Effect of Hyperlipidemia on Functional Activity of Peritoneal Macrophages in CBA and C57Bl/6 Mice

E. P. Kiseleva, V. P. Puzyreva, R. P. Ogurtsov, and I. G. Kovaleva

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The effect of experimental hyperlipidemia on functional activity of macrophages was studied in CBA and C57Bl/6 mice resistant and sensitive to the formation of aorta lesions, respectively. Two-month atherogenic diet increased the content of cholesterol in the serum and cells of peritoneal exudate in mice of both strains. In parallel, production of nitrites and 5'-nucleotidase activity in peritoneal macrophages increased, while parameters of phagocytosis, pinocytosis, and NBT test remained unchanged. Changes in the state of macrophages can be explained by increased cholesterol content. The absence of differences in functional activity of macrophages in CBA and C57Bl/6 mice indicates that the observed shifts are insignificant for the development of fatty streaks in the aorta.

Key Words: *experimental hyperlipidemia; inbred mice; macrophages; nitrites; 5'-nucleotidase*

The pathogenesis of atherosclerosis includes intricate interactions of arterial walls, smooth muscle elements, blood cells, and circulating lipoproteins. Macrophages play an important role in atherogenesis, because they absorb and metabolize lipids, release proteases, apolipoproteins, cytokines and growth factors, oxygen radicals [8]. There are data on different sensitivity of inbred mice maintained on atherogenic diets to the development of fatty streaks in the aorta: C57Bl/6 mice are prone and CBA mice are resistant to these changes.

Many investigators used sensitive and resistant mouse strains for identification of the most significant factors of aorta lesion [8]. Apart from genetic differences in the spectra of circulating lipid, differences in lipoproteinase production by macrophages [14] and in expression of some genes responsible for the development of inflammation and activation of NF-kB transcription factor in sensitive mice were demonstrated [11].

Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg. ***Address for correspondence:*** Aleksei.Kiselev@pobox.spb.ru, immun@immun.iem.ras.spb.ru. Kiseleva E. P.

We previously detected differences in the functional activities of T and B lymphocytes in CBA and C57Bl/6 mice maintained on atherogenic diets [2, 4]. Here we studied the effects of experimental hyperlipidemia on functional activity of peritoneal macrophages in these mouse strains.

MATERIALS AND METHODS

Male CBA and C57Bl/6 mice (18-20 g) from Rappolovo Breeding Center of Russian Academy of Medical Sciences were used. Experimental animals were fed atherogenic diets consisting of standard granulated fodder (67%), cholesterol (3%), sunflower oil (28.8%), and cholic acid (0.2%) for 2 months. Controls received a standard ration. Cholesterol and triglycerides were measured on an AA-2 autoanalyzer (Technicon), lipid composition of peritoneal exudate cells (PEC) was studied by gas chromatography as described previously [3]. Activity of PEC 5'-nucleotidase, parameters of NBT test (spontaneous and stimulated with zymosan or 1 μg/ml phorbol ether), and intensity of neutral red pinocytosis were evaluated spectrophotometrically [1]. Nitrite production (spontaneous or stimulated with

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10 ng/ml *E. coli* O55B5 LPS) was evaluated using Griss reagent [12]. Phagocytic activity of macrophages was studied in PEC suspension after 30-min incubation at 37°C with heat-killed *Saccharomyces cerevisia* under a phase contrast microscope. The percentage of phagocytosed cells per 100 macrophages was evaluated in each preparation. PEC composition was studied in smears stained after Romanowskii—Giemsa.

Serum lipids were assayed in each mouse separately, PEC from 4 mice were pooled, each experiment was repeated 2-3 times. The results were statistically processed using Student's *t* test.

RESULTS

Before the experiment CBA mice had lower LDL cholesterol content and higher serum content of triglycerides compared to C57Bl mice. After 2-month atherogenic diet the mice of both strains developed hyperlipidemia (Table 1), the content of total and LDL cholesterol increased. The content of HDL cholesterol more markedly increased in CBA mice compared to C57Bl/6 mice (by 1.9 and 1.2 times, respectively), which can be a factors determining resistance of these animals to the formation of fatty lesions.

The increase in cholesterol content in PEC membranes was observed only in CBA mice, but the content of cholesterol esters reflecting cholesterol content in the cytoplasm increased in mice of both strains. The possibility of cholesterol accumulation in cell membranes in hypercholesterolemia is very important, because in normal cells cholesterol entry and production are strictly regulated [7]. Our data are in line with the results of other scientists who demonstrated that hypercholesterolemia in rats fed atherogenic diets led to accumulation of cholesterol in macrophage membranes [13].

