Modification and Clearance of Low Density Lipoproteins during the Formation of Endotoxin-Lipoprotein Complexes

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Changes in electrical charge and clearance rate of LDL after the formation of their complexes with bacterial LPS were studied in experiments on Wistar rats. It was found that binding of *S. minnesota* R595 LPS with ^{125}I-LDL sharply accelerated clearance of the greater part of LDL complexes, but on the other hand induced the appearance of an LDL—LPS subfraction with slower elimination rate compared to free LDL. Electrophoresis showed that after binding of LPS, LDL acquired a negative charge. These data suggest that the formation of LDL—LPS complexes is accompanied by modification of LDL due to which they acquire atherogenic properties.

Key Words: *bacterial lipopolysaccharides; low density lipoproteins; lipopolysaccharide-low density lipoprotein complexes*

Bacterial LPS (endotoxins) are the major components of the outer membrane of gram-negative bacteria. Multiplication and lysis of these bacteria in the organism lead to the release of LPS into systemic circulation, which induces systemic endotoxemia and its various pathological consequences.

LPS are not present in the circulation in the free form, but rapidly form complexes with special proteins characterized by high affinity to lipid A and with lipoproteins (LP) of all classes [1,2,7]. In the serum, these high affinity proteins bind LPS more rapidly than LP [12]. However, under conditions of normo- and hyperlipidemia and in the presence of sufficient concentrations of endotoxin in the blood, the contribution of LP into LPS binding attains 60-80% [2,4,5]. This is a protective mechanism, because endotoxic activity of LPS is minimum in LPS—LP complexes [5].

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The rate of LDL clearance is of principal importance in hypercholesterolemia, because long-term circulation of LDL is one of the major factors of atherogenesis [9]. apo-B-LDL modified in the circulation acquire a negative charge and are captured via not apo-B,E receptors, but scavenger receptors (ScR); these modified LDL are uncontrollably captured by macrophages of the arterial wall with the formation of foam cells [6].

Clearance of endotoxin is realized by the macrophage system. It should be noted that LPS in low concentrations (<10 ng/ml) are captured via LBP-CD14 pathway, while high LPS concentrations (>100 ng/ml) are scavenged via ScR [10], which are highly expressed on macrophages. It is known that LP in LPS—LP complexes mask LPS from CD14, thus reducing their binding with macrophages, therefore clearance of ^{125}I-LPS bound to LP decreases [4]. However, changes in LP clearance in LPS—LP complexes compared to clearance of free LP (*i.e.* the fate of LPS—LP complexes on the whole) were never studied. It is also unknown, whether LDL

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after complexation with LP acquire a negative charge, thus becoming more atherogenic.

Here we studied clearance of labeled LDL, components of LPS—LDL complexes, and evaluated electrical charge of these complexes.

MATERIALS AND METHODS

LDL fraction (floating density *d*=1.019-1.063 g/ml) was isolated from pooled serum (from conventionally healthy individuals) containing 0.1% EDTA by sequential ultracentrifugation in KBr gradient (after Lindgren). LDL were concentrated by repeated ultracentrifugation and were used for preparing LDL—LPS complexes together with LP-free serum (*d*>1.21 g/ml). Centrifugation was carried out on a Beckman L8-M ultracentrifuge with a Ti-80 angle rotor (105,000*g*, 20 h at 4°C). LDL fraction was purified from KBr by gel-filtration on a Sephadex G-25 chromatography column, protein content was measured after Lowry, and then LDL were labeled with ^{125}I using the iodine monochloride method proposed by McFarlane in modification of Bilheimer *et al*. using Na^{125}I (Amersham Pharmacia Biotech UK Ltd, Buckinghampshire England, specific activity 100 mCi/ml, without carrier). After labeling, trichloroacetic acid-precipitated radioactivity of labeled LDL was 98%, specific activity of ^{125}I-LDL was 25 μCi/mg LDL protein. ^{125}I-LDL and LP-free serum were sterillized by filtration and ^{125}I-LDL—LPS complexes were prepared. To this end, 1 ml 0.5% aqueous solution of highly purified LPS from *Salmonella minnesota* R595 (kindly provided by Dr. M. Freudenberg and Dr. C.Galanos, Max-Planck-Institut fur Immunobiologie, Freiburg, Germany) was incubated for 30 min with 0.5 ml LP-free medium, an equal volume of ^{125}I-LDL was added, and the mixture was incubated for 2 h at 37°C. To remove unbound LPS, LPS—LDL complexes were isolated by ultracentrifugation at *d*=1.063. In control samples, LPS was replaced with an equal volume of buffered saline.

For evaluation of the rate of ^{125}I-LDL and ^{125}I-LDL—LPS clearance from the blood, labeled LDL or LDL—LPS complexes were injected into the caudal vein of male Wistar rats in a dose of 10 μCi/100 g body weight and after certain time intervals radioactivity of the blood samples (0.1 ml) from the retroorbital sinus was measured. Label half-elimination time ($T_{1/2}$) was calculated in exponential zones of the clearance curves after subtraction of a component determined by LDL iodination by the formula:

$$T_{1/2}=\frac{0.693t}{\ln C_1-\ln C_2},$$

where $0.693=\ln 2$, C_1 and C_2 are initial and subsequent label concentrations in the blood, and *t* is the time between these measurements. Clearance rate constant (K index) was calculated by the formula: $K=0.693/T_{1/2}$.

