Interaction of Low-Density Lipoproteins and Elastins from the Intima and Media of Human Intact and Atherosclerotic Aorta

V. V. Tertov, I. V. Suprun, L. A. Medvedeva, T. A. Skalbe, and A. N. Orekhov

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Binding of low-density lipoproteins to elastins from the media and proteoglycan and muscle sublayers of human intact and atherosclerotic aorta was studied. Circulating modified low-density lipoproteins intensively bound to elastin due to the loss of sialic acid.

Key Words: *low-density lipoproteins; elastin; aorta*

Accumulation of intra- and extracellular lipids is typical of atherosclerosis in human vessels. N. A. Anichkov *et al.* described primary lipid deposits along aortic elastic fibers in humans and rabbits fed a cholesterol-rich diet [1]. Association of vascular lipids and elastin was described [10]. Elastin fractions isolated from the intima and media of human aorta with elastase can bind low-density lipoproteins (LDL) [15]. Elastin obtained from the intima of human atherosclerotic aorta binds lipids of very-low-, intermediate-, low-, and high-density lipoproteins isolated from the plasma [6,7]. However, binding of plasma lipoproteins to elastins from various sublayers of the intima and media of human intact and atherosclerotic aorta received little attention. Our recent studies revealed circulating modified LDL in human blood (cmLDL or desialylated LDL). Unlike native LDL (nLDL), cmLDL induce accumulation of cholesterol and cholesterol esters in the intima of intact human aorta [12] and are characterized by low contents of sialic acid, neutral sugars, and neutral lipids, high density, large negative charge, small size, and high susceptibility to oxidation [13]. Here we studied binding of nLDL and cmLDL to elastins isolated from the media and proteoglycan and muscle sublayers of human intact and atherosclerotic aorta.

Group of Atherosclerotic Cell Pathology, Institute of Experimental Cardiology, Russian Cardiology Research-and-Production Complex, Russian Ministry of Health, Moscow. **Address for correspondence:** inat@cardio.ru. V. V. Tertov

MATERIALS AND METHODS

LDL were isolated by two-stage ultracentrifugation in NaBr density gradient [11]. nLDL and cmLDL were separated by lectin chromatography on a column packed with *Ricinus communis* agglutinin agarose (Boehringer Mannheim) [14] and then labeled with ^{125}I [3]. nLDL were treated with 0.02 U neuraminidase from *Arthrobacter ureafaciens* (Oxford GlycoSystems) at 37°C. Purified elastin was obtained from 0.2-2.5 g tissue as described previously [7].

^{125}I-LDL binding to elastin was studied in 96-well plates (Nunc). Wells containing 100 μl elastin in phosphate-saline buffer (pH 7.2) were incubated at 4°C for 24 h, washed with phosphate buffer containing 0.2% bovine serum albumin, and incubated with iodinated LDL at 37°C for 2 h. Iodinated LDL bound to elastin were dissolved in 200 μl 0.1 N NaOH, and radioactivity was measured on a RackGamma II γ-counter (Wallac OY).

The results were analyzed by Student's *t* test.

RESULTS

Table 1 shows binding of nLDL and cmLDL to elastins from the intima of human intact and atherosclerotic aorta. cmLDL more effectively bind to elastin

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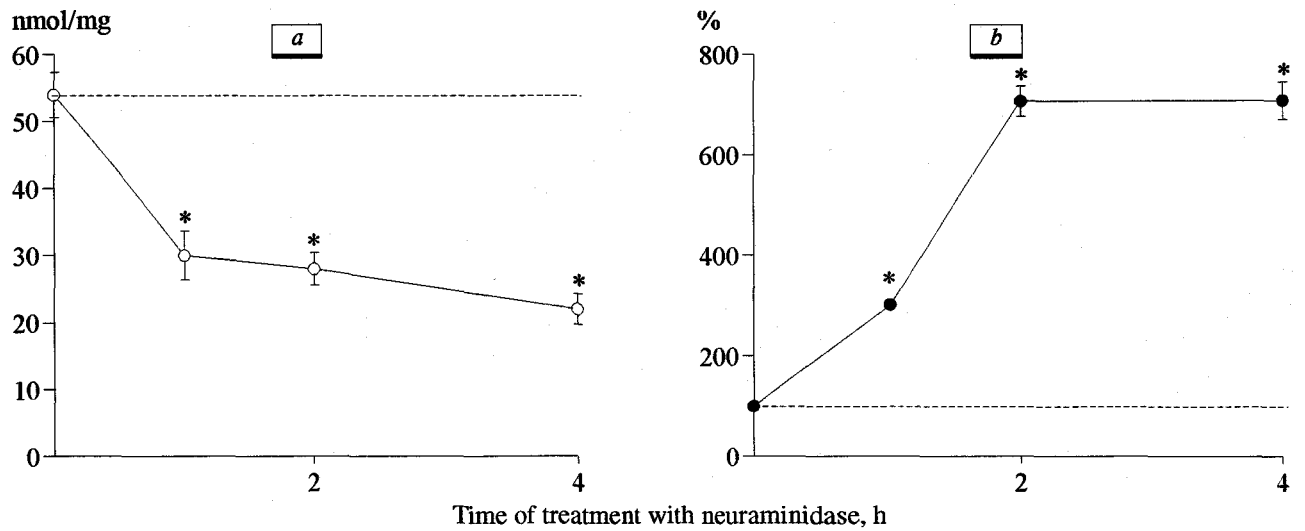

Fig. 1. Effect of neuraminidase treatment on the content of sialic acid in LDL (*a*) and LDL binding to elastin from proteoglycan layer of fatty streaks (*b*). Ordinate: *a*) content of sialic acid in LDL, nmol/mg protein; *b*) LDL bound to elastin, % of control. *$p<0.05$ compared with the control (dotted line).

TABLE 1. Content of Elastin-Associated ApoB (pg/well) in Sublayers of Human Intact and Atherosclerotic Aorta (*M±m*)

Aortic region	Layer					
	proteoglycan		musculoelastic		media	
	nLDL	cmLDL	nLDL	cmLDL	nLDL	cmLDL
Intact	595±44	964±74*	287±14	446±50*	244±24	289±18
Infiltration	634±52+	1269±60*	339±14+	497±32*	319±32	378±27
Fatty streaks	1181±104+	1826±137*+	561±20+	969±18*+	333±30	396±43
Fibroatheroma	418±11+	637±51*+	389±8*	829±15*+	296±24	317±27

Note. *$p<0.05$ compared with nLDL and +$p<0.05$ compared with intact regions.

than nLDL. We proposed that binding of modified LDL to elastin depends on the presence or absence of sialic acid in carbohydrate chains of LDL. To test this hypothesis, we removed sialic acid with neuraminidase and studied on LDL binding to elastin from the proteoglycan layer of fatty streaks displaying the maximum LDL-binding capacity.

The treatment of nLDL with neuraminidase for 1-4 h led to a monotonous decrease in the content of sialic acid (Fig. 1). The binding of LDL treated with neuraminidase for 4 h to elastin 5-fold surpassed the control level.

Thus, *in vitro* removal of sialic acid stimulated binding of LDL to elastin. The role of sialic acid in LDL binding to other matrix components, proteoglycans, was reported previously [2,4].

The content of cmLDL in the intact and, especially, in the atherosclerotic intima surpassed the content of sialylated LDL [13]. Furthermore, the content of sialic acid in LDL from fatty streaks is lower than in LDL from intact intima. Enhanced binding of cmLDL to elastin and other components of the connective tissue matrix *in vivo* prolongs their presence in the vascular wall. Our previous experiments showed that cmLDL induce cholesterol accumulation in intima cells of human aortic [9], and that cholesterol accumulation in cells inversely correlated with the content of sialic acid in LDL [8]. Therefore, the elevation of cmLDL concentration and high degree of their desialylation in the vascular wall promote the formation of cholesterol deposits in the intima. Enhanced binding of cmLDL to elastin probably leads to the formation of extra- or intracellular lipid deposits in the aorta.

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