Antigenic Differences between apo-B in Native and Circulating Modified Low-Density Lipoproteins

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The state of apo-B in native and circulating modified low-density lipoproteins was studied by solid-phase enzyme immunoassay. We studied the interaction of these particles with monoclonal antibodies to apo-B of low-density lipoproteins. Native and circulating modified low-density lipoproteins had different affinity for the studied antigens. Our results illustrate conformational changes in apo-B of circulating modified low-density lipoproteins compared to native low-density lipoproteins. These changes probably contribute to increased accumulation of particles in vascular cells and their transformation into foam cells giving way to atherosclerotic vascular lesions.

Key Words: *low-density lipoproteins; modified low-density lipoproteins; apo-B; atherosclerosis*

We previously detected and isolated human blood low-density lipoproteins (LDL) differing from native LDL (nLDL) by reduced content of sialic acid (terminal sugar in biantennary carbohydrate chains of the lipoprotein particle). They were named circulating modified LDL (cmLDL) [3,9]. cmLDL induce accumulation of lipids (*e.g.*, cholesterol esters) in human aortic intima cells and macrophages and, therefore, possess atherogenic activity [8]. The content of sialic acid in cmLDL from the blood of patients with atherosclerosis was 2-3 times lower than in nLDL [5,8]. A strong negative correlation was revealed between the content of sialic acid in LDL and ability of these particles to induce lipid accumulation *in vivo* in cultured cells [4]. cmLDL differ from nLDL by not only reduced content of sialic acid, but also other characteristics [8]. For example, the diameter of cmLDL is 10-15% lower than that of nLDL. The density of cmLDL is higher compared to nLDL. cmLDL have a higher electrophoretic mobility than nLDL. Therefore, cmLDL have high negative surface charge [7]. Further studies revealed significant differences in the lipid composition of nLDL and cmLDL [1,8]. Moreover, the content of lipid-soluble vitamins A and E (natural antioxidants) in cmLDL is much lower than in nLDL. These differences probably contribute to the ability of cmLDL to initiate lipid peroxidation [8]. Although much attention was given to cmLDL, changes in apo-B of this LDL fraction remain unknown. Here we studied differences in the localization of apo-B on the surface of nLDL and cmLDL by means of solid-phase enzyme immunoassay with monoclonal antibodies to this protein.

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MATERIALS AND METHODS

LDL were isolated by two-step ultracentrifugation in a NaBr density gradient [5]. nLDL and cmLDL were separated by lectin chromatography on a column packed with *Ricinus communis* agglutinin agarose (Boehringer Mannheim GmbH) [8].

Monoclonal mouse antibodies to human apo-B were obtained by the method of hybridization [2]. We

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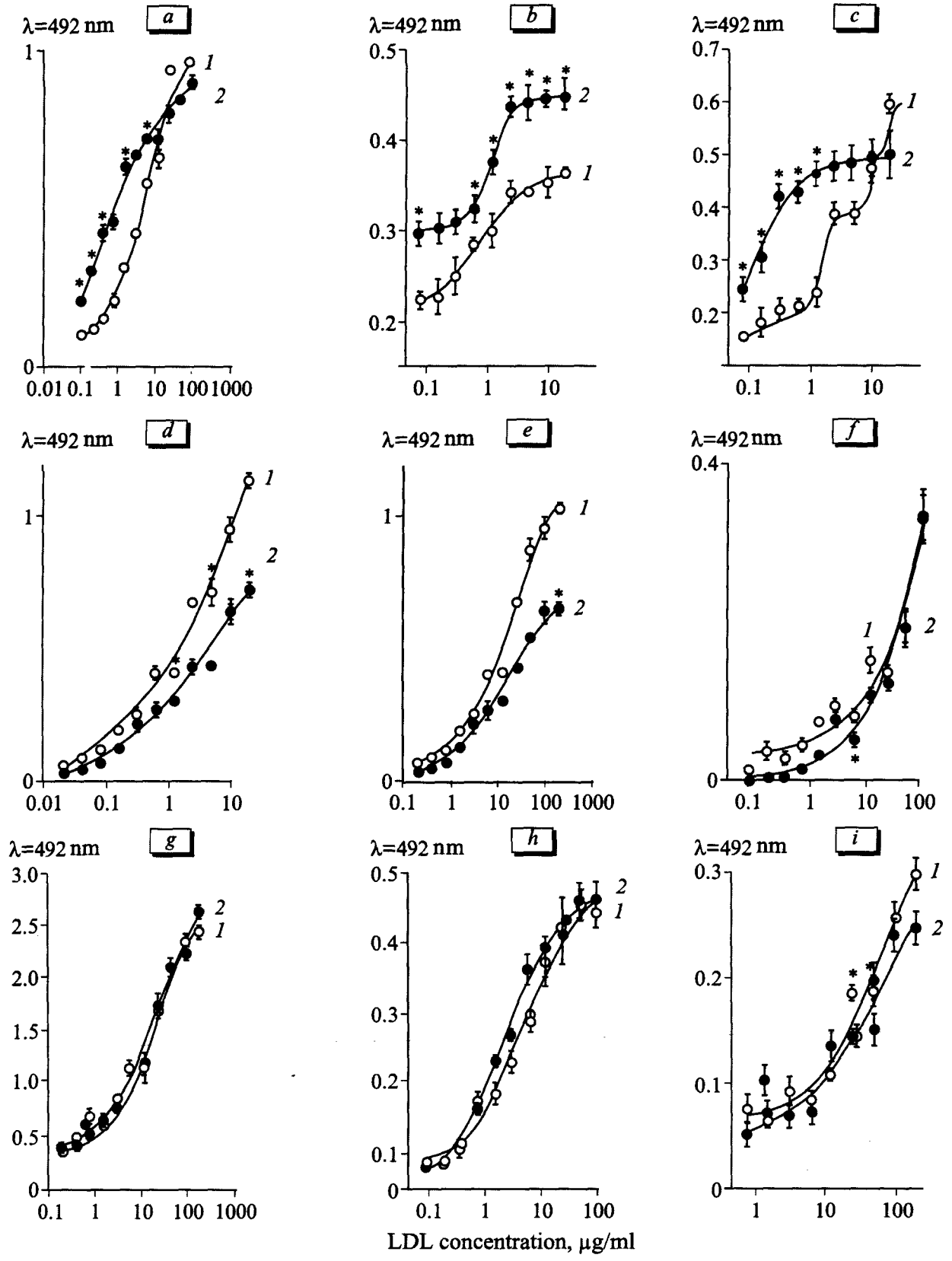