Electrophoresis of LPS—LDL complexes was performed in 1% agarose gel in tris-barbiturate buffer at pH 8.6 (after L. A. Osterman) The gels were then fixed in methanol-acetic acid-water (60:10:30, v/v/v) mixture, stained with 0.1% Coomasie G-250, washed, and relative electrophoretic mobility (*R*m) was determined as the ratio of the distance from the start to the middle of the band for LDL—LPS complexes to that for free LDL.

The data were processed by routine methods of variation statistics.

RESULTS

The mean half-elimination time for ^{125}I-LDL was 11±2 h, while K index was 0.061±0.010 h^{-1}, which corresponded to the time of the presence of LDL particles in the circulation. Clearance of ^{125}I-LDL—LPS was considerably accelerated compared to that of free LP particles (Fig. 1). The mean $T_{1/2}$ for ^{125}I-LDL—LPS complexes was 55±10 min and K-index 0.76±0.14 h^{-1}.

The dynamics of LDL—LPS clearance was biphasic and was described by two different exponential curves (Fig. 1): 56±10% complexes were eliminated during the first hour ($T_{1/2}$=36±5), but then elimination was decelerated and became slower than elimination of free LDL ($T_{1/2}$=40±10 h). Thus, the formation of LDL—LPS complexes accelerates clearance of LDL incorporated into these complexes, but simultaneously induced the appear-

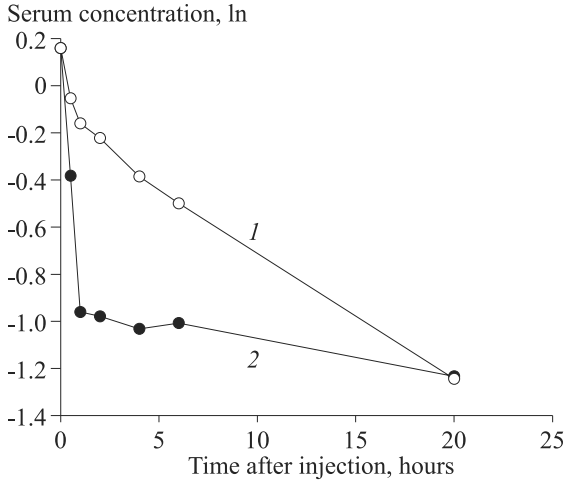

Fig. 1. Clearance of free ^{125}I-LDL (*1*) and ^{125}I-LDL—LPS complexes (*2*) in rats.

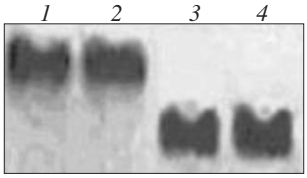

Fig. 2. Electrophoretic mobility of free LDL particles (*1,2*) and LDL—-LPS complexes (*3,4*) in 1% agarose gel.

ance of a subfraction of LDL—LPS complexes, which are eliminated slower than free LDL. Judging from quantitative differences in $T_{1/2}$ (by more than 1.5 orders of magnitude), the rapidly and slowly eliminated subfractions of LDL—LPS complexes are eliminated via different receptor systems. $T_{1/2}$ for rapidly eliminated subfraction is close to that for modified particles captured by scavenger receptors. It can be hypothesized that different LDL subfractions form complexes with LPS in different ways: the major part of LDL particles after binding with LPS can interact with ScR due to the presence of negatively charged phosphate groups of lipid A and 2-keto-3-deoxyoctonic acid (KDO) residues of LPS [8,10], while slowly eliminated subfraction of LDL—LPS complexes is masked from apo-B,E-receptors.

In special experimental series, changes in electrical charge of LDL particles after binding with LPS were evaluated by agarose-gel electrophoresis. Indeed, negative electrical charge should increase the rate of migration of LDL particles towards anode. After the formation of LDL—LPS complexes, electrical mobility of LDL considerably increased (Fig. 2). The relative electrophoretic mobility of LDL—LPS complexes in our experiments was 2.63. Since, binding of LPS to LDL is realized via interaction between lipid A and apo-B [3,11], lipid A masks/modifies the positively charged arginine and lysine residues in apo-B via ionic interaction.

Thus, after formation of LDL—LPS complexes, the major part of LDL is modified and is rapidly eliminated from the circulation. Since uncontrollable capture of modified LDL by macrophages leads to their transformation into foam cells, the proatherogenic role of this subfraction of LDL—LPS complexes is beyond doubts. LDL of slowly eliminated subfraction of LDL—LPS complexes are evidently masked from apo-B,E receptors. It cannot be excluded the long-term presence of this subfraction in the circulation can also lead to oxidative and/or enzymatic modification of LDL and play an atherogenic role.

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