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Binding of LPS and LPS—LDL Complexes to Rat Hepatocytes

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We studied binding of ^{3}H-LPS toxin and LPS complexes with serum ^{125}I-LDL to primary culture of rat hepatocytes. Receptor binding of LPS and LDL—LPS complex was ~50 and 77% of the total, respectively. Scatchard plot was linear in both cases. LDL and LDL—LPS complexes were inessential for LPS binding, while LDL—LPS binding was appreciably suppressed by LPS (-30%) and LDL (-65%).

Key Words: *LPS; serum lipoproteins; hepatocytes; receptors; binding*

LPS toxin can specifically bind to some membrane proteins on hepatocyte (HC) surface, primarily to CD14 [1]. This protein interacts with LPS associated with LPS-transporting protein. Expression of CD14 receptor on hepatocytes and secretion of LPS-transporting protein sharply increase during endotoxemia [6,11]. In addition, lipid A fatty acid chains of some LPS molecules can nonspecifically incorporate into phospholipid bilayer of hepatocyte plasma membrane.

The aggregate state of LPS (free or associated with serum proteins and lipoproteins) is an important factor for its interactions with cell receptors [3,10]. Free LPS is present as monomers (at micelloformation concentration below a critical level [8], *i.e.* in the absence of intoxication) or as large aggregations (micelles) with a molecular weight of up to 2 MDa [8] forming in the presence of high concentrations of LPS.

The structure of LPS—LDL complexes is well studied [4,10]. Potentially LDL—LPS complexes on hepatocytes surface can react with three receptor types: LDL receptors (apo-B/E) [10], LPS receptors, and scavenger receptors. However, scavenger receptors binding modified lipoproteins are absent on hepatocytes, and their function is most likely performed by LDL receptors [5], while binding to type 1 class B scavenger receptors expressed on hepatocytes is hardly possible, because their functions are different [7].

We compared for the first time the binding of LPS and LDL—LPS complexes to primary hepatocyte culture.

MATERIALS AND METHODS

Purified lyophilized wild type *Salmonella typhimurium* LPS (Sigma) was used; ^{3}H-LPS (specific activity 22 800 cpm/μg) was obtained by the reaction with NaB-$^{3}H_4$.

Human LDL were isolated as described previously [10], ^{125}I-LDL (^{125}I-apo-B 100) were prepared [9]. ^{125}I-LDL—LPS (135,600 cpm/μg protein; 0.1 μg LPS/μg apoprotein) complexes were prepared in the presence of serum proteins [10].

Hepatocytes were isolated from Sprague-Dawley male rats (250-300 g) [6]. According to light microscopy, the purity of hepatocytes was 97-98%; at least 97% isolated cells were viable (not incorporated trypan blue). The cells were cultured in 100-ml gelatin-coated plastic dishes at a density of 5×10^{6} cells/dish in 5 ml Williams E medium containing 10% FCS.

The measurements were carried out at low temperature (4°C, 4 h) in order to differentiate between the processes of LPS and LDL—LPS complexes binding to cell surface and their internalization.

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RESULTS

Binding of LPS to hepatocytes was saturable (Fig. 1, *a*); nonspecific binding was approximately equal to specific and played an important role. Scatchard plot for specific binding of LPS to hepatocytes was linear, which suggests the presence of one endotoxin binding site on hepatocyte membrane with the following parameters: number of binding sites n=48 ng LPS/cell and K_d=2.55 μg/ml. For tentative estimation of the number of toxin molecules bound to the cell, the molecular weight of wild type LPS monomer can be taken for 13 KDa [10]: $n=2.2\times10^6$ LPS molecules/cell. If the monomer weight is replaced by the mean weight of LPS aggregations (about 2 MD [10]), the above value decreases by more than 2 orders of magnitude.

The receptor interaction predominated during LDL—LPS complex binding (Fig. 1, *b*): 75-80% of total binding. Scatchard plot was approximately linear, this giving the following parameters of one binding site: n=120 ng protein/cell and K_d=3.2 μg protein/ml. If we take the weight of LDL—LPS complexes for about 3 MDa [10], the result is 24×10^3 binding sites per cell. These parameters, similarly as for LPS, are presumably averaged.

LPS binding to hepatocytes was suppressed by adding an excess of LDL—LPS complex (-15%), while LDL had no effect on this process (Fig. 2, *a*). The interaction between LDL—LPS complexes and hepatocytes was appreciably blocked by LDL (-65%), while the inhibitory effect of LPS was less pronounced (-30%; Fig. 2, *b*). These data suggest that LDL—LPS com-