Fig. 1. Concentration dependences for the interaction of native (*1*) and circulating modified LDL (*2*) with monoclonal antibodies to human apo-B. Antibodies of 2E3 (*a*), 2G1 (*b*), 7C2 (*c*), 3C8 (*d*), 6E2 (*e*), 2G8 (*f*), 5F8 (*g*), 4C11 (*h*), and 3G4 (*i*) clones. The results are expressed as means and standard deviations (3 measurements). *$p<0.05$ compared to native LDL.

used antibodies produced by 2E3, 2G1, 7C2, 3C8, 6E2, 2G8, 5F8, 4C11, and 3G4 cells.

Binding of LDL to monoclonal antibodies was assayed in a 96-well plate (Nunc. Roskilde). Polyclonal goat antibodies to human apo-B (100 μl, IMTEK) in isotonic phosphate buffer (IPB, pH 7.2) were placed in wells and incubated at 4°C for 24 h. The wells were washed with IPB containing 0.2% bovine serum albumin (BSA). BSA-IPB (2%, 100 μl) was placed in wells and incubated at room temperature for 1 h. The wells were washed 5 times with 100 μl 0.2% BSA-IPB. LDL were placed in wells and incubated at 37°C for 2 h. The wells were washed. Monoclonal mouse antibodies to human apo-B (100 μl) were placed in wells and incubated at room temperature for 1 h. The wells were washed. Peroxidase-labeled monoclonal goat antibodies to mouse immunoglobulins (100 μl) were placed in wells, incubated at room temperature for 1 h, and developed with *o*-phenylenediamine. After washout the substrate buffer (100 μl) containing 12 ml 0.1 M citrate buffer (pH 4.5), 5 mg *o*-phenylenediamine, and 18 μl 3% H_2O_2 was added and incubation was performed at 37°C for 20-30 min. The reaction was stopped with 20 μl 50% H_2SO_4. Optical density was measured on a Multiscan Bichromatic multichannel spectrophotometer (Labsystems OY) at 492 nm.

Monoclonal antibodies were assayed in preliminary experiments to exclude the possibility of nonspecific binding to bottom layer antibodies (polyclonal goat antibodies to human apo-B) and evaluate the optimal dilution of antibodies.

Differences in binding of LDL were estimated by means of variational analysis. The differences were significant at $p<0.05$.

RESULTS

cmLDL more effectively than nLDL bound to antibodies of 2E3, 2G1, and 7C2 clones (Fig. 1, *a-c*). We compared the interaction of nLDL and cmLDL with 2E3 antibodies. Binding of LDL to antibodies did not differ in the asymptotic maximum range. However, cmLDL in a concentration of 0.1-12 μg/ml were more potent in binding to 2E3 antibodies than nLDL (by 3-8 times, Fig. 1, *a*). We revealed no differences in binding of nLDL and cmLDL in the asymptotic maximum range, which was probably related to sensitivity limits of the method. cmLDL in various concentrations more effectively interacted with 2G1 antibodies than nLDL (Fig. 1, *b*). Similarly to experiments with 7C2 antibodies (Fig. 1, *a*), we found no differences in binding of nLDL and cmLDL to 7C2 antibodies in the asymptotic maximum range (Fig. 1, *c*). cmLDL in a concentration of 0.1-1.2 μg/ml more effective interacted with 7C2 antibodies than nLDL. However, binding of nLDL to 3C8 and 6E2 antibodies was more significant compared to cmLDL (Fig. 1, *d*, *e*).

cmLDL are characterized by changes not only in the composition and properties of lipid and carbohydrate components [1,8], but also in the macromolecular structure of apo-B.

apo-B probably underwent local structural modification. This suggestion was confirmed by the results of studying the interaction of nLDL and cmLDL with antibodies of 2G8, 5F8, 4C11, and 3G4 (Fig. 1, *f-i*). No significant differences were revealed in the interaction of nLDL and cmLDL with antibodies produced by these clones.

Our results indicate that changes in the carbohydrate and lipid composition of cmLDL are accompanied by structural modification of apo-B. It determines changes in the density of LDL and increase in the negative surface charge of particles [8]. Conformational changes in apo-B may suppress binding of cmLDL to apo-B and E-receptor for nLDL. cmLDL gain the ability to bind to the scavenge receptor for modified LDL, asialoglycoprotein receptor, and/or cell wall glycoproteins. These changes probably contribute to increased accumulation of cmLDL in vascular wall cells [4,5] and their transformation into foam cells, which produces atherosclerotic vascular damage.

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