Effect of Hyperlipoproteinemia on Functional Activity of Peritoneal Macrophages during Tumor Growth

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The role of hypercholesterolemia as a factor modulating functional activity of macrophages during the growth of syngeneic transplanted 22a hepatoma in mice was studied. Starting from day 21 after inoculation of tumor cells we observed the development of hyperlipoproteinemia paralleled by an increase in macrophage activity parameters. The total serum cholesterol content and production of nitroxide anions by macrophages were in positive correlation on days 14-35 of tumor growth. We hypothesized that the development of hypercholesterolemia at the late stages of some tumor growth is a factor stimulating production of nitrites and 5'-nucleotidase activity.

Key Words: *experimental tumors; cholesterol; macrophages; nitrites; 5'-nucleotidase*

Activated macrophages play an important role in the regulation of tumor growth [11]. The growth of experimental tumors is accompanied by changes in functional activity of macrophages, *e.g.* increased production of nitroxide anions [4,5] and activation of 5'-nucleotidase ectoenzyme [3,15]. The mechanisms of these changes are unknown. We observed similar shifts in functional activity of macrophages in mice without tumors receiving on atherogenic diet [2]. The growth of some experimental tumors is paralleled by the development of hypercholesterolemia [12], and we therefore hypothesized that this factor can also be essential for macrophages under conditions of tumor growth.

5'-Nucleotidase (CD73) is one of the main enzymes of purine metabolism providing substrates for macrophage activation (14) and a factor of cell adhesion and mobility [13]. Nitroxide anions possess cytotoxic effects and ensure the tumoricidal activity of macrophages. Their effect on apoptosis [6] and angiogenesis [10] depends on their concentration.

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Here we analyze the correlation between changes in serum content of neutral lipids, on the one hand, and 5'-nucleotidase activity, nitrite production, and other parameters of functional activity of peritoneal macrophages, on the other, during the growth of transplanted 22a hepatoma in mice.

MATERIALS AND METHODS

Experiments were carried out on male C3HA mice (18-20 g) from Rappolovo Breeding Center, Russian Academy of Medical Sciences. The animals received 10^5 cells of syngeneic continuous 22a hepatoma (0.2 ml). 22a hepatoma cell strain was kindly provided by Dr. O. N. Pogodina from Institute of Cytology, Russian Academy of Sciences. Controls received the same volume of normal saline. The mice were sacrificed by cervical dislocation. Serum levels of total cholesterol, α-cholesterol, and triglycerides were measured on a Keysys autoanalyzer (Boehringer Mannheim). Resident peritoneal macrophages were obtained by peritoneal lavage (cells from 4-5 animals were pooled) and incubated in 96-well plates in RPMI-1640 with 10% ETC for 2 h. The monolayer was washed from nonadhesive cells and used for evaluation of 5'-nucleo-

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tidase activity, neutral red pinocytosis, LPS-stimulated nitrite production, and phorbol ester-stimulated NBT reduction test by spectrophotometrical methods as described previously [2]. Peritoneal exudation cell composition was evaluated in smears stained by the method of Romanowsky—Giemsa. At all stages of the study the smears consisted of 30-40% macrophages, 60-65% lymphocytes, 1-3% mast cells, and solitary neutrophils.

The data were processed using Student's *t* test and analysis of paired correlations.

RESULTS

No changes in serum levels of neutral lipids were observed during the first 2 weeks after hepatoma cell inoculation. Total cholesterol content increased starting from day 21, and the content of α-cholesterol and triglycerides starting from day 28 (Fig. 1, *a*).

The study of functional activity of macrophages on days 14-35 of hepatoma growth revealed an appreciable increase in all parameters with the peak on day 28 (Fig. 1, *b*, *c*). Increased production of nitroxide and superoxide anions and pinocytosis stimulation are characteristic signs of macrophage activation. The mechanisms of 5'-nucleotidase activation in these cells are little studied. Some scientists regard these changes as a sign of immunosuppression [1], because the state of "classical" activation of macrophages is associated with a drop of enzyme activity [14].

We observed increased 5'-nucleotidase activity in cells with increased functional activity of three other parameters studied, and hence, this macrophage phenotype can be regarded as a variant of their alternative activation.

Analysis of functional activity of macrophages and parameters of lipid metabolism showed their simultaneous increase at the late stages of hepatoma growth. Changes in serum level of total cholesterol and nitrite production in macrophages positively correlated on days 14-35 ($r \pm m = 0.98 \pm 0.14$ at $p < 0.01$).

