ONCOLOGY

Effect of Metabolic Factors on Apoptosis in Thymocytes during Tumor Growth

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Intensive apoptotic death of thymocytes is a possible mechanism of thymus involution during tumor growth. We studied the role of hypercholesterolemia and lactate acidosis in the induction of increased sensitivity of thymocytes to apoptosis during growth of transplanted hepatoma 22a in mice. Spontaneous apoptosis in thymocytes during tumor growth in mice was studied *in vitro* by acridine orange/ethidium bromide staining and diphenylamine test. Plasma levels of lactate, total cholesterol, α-cholesterol, and triglycerides were measured. A positive correlation was found between intensification of apoptosis (diphenylamine test) and increased concentration of total plasma cholesterol on days 21 and 28 after inoculation of tumor cells. Plasma lactate content did not increase at this term. We hypothesize that hypercholesterolemia accompanying tumor growth acts as a factor increasing thymocyte sensitivity to apoptosis.

Key Words: *thymus; tumors; cholesterol; lactate; apoptosis*

Recent studies indicate that although the weight of the thymus decreases in adult organisms, this organ actively functions and produces T lymphocytes [6]. Normal maturation of thymocytes is very important during tumor growth, chemotherapy, and X-ray therapy. The development of neoplasms in humans and animals is accompanied by involution of the thymus. The mechanisms of these changes remain unclear. Previous studies showed that this process is not associated with increased glucocorticoid concentration. In our previous studies we showed that the growth of hepatoma 22a is associated with intensive intrathymic death of lymphocytes and their increased sensitivity to apoptosis [2]. Similar changes were observed in breast adenocarcinoma in mice [5].

We hypothesized that apoptosis in thymocytes is induced by increased blood lactate concentration. Tumor growth is accompanied by pronounced changes in lipid and carbohydrate metabolism. Tumor cells produce considerable amounts of lactate. Plasma concentrations of lactate considerably increases at the late stage of experimental tumor growth. Lactate *in vitro* intensifies apoptosis in thymocytes [9].

Changes in blood cholesterol concentration also modulate apoptosis. The growth of most tumors is followed by the development of hypercholesterolemia. However, this is not typical of hepatomas synthesizing the required growth substrates and histogenetically different experimental tumors accompanied by hypercholesterolemia [8]. *In vitro* enrichment of thymocyte membranes with cholesterol [5], hyperlipoproteinemia, and *in vivo* increase in thymocyte cholesterol content affect apoptosis in thymocytes [1].

Here we studied changes in the intensity of apoptosis in mouse thymocytes during growth of transplanted hepatoma 22a and evaluated the relationship between this parameter and plasma levels of lactate and lipoproteins.

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MATERIALS AND METHODS

Experiments were performed on C3HA mice weighing 18-20 g and obtained from the Rappolovo nursery (Russian Academy of Medical Sciences). The animals were subcutaneously inoculated with 10^5 syngeneic hepatoma 22a cells. Control mice received an equivalent volume of physiological saline. The mice were killed by cervical dislocation at fixed time intervals after inoculation of tumor cells. Plasma levels of total cholesterol, α-cholesterol, and triglycerides were measured on a Keysys automatic analyzer (Boehringer Mannheim). Lactate concentration was determined spectrophotometrically using Vital Diagnosics kits. The thymuses were weighted, and the mean number of cells per thymus was estimated. Thymocyte suspension was incubated in RPMI-1640 medium containing 10% fetal bovine serum at 37°C for 3 h and stained with acridine orange and ethidium bromide. The ratio of apoptotic cells was determined morphologically under a luminescence microscope [4]. DNA fragmentation was studied in the diphenylamine (DPA) test after 24-h incubation under similar conditions [4].