TABLE 1. Effect of Atherogenic Diet on Lipid Content in the Serum and PEC in CBA and C57Bl/6 Mice (*M*±*m*, *n*=8-16)

Parameter	CBA		C57Bl/6	
	control	experiment	control	experiment
Serum lipids, mmol/liter				
total cholesterol	2.40±0.13	6.03±0.31*	2.33±0.13	3.75±0.21*
LDL cholesterol	0.10±0.04	2.22±0.08**	0.72±0.04	1.63±0.10**
VLDL cholesterol	0.72±0.04	0.78±0.05	0.36±0.02	0.49±0.03**
HDL cholesterol	1.58±0.13	3.03±0.18*	1.24±0.08	1.47±0.08***
triglycerides	1.60±0.09	1.76±0.10	0.83±0.03	1.09±0.06**
PEC lipid composition				
total cholesterol, μg/ml protein	38.90+1.35	56.01±1.98**	22.50±0.82	20.81±1.38
free cholesterol, μg/mg protein	35.85±1.07	46.82±1.82**	20.45±0.68	16.65±1.17***
cholesterol esters, %	7.25±0.28	18.05±0.33*	9.00±0.33	14.55±2.16***

Note. Here and in Table 2: *p<0.001, **p<0.01, ***p<0.05 compared to the control.

TABLE 2. Effects of Hyperlipidemia on Functional Activity of Macrophages in CBA and C57Bl/6 Mice (*M*±*m*)

Parameter	CBA		C57Bl/6	
	control	experiment	control	experiment
5'-Nucleotidase, nmol/h/10^6 PEC	74.2±6.3	121.5±7.2*	133.9±9.1	164.3±2.9**
NBT test, OD_{620}				
spontaneous	0.040±0.006	0.048±0.006	0.048±0.008	0.045±0.007
zymosan-induced	0.159±0.010	0.163±0.008	0.185±0.025	0.206±0.028
phorbol ether-induced	0.163±0.005	0.186±0.012		
Neutral red pinocytosis, OD_{540}	0.642±0.042	0.742±0.031	0.424±0.030	0.397±0.056
NO_2^- production, nmol/10^6 PEC				
spontaneous	1.08±0.16	3.05±0.15*	0.87±0.04	4.72±0.55*
LPS-induced	1.81±0.20	7.35±0.33*	1.47±0.18	7.28±0.13*
Yeast phagocytosis, %	24.8±6.2	21.3±1.7	14.7±1.4	16.0±3.1

The count and composition of PEC did not change in experimental animals of both strains (~30% macrophages, 65% lymphocytes, 5% mast cells and neutrophils). Since the contribution of non-macrophage cells in NBT test, 5'-nucleotidase activity, NO_2^- production, and pinocytosis is negligible, functional activity of macrophages can be evaluated by these parameters.

The initial immunological parameters in the studied mouse strains were different. Activity of 5'-nucleotidase and parameters of phagocytosis and pinocytosis in control CBA mice were lower than in C57Bl/6 mice. Hyperlipidemia produced similar changes in functional activity of PEC in both strains. Activity of 5'-nucleotidase and spontaneous and LPS-induced production of nitrites in macrophages markedly increased (Table 2). On the other hand, the production of superoxide anions (NBT test), parameters of pinocytosis and phagocytosis did not differ from the control.

The effects of atherogenic diet on NO production and 5'-nucleotidase activity in macrophages were not studied; however, there is indirect evidence on the important role of lipids in the regulation of this enzyme. 5'-Nucleotidase is a surface membrane enzyme anchor to the lipid bilayer through a glycosylphosphatidylinositol chain [15]. It is currently accepted that organization of this type of proteins on the membrane, signal transduction, and regulation of recirculation is realized in rafts, membrane sites enriched with cholesterol and sphingolipids [5]. Changes in cholesterol content in cell membrane can modulate activity of this ectoenzyme.

Hence, we demonstrated enhanced production of nitroxide anion and high 5'-nucleotidase activity in macrophages of mice maintained on an atherogenic diet. Activation of 5'-nucleotidase on cell surface can increase local concentration of intracellular adenosine. This compound can induce apoptosis, enhance vascular permeability, and produce angiogenic and immunomodulating effects [6,10]. It can be hypothesized that macrophages with this functional phenotype stimulate inflammatory reaction and are involved in the formation of fatty streaks in the aorta, but they do not play the key role in this process. This is confirmed by the absence of difference in macrophage activity in CBA and C57Bl/6 mice with different genetic predisposition to the development of fatty injuries.

On the other hand, mice of these strains maintained on an atherogenic diet demonstrated opposite types of lymphocyte reaction: suppression of the immune response, proliferation, and apoptosis in CBA mice and activation of these processes in C57Bl/6 mice [2,4]. Intracellular content of cholesterol increased both in lymphocytes and macrophages, but the difference between the strains was detected only in lymphocytes. It can be hypothesized that, the immunopathogenesis of fatty streaks depends primarily on lymphocytes, *e.g.* natural killer cells of γδT cells recognizing lipid antigens [9].

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