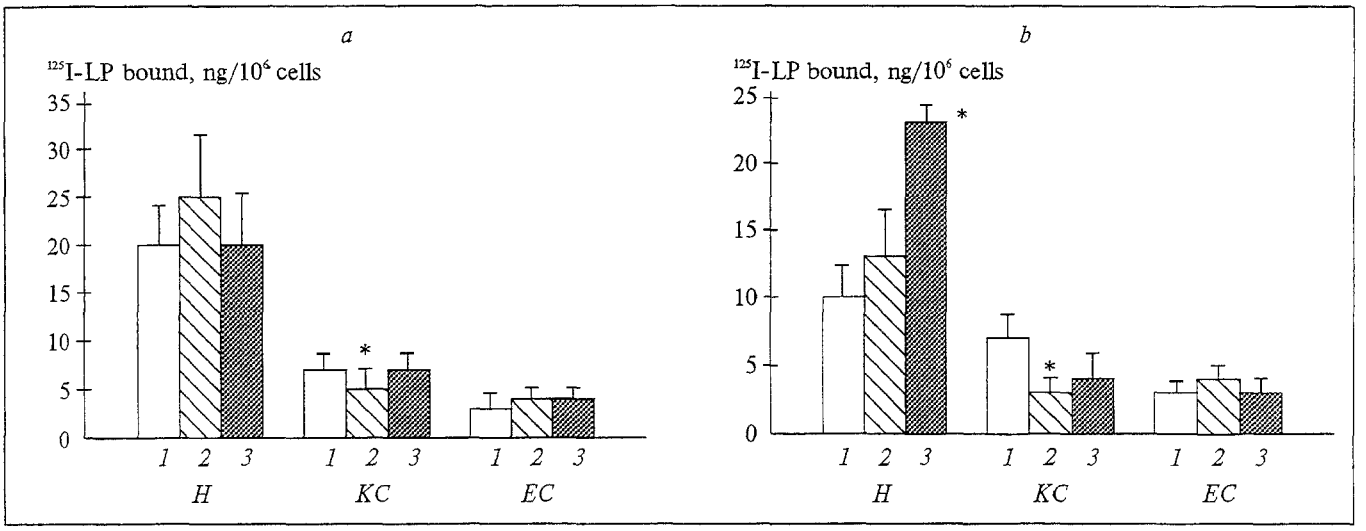

Fig. 2. ^{125}I–HDL (*a*) and ^{125}I–LDL (*b*) binding to hepatocytes (H), Kupffer cells (KC) and endothelial cells (EC) isolated from intact animals (*1*) and 24 h (*2*) and 72 h (*3*) after LPS administration. * $p<0.05$; ** $p<0.01$ in comparison with the control ($n=5-10$).

(K_d) for high-affinity binding of ^{125}I-LP are presented in Fig. 1.

In the intact rats the maximal LP-binding capacity was found in hepatocytes. In Kupffer cells ^{125}I-HDL and ^{125}I-LDL binding was 1.6- and 2.5-fold weaker than in hepatocytes, respectively (Fig. 2.). In endotheliocytes these parameters were even lower. The nonparenchymal cells are considerably smaller than the hepatocytes, and therefore when the binding is calculated per milligram of cell protein, the Kupffer cells turn out to bind 7.2-fold more ^{125}I-HDL and 5-fold more ^{125}I-LDL than do the hepatocytes. Thus, Kupffer cells play an important role in the metabolism of not only LDL [12] but also HDL.

The prodigiosane stimulation of the MPS resulted in a marked alteration of receptor activity in the liver cells. For example, the ^{125}I-HDL binding to Kupffer cells decreased 4.5-fold 24 hours after LPS administration (Fig. 2) and returned to the initial level after 72 hours. Similar, though less pronounced, changes were observed for ^{125}I-LDL binding in Kupffer cells. On the other hand, ^{125}I-HDL binding in hepatocytes rose for LPS activation of the MPS, being twice as high in stimulated than in intact animals 72 hours after stimulation (Fig. 2). No reliable differences were found in ^{125}I-LDL binding to hepatocytes. The ^{125}I-LP binding to endotheliocytes also remained unchanged under these conditions. Thus, activation of the MPS is accompanied by a marked increase of the number of HDL receptors in hepatocytes.

It is well known that LPS, when injected intravenously, binds mainly to HDL [14]. The considerably reduced number of receptors in Kupffer cells observed here is probably related to an active receptor-mediated endocytosis of the LPS-HDL complex during the earliest stages after LPS administration, whereas activation of the receptor apparatus in the hepatocytes is a result of LPS stimulation of the Kupffer cells. The role of Kupffer cells in intercellular cooperation is still poorly understood [4]. However, the functional state of the MPS and, particularly, of its hepatic element, Kupffer cells, largely determines the "atherogenic potential" of the blood.

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