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

RESULTS

In mice with hepatoma 22a body weight and cellularity of the thymus progressively decreased from the 21st day after inoculation of tumor cells (Fig. 1, *a*). Fluorescent test revealed intensification of spontane-

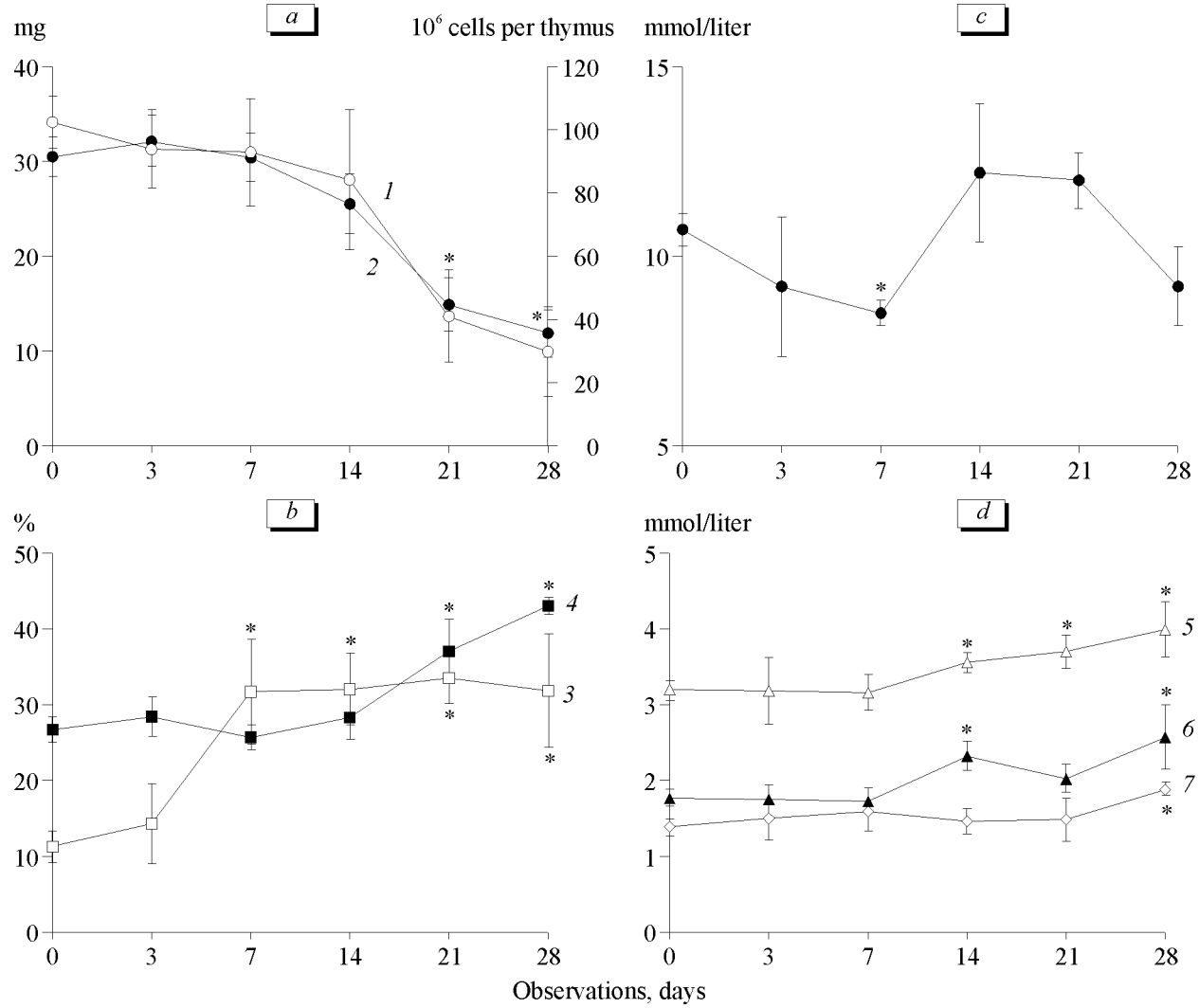

Fig. 1. Effects of hepatoma 22a growth on cellularity of the thymus (*a*), spontaneous apoptosis in thymocytes (*b*), and plasma levels of lactate (*c*) and lipoproteins (*d*). Abscissa: time after inoculation of tumor cells, days; 0, control (injection of physiological saline). Weight of the thymus (*1*); number of cells in the thymus (10^6 cells per thymus, *2*); spontaneous apoptosis, fluorescence study (%, *3*) and DPA test (%, *4*); and contents of total cholesterol (mmol/liter, *5*), triglycerides (mmol/liter, *6*), and α-cholesterol (mmol/liter, *7*). Each experimental group includes 8-10 mice. The control group included 40-50 mice. *$p<0.05$ compared to the control.

ous apoptosis in thymocytes from day 7, *i.e.* 2 weeks before the appearance of morphological changes (Fig. 1, *b*). A less sensitive test with DPA showed that the intensity of apoptosis increased from day 21 (Fig. 1, *b*). Dexamethasone in a concentration of 5 μM served as the standard inductor of apoptosis in thymocytes *in vitro*. Fluorescent and DPA test showed that dexamethasone caused apoptosis in 40-50% cells from control mice.