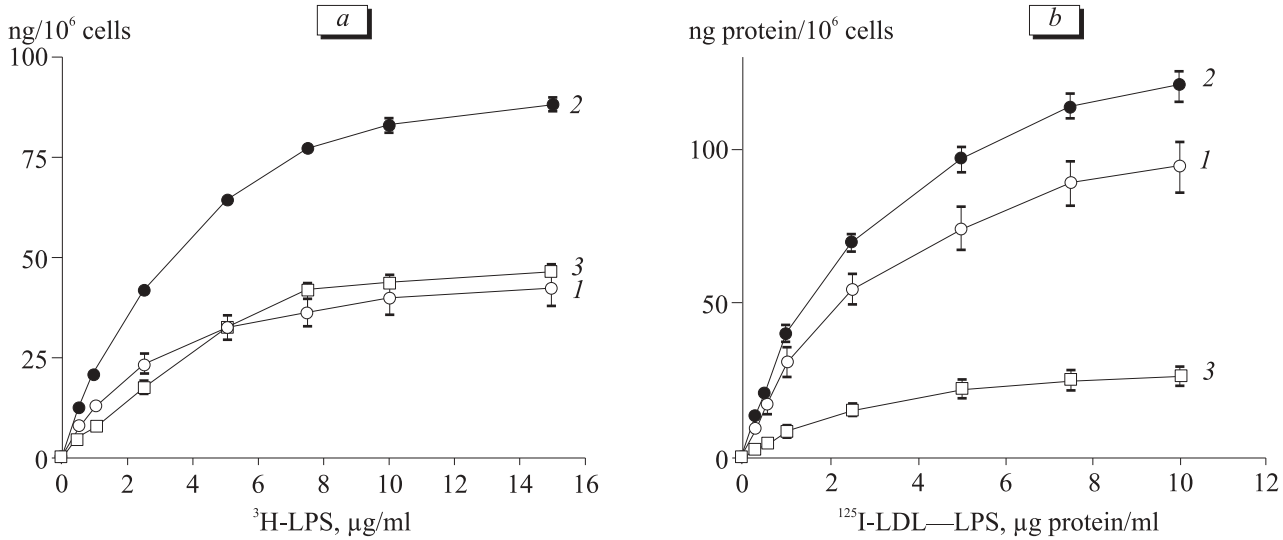

Fig. 1. Parameters of binding of ^{3}H-LPS (*a*) and ^{125}I-LDL—LPS (*b*) to hepatocyte culture. Specific (*1*) binding (ordinate) is calculated by the difference between total (*2*) and nonspecific binding (*3*), which was determined in the presence of 30-fold excess of unlabeled agent.

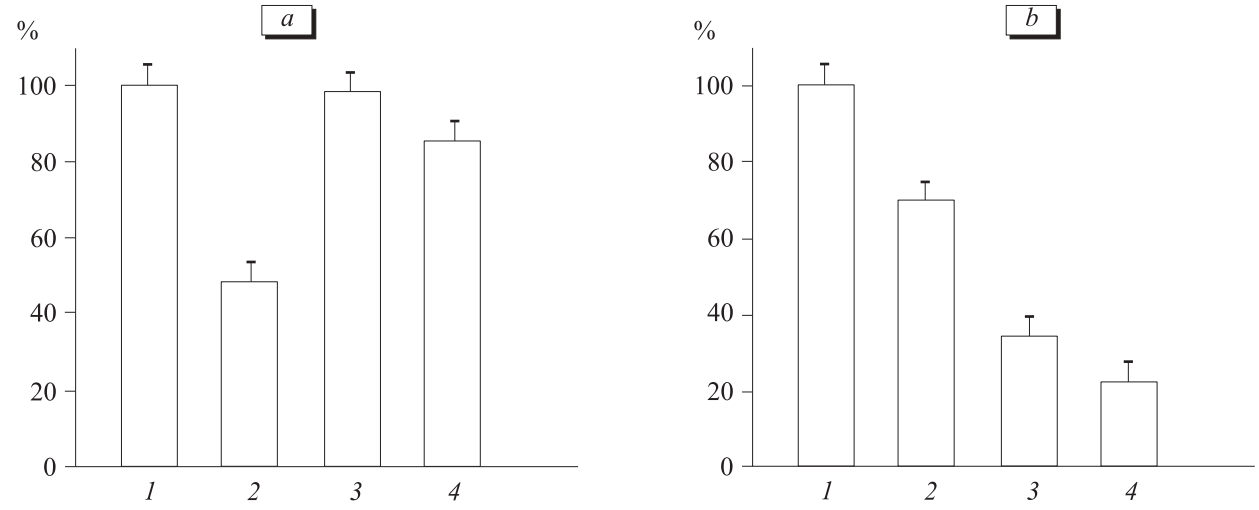

Fig. 2. Effect of 30-fold excess of unlabeled agent on binding of ^{3}H-LPS (2.5 μg/ml; *a*) and ^{125}I-LDL—LPS (2.5 μg protein/ml; *b*) to hepatocyte culture (10^6 cells/ml). *1*) control (no addition); *2*) addition of LPS; *3*) LDL; *4*) LDL—LPS.

plexes bind mainly to LDL receptors and less so to LPS receptors on hepatocyte surface.

A principal result of this study is the demonstration of binding of appreciable amount of LPS alone and LDL—LPS complexes to hepatocytes. Taking into account great size of these cells and their high number in the liver, we conclude that appreciable percent of LPS (LPS monomers/aggregates and complexes with LDL) can be directly bound by parenchymal cells, particularly under conditions of suppressed functions of Kupffer and/or endothelial cells [2].

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