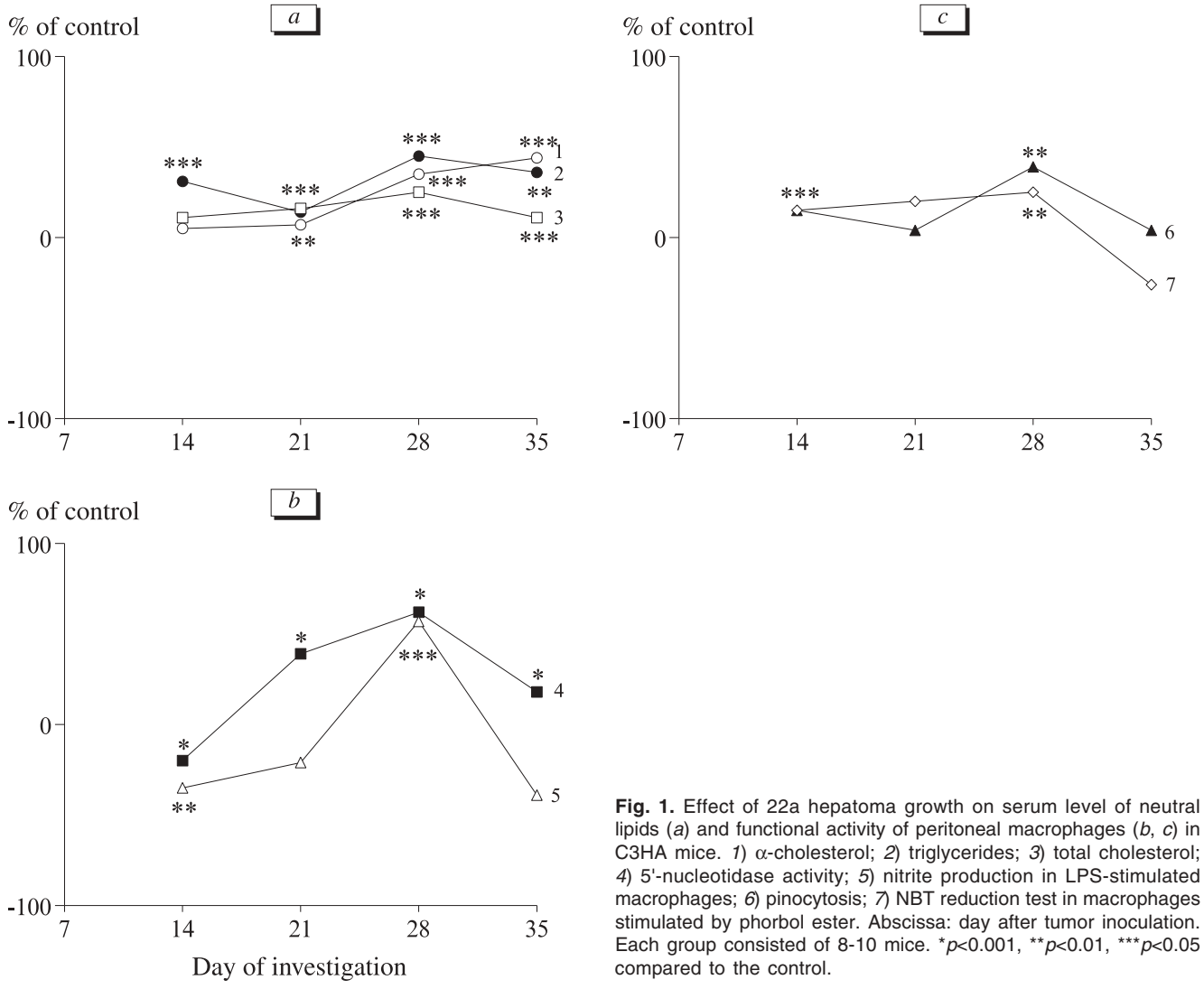

Fig. 1. Effect of 22a hepatoma growth on serum level of neutral lipids (*a*) and functional activity of peritoneal macrophages (*b*, *c*) in C3HA mice. *1*) α-cholesterol; *2*) triglycerides; *3*) total cholesterol; *4*) 5'-nucleotidase activity; *5*) nitrite production in LPS-stimulated macrophages; *6*) pinocytosis; *7*) NBT reduction test in macrophages stimulated by phorbol ester. Abscissa: day after tumor inoculation. Each group consisted of 8-10 mice. *$p<0.001$, **$p<0.01$, ***$p<0.05$ compared to the control.

We previously showed that nitrite production and 5'-nucleotidase activity increased in animals without tumors receiving atherogenic diet [2]. Other authors described increased production of nitroxide anions by cholesterol-loaded neutrophils [9].

Hence, hyperlipoproteinemia developing in animals at the late stages of hepatoma growth can be (similarly as in mice receiving atherogenic diet) a factor modulating production of nitroxide anions and activity of 5'-nucleotidase in macrophages. However, comparison of both experimental models revealed essential differences in the functional phenotype of macrophages: all the studied parameters increased in mice with tumors, while in animals receiving atherogenic diet the production of nitrites and 5'-nucleotidase activity increased against the background of unchanged pinocytosis intensity and NBT reduction rate [2]. Presumably, these changes were caused by other factors influencing macrophages in animals with tumors. For example, the increase of 5'-nucleotidase activity on macrophage surface can depend on the release of cell degradation products, specifically adenosine phosphates into the blood flow [8]. Extracellular adenosine forming by dephosphorylation can stimulate pinocytosis [7] and production of superoxide anions in macrophages [14].

Presumably, the pattern of changes in the functional activity of macrophages during tumor growth is determined by tumor cell products, while hyperlipoproteinemia is just an extra factor.

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