Plasma lactate level remained unchanged over 4 weeks after inoculation of tumor cells, but increased on day 35 (Fig. 1, *c*). Thus, intensification of apoptosis was not related to the increase in blood lactate concentration.

In tumor-bearing animals the plasma levels of total cholesterol and α-cholesterol increased on days 21 and 28 after inoculation of hepatoma cells, respectively (Fig. 1, *d*). Intensification of apoptosis and involution of the thymus in mice correlated with the increase in plasma cholesterol level. A positive correlation was found between the results of the DPA test and the concentration of total cholesterol (0.93 ± 0.19, $p<0.01$).

It should be noted that intensification of spontaneous apoptosis in thymocytes (judging from fluorescence data) preceded hyperlipidemia. It was probably associated with the increase in blood corticosterone concentration in hepatoma-bearing mice on day 7 after inoculation, which can be regarded as a trigger mechanism of apoptosis [2]. However, at later stages of tumor growth corticosterone level did not differ from the control and did not correlate with signs of thymus involution and increased sensitivity of thymocytes to apoptosis.

Cholesterol is a precursor of glucocorticoid hormones, well-known inductors of apoptosis. Epithelial cells of the thymus express enzymes catalyzing biosynthesis of corticosterone from cholesterol. Coculturing of these cells with thymocytes triggers apoptosis [7]. Theoretically, hypercholesterolemia can promote local activation of glucocorticoid synthesis in the thymus, but does not increase their concentration in the circulation.

Hyperlipoproteinemia can promote the increase in cholesterol content in lymphocyte membranes, which is observed in patients with breast cancer. An impressive body of evidence indicates that incorporation of cholesterol into thymocyte membranes modulates apoptosis.

Our previous studies showed that hyperlipoproteinemia produces opposite effects on apoptosis in thymocytes from non-tumor CBA or C57Bl/6 mice feeding an atherogenic diet [1]. C3HA mice with hepatoma and CBA mice feeding an atherogenic diet have the same H-2^k haplotype. However, these animals differed in parameters of apoptosis. The development of hypercholesterolemia was accompanied by intensification of thymocyte apoptosis in mice with tumors and suppression of this process in animals without tumors. It should be emphasized that in mice feeding an atherogenic diet for 2 months hyperlipoproteinemia was more pronounced than in animals examined 28 days after inoculation of hepatoma cells. In these mice the total plasma cholesterol increased by 2.5 and 1.25 times, respectively.

In vitro experiments showed that changes in thymocyte apoptosis depend on cell saturation with cholesterol [5]. Incubation of thymocytes with exogenous cholesterol in increasing concentrations increased the ratio of apoptotic cells. However, further increase in membrane cholesterol content was followed by suppression of apoptosis. Previous experiments showed that lipoproteins produce a dose-dependent effect on proliferative activity of mouse splenocytes. These data suggest that mild hypercholesterolemia in tumor-bearing animals promotes intensification of apoptosis. However, severe hypercholesterolemia in mice feeding an atherogenic diet suppresses apoptosis in thymocytes.

We revealed a correlation between the total cholesterol level and intensity of apoptosis in thymocytes of tumor-bearing mice. Published data and results of our experiments indicate that hyperlipoproteinemia developed at the late stage of hepatoma growth can modulate apoptosis in thymocytes.

Intensification of apoptosis in thymocytes plays an important role in differentiation of these cells. This process is considered as a possible mechanism of thymus involution during tumor growth [2,3]. In non-tumor mice feeding an atherogenic diet the increased or decreased sensitivity of thymocytes to apoptosis is not associated with involution of the thymus. In these animals the weight of the thymus does not differ from the control [1]. Probably, other metabolites and products of tumor cells play a major role in thymus involution during tumor growth, while hyperlipoproteinemia acts as an additional factor promoting apoptosis in thymocytes.

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