Metabolism of Lymphocytes in Mice with Ehrlich Ascites Carcinoma

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The pattern of metabolic processes in lymphocytes of mice with Ehrlich ascites carcinoma depended on the stage of tumor growth.

Key Words: *lymphocytes; Ehrlich ascites carcinoma; metabolism; enzymes*

Lymphocytes play a major role in organism's response to cancer. Functional activity of immunocompetent cells depends on the intensity of metabolic processes. The tumor affects activity of cells in various tissues, including immunocompetent cells. This effect depends on tumor size (*i.e.*, stage of tumor growth) [2,4,10]. It is interesting to evaluate whether the tumor can modulate the state and metabolic processes in immunocompetent cells.

Here we studied changes in activity of key enzymes of lymphocyte metabolism in mice with Ehrlich ascites carcinoma during tumor growth.

MATERIALS AND METHODS

Experiments were performed on male and female outbred albino mice weighing 20-25 g and aging 2.0-2.5 months. The animals were maintained in a vivarium under standard conditions. Tumor cells (3×10^6) in 0.2 ml physiological saline were inoculated into the abdominal cavity. Before inoculation the cells were washed 3 times by centrifugation in physiological saline to remove ascitic fluid of the donor animal.

Blood lymphocytes were isolated by centrifugation in a Ficoll-Verografin density gradient (ρ=1.087 g/ml) on days 5, 7, 9, 11, 13, and 15 after tumor inoculation. The day of inoculation was considered to be the 1st day of tumor growth. On day 15 the mortality rate of mice was 20%. The control group included intact animals. Lymphocytes were washed with cold physiological saline to remove plasma (repeated centrifugation and resuspension) and the cell suspension obtained after the 3rd centrifugation was frozen. After defrosting the suspension was homogenized [6].

Activities of NAD-dependent isocitrate dehydrogenase (NAD-ICDH), malate dehydrogenase, NADPH-dependent ICDH (NADPH-ICDH), anaerobic lactate dehydrogenase, glucose-6-phosphate dehydrogenase (G-6-PDH), and glycerol-3-phosphate dehydrogenase (G-3-PDH) were measured by the bioluminescent method [8]. Enzyme activity was expressed in enzymatic units per 10,000 cells (1 U=1 μmol/min) [3]. The contents of oxaloacetate, lactate, pyruvate, and NAD^+ were estimated by the bioluminescent method.

Enzyme activity was measured in each experimental sample, which included homogenates of lymphocytes from 20-22 animals. The arithmetic means and errors were calculated and the differences between samples were evaluated using Mann—Whitney test [7].

RESULTS

NAD-ICDH activity in lymphocytes underwent changes during the growth of Ehrlich ascites carcinoma (Fig. 1, *a*). Along with the citrate synthase-catalyzed reaction, the reaction catalyzed by NAD-ICDH is the slowest steps in the tricarboxylic acid cycle (TCA

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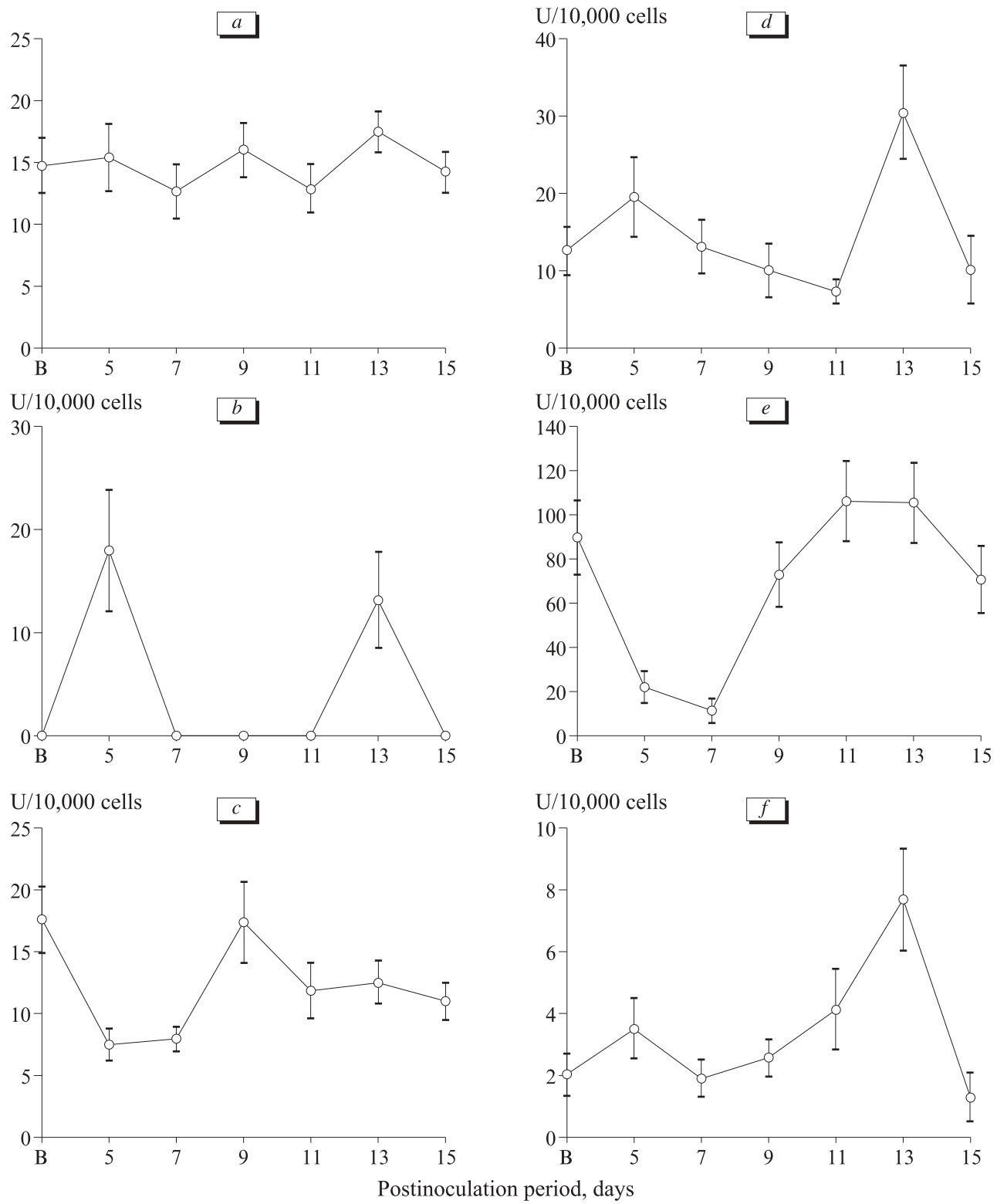

Fig. 1. Activity of key enzymes in lymphocytes of mice with Ehrlich ascites carcinoma: NAD-isocitrate dehydrogenase (*a*), NADPH-isocitrate dehydrogenase (*b*), glucose-6-phosphate dehydrogenase (*c*), malate dehydrogenase (*d*), lactate dehydrogenase (*e*), and glycerol-3-phosphate dehydrogenase (*f*). Ordinate: *a*, thousands U/10,000 cells. Here and in Figs. 2 and 3: B, basal level.

cycle) and can limit the rate of metabolic conversion. Even minor changes in activity of NAD-ICDH can modulate the rate of substrate transformation in the TCA cycle (at least, in this stage). On days 5 and 13 we observed an increase in activity of another TCA enzyme - malate dehydrogenase (Fig. 1, *b*), but the

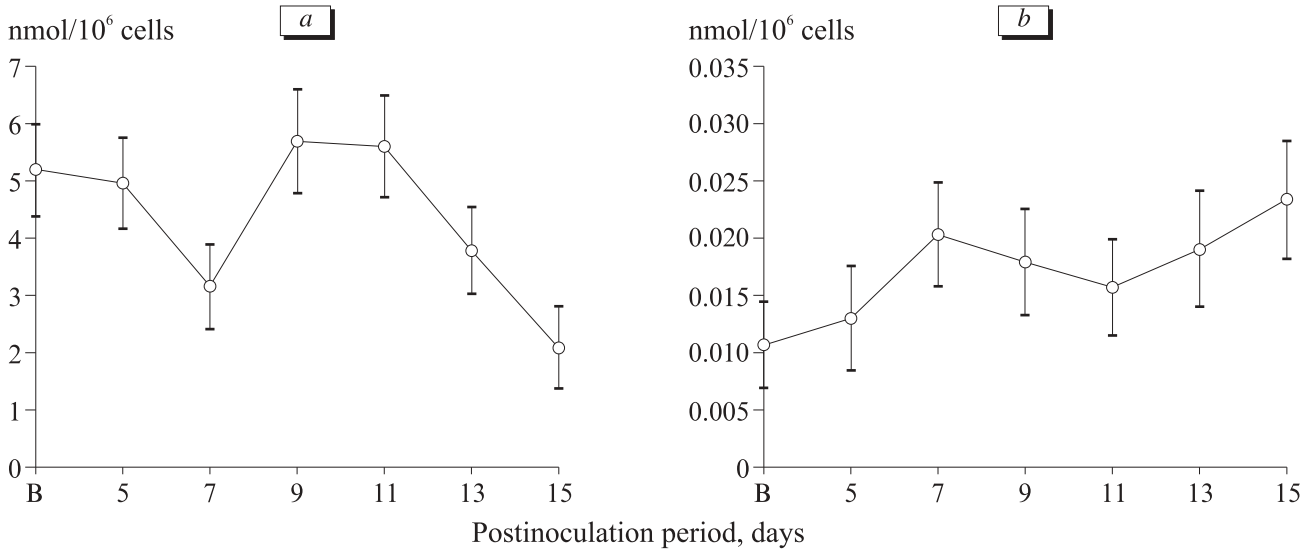

Fig. 2. Contents of lactate (*a*) and NAD^+ (*b*) in lymphocytes of mice with Ehrlich ascites carcinoma.

product of this reaction oxalacetate was not detected by highly sensitive bioluminescence method. Therefore, the rate of late reactions of the CTA cycle is sufficiently high and this product did not accumulate.

NADPH-ICDH is a subsidiary enzyme of the Krebs cycle. Activity of this enzyme is normally low. Our study showed that NADPH-ICDH activity increases on days 5 and 13 (Fig. 1, *c*). These terms correspond to the end of the lag phase of tumor growth and beginning of the terminal period [9] and are critical for the metabolism of immunocompetent cells.

Activity of lactate dehydrogenase catalyzing anaerobic reaction sharply decreased by the 7th day (Fig. 1, *d*), which led to a sharp decrease in lactate content in lymphocytes (Fig. 2, *a*). By day 11 lactate dehydrogenase activity increased, which was paralleled by an increase in lactate concentration. However, the concentration of lactate decreased on day 13 and on day 15 this was attended by a decrease in lactate dehydrogenase activity. The increase in intracellular NAD^+ concentration probably plays a role in the observed decrease in lactate content during the late stage of tumor growth (Fig. 2, *b*). The ratio between intracellular lactate and pyruvate concentrations progressively decreased after inoculation of tumor cells, which reflects activation of cell energy metabolism during tumor growth (Fig. 3).

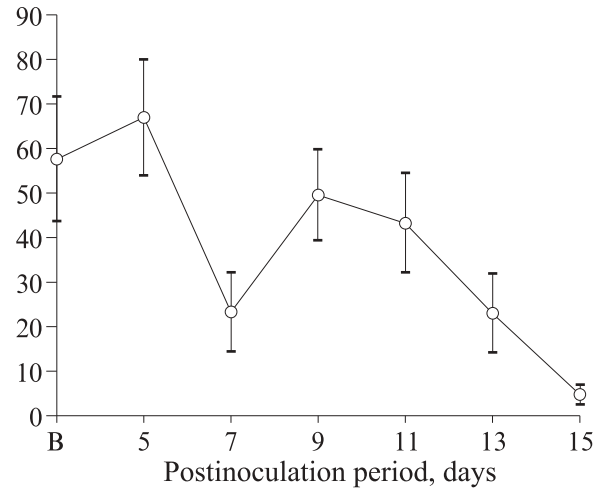

Fig. 3. Lactate/pyruvate ratio in lymphocytes of mice with Ehrlich ascites carcinoma.

Activity of G-3-PDH significantly increased from the 7th to 13th day after inoculation of tumor cells (logarithmic growth phase). This enzyme catalyzes conversion of glycerol-3-phosