Effect of Syngeneic and Xenogeneic Apolipoprotein B-Containing Lipoproteins on Immune Response and Phagocytosis in Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 3, pp. 293-296, March, 1998
Original article submitted January 8, 1997

Apolipoprotein B-containing lipoproteins injected parenterally to CBA mice exhibit immunomodulatory activity. This activity depends on the class (very low or low density lipoproteins) and origin (syngeneic or xenogeneic) of injected lipoproteins. Lipoproteins also modulate phagocytic activity of neutrophils. *In vitro* lipid peroxidation impairs the immunomodulatory activity of lipoproteins.

Key Words: *apolipoprotein B-containing lipoproteins; immune response; phagocytosis; lipid peroxidation*

Immunomodulatory activity of plasma lipoproteins (LP) attracts great attention. It has been shown that apolipoprotein (apo) B-containing LP, both very low (VLDL) and low density (LDL), suppress mitogen-induced lymphocyte proliferation *in vitro* and immune response to thymus-dependent antigens *in vivo* [4,5]. However, xenogeneic LP with pronounced immunogenic properties have been generally used, hence immunosuppression due to antigen competition cannot be ruled out. It has been recently hypothesized that immunosuppressive activity of LP is due to the presence of lipoperoxides [7]. In the present study we compare the effects of syngeneic and xenogeneic apoB-containing LP of different classes (native and oxidized *in vitro*) on immune response and phagocytosis.

MATERIALS AND METHODS

Syngeneic LDL and VLDL from blood of CBA male mice (14-16 g) and xenogeneic LP from donor blood were isolated by ultracentrifugation [8] in the presence of protease inhibitors and antioxidants. LP were oxidized by incubation with 10 μM $CuSO_4$ at 37°C for 4-6 h. The reaction was stopped by adding EDTA and cooling (4°C). The degree of oxidation was assessed by accumulation of thiobarbituric acid-reactive substances [9]. LP were used no later than 48 h after isolation.

Different doses of LP (μg protein/animal) were injected subcutaneously or intravenously (into the caudal vein) 24 h prior to immunization with sheep erythrocytes (10^7 cells/animal) or prior to isolation of peritoneal neutrophils. Control animals received apyrogenic physiological saline. On day 4 after intravenous immunization with sheep erythrocytes, anti-erythrocyte IgM-producing cells were counted in the spleen of each animal [6]. Phagocytic activity of peritoneal neutrophils *in vitro* [1] was expressed as phagocytic index, i.e., the percentage of phagocytizing neutrophils. One-day-old culture of *St. aureus* was used as the test microorganism.

In vitro expression of Thy-1 antigen on bone marrow T precursors was assessed using rabbit antiserum against brain cortex of CBA mice adsorbed by mouse liver homogenate and mouse and sheep erythrocytes [2]. This antiserum in the presence of fresh guinea pig complement (1:3) induced lysis of 88% thymocytes and does not interact with bone marrow

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0007-4888/98/0003-0259$20.00

cells of CBA mice. Cytotoxicity of LP was evaluated by the number of live lymphoid cells after 30-min *in vitro* incubation (3×10^7 cells/ml) with various concentrations of LP. Cell viability was assessed by Trypan Blue exclusion (0.2% aqueous solution) [1].

RESULTS

Effect of LP on immune response. Intravenous injection of syngeneic or xenogeneic VLDL in a dose of 80 μg/animal 24 h prior to immunization with sheep erythrocytes increased the number of antibody-producing cells 1.6- and 2.9-fold, respectively (Table 1). Oxidation diminished the immunostimulating effect of VLDL; the effects of syngeneic and xenogeneic VLDL differed little. Reduced immunostimulating activity of oxidized syngeneic VLDL was also observed after decreasing the dose of injected LP: in a dose of 10^{-4} μg native LP increased the number of antibody-producing cells, while oxidized LP were effective only in a dose of 1 μg and had no effect on immune response in doses 10^{-2}-10^{-4} μg (Table 2).

The effect of LDL was different. We found considerable differences in the effect of LDL of various origins (Table 1). Regardless the administration route (intravenous or subcutaneous), native xenogeneic LDL in a dose of 80 μg had no effect, while oxidized LDL inhibited immune response to sheep erythrocytes in CBA mice. In contrast, native and oxidized syngeneic LDL injected intravenously exerted a slight immunostimulating effect. When injected subcutaneously, native syngeneic LDL increased, while oxidized LDL had no effect on the number of antibody-producing cells.

Surprisingly, a decrease in the dose of syngeneic LDL from 80 to $1\text{-}10^{-4}$ μg abolished their immunostimulating effect and led to pronounced inhibition of immune response (Table 2). Being injected subcutaneously, syngeneic LDL also exhibited immunosuppressive activity (data not shown). Oxidation reduced the immunosuppressive effect of LDL: oxidized LDL in a dose of 10^{-4} μg had no effect on immune response, while native LDL were effective in all doses.

Effect of LP on phagocytic activity of neutrophils. Native syngeneic and xenogeneic VLDL in a dose of 80 μg had no effect on phagocytosis (Table 1), whereas oxidized VLDL significantly (xenogeneic LP) or slightly (syngeneic LP) suppressed phagocytosis. On the other hand, in low doses native and oxidized syngeneic VLDL equally stimulated phagocytic activity of neutrophils (Fig. 2).

Regardless the administration route, syngeneic and xenogeneic native LDL in a dose of 80 μg had no effect, while oxidized LDL suppressed phagocytic activity of neutrophils (Table 1). In low doses syngeneic LDL had practically no effect on phagocytosis (Table 2).

LP did not change phagocytic number (mean number of microbial cells per phagocyte), which varied from 1.7 to 2.0.

TABLE 1. Effect of Syngeneic and Xenogeneic VLDL and LDL (80 μg/mouse) on Immune Response and Phagocytosis in Mice (*M±m*)

Injected LP	Modification of LP	Intravenous injection		Subcutaneous injection	
		number of APC per 10^6 spleen karyocytes	phagocytic index, %	number of APC per 10^6 spleen karyocytes	phagocytic index, %
Syngeneic VLDL	Control	47.6±3.8 (32)	25.3±1.9 (11)	—	—
	Native	76.2±8.9 (19)*	27.4±2.1 (10)	—	—
	Oxidized	64.2±4.0 (14)*	22.6±2.3 (10)	—	—
Xenogeneic VLDL	Control	38.6±5.9 (9)	25.3±1.2 (8)	—	—
	Native	112.6±12.6 (9)*	25.9±0.5 (8)	—	—
	Oxidized	65.6±11.9 (8)	20.9±0.1 (8)*	—	—
Syngeneic LDL	Control	45.8±4.1 (27)	25.7±1.6 (39)	42.0±7.9 (28)	20.6±0.3 (8)
	Native	61.2±9.3 (20)	25.7±1.7 (29)	66.7±11.0 (27)*	21.2±0.7 (8)
	Oxidized	62.7±7.5 (21)	20.2±1.3 (33)*	41.9±4.2(26)	16.6±0.7 (8)*
Xenogeneic LDL	Control	48.0±2.5 (17)	25.4±1.2 (19)	48.0±3.6 (14)	21.0±0.4 (8)
	Native	49.3±7.0 (15)	27.6±1.3 (9)	52.0±4.5 (14)	20.4±1.7 (8)
	Oxidized	34.7±5.9 (9)*	21.0±0.8 (9)*	22.2±3.5 (14)*	17.1±1.4 (8)*

Note. Here and in Table 2: APC: antibody-producing cells. *$p<0.01$, *$p<0.05$ compared with the control. Number of animals is shown in parentheses; dash: not determined.

TABLE 2. Effect of Intravenous Injection of Low Doses of Syngeneic VLDL and LDL on Immune Response and Phagocytosis in Mice (M±m)

Class of LP	Modification of LP	Number of APC per 10^6 spleen karyocytes after injection of LP in doses, μg/mouse			Phagocytic index (%) after injection of LP in doses, μg/mouse		
		1	10^{-2}	10^{-4}	1	10^{-2}	10^{-4}
Control		45.0±2.4 (17)	42.7±5.0 (10)	42.7±5.0 (10)	25.3±1.9 (11)	20.4±1.4 (8)	20.4±1.4 (8)
VLDL	Native	64.2±4.0 (14)*	64.9±6.9 (7)*	71.8±11.1 (7)*	41.8±1.0 (11)*	31.3±1.5 (9)*	30.3±1.6 (8)*
	Oxidized	87.9±6.2 (14)*	41.3±3.5 (8)	42.2±4.1 (7)	42.0±3.5 (11)*	—	33.6±3.0 (8)*
LDL	Native	24.9±5.8 (14)*	13.6±1.9 (7)*	19.0±5.6 (7)*	29.6±3.1 (10)	21.2±1.3 (8)	21.2±1.4 (8)
	Oxidized	22.8±6.3 (14)*	18.2±4.4 (8)*	43.9±7.2 (8)	26.9±1.9 (10)	23.6±0.5 (8)	22.0±1.6 (8)

It should be noted that the observed modulation of immune response and phagocytosis was not due to the presence of low-molecular-weight nonlipid substances, since dialysis buffer exhibited no immuno- and phagocytosis-modulating activities.

Cytotoxicity of LDL. Unlike native LDL, *in vitro* oxidized xenogeneic and syngeneic LDL were toxic for mouse lymphoid cells, which is consistent with published data [3]. *In vitro* incubation of thymocytes, splenocytes, bone marrow cells, and neutrophils with oxidized LDL (40-80 μg/ml) induced lysis of 5.6±1.6 to 37.4±3.4% cells. Peritoneal neutrophils were most susceptible cells: even in a concentration of 10 μg/ml (nontoxic for other cells) oxidized LDL induced lysis of 24.2±3.0 neutrophils.

Effect of LP on maturation of T precursors. Incubation of mouse bone marrow cells (37°C, 30 min) with 0.0001-5 μg/ml apoB-containing LP regardless their state (native or oxidized) and origin (syngeneic or xenogeneic) increased the number of Thy-1^+ cells from 0% (control) to 5±1.1-16.3±2.6% (experi-ment).

Thus, we observed opposite effects of low doses of two apoB-containing lipoprotein fractions: VLDL stimulate, while LDL inhibit immune response in CBA mice. The absence of the immunosuppressive activity (and even immunostimulating effect) of high LDL doses is probably due to minor admixture of "immunostimulating" LP in the studied fraction (1.006-1.063 g/ml density range). After 80- to 800,000-fold dilution of the LDL preparation, the effect of this stimulating subfraction became negligible and LDL exerted a pronounced immunosuppressive effect. *In vitro* oxidation reduced immunomodulatory activity of LP, the immunostimulating activity of apoB-containing LP was more susceptible to oxidation than their immunosuppressive activity. This probably explains suppression of immune response in mice injected with high doses of oxidized xenogeneic LDL.

The mechanisms of immunomodulatory effect of apoB-containing LP remain unclear. They can be associated with the effect of LP on maturation of T precursors. Suppression of phagocytosis probably results from direct toxic effect of large doses of oxidized LP on neutrophils, which are most susceptible to the toxic action of oxidized LDL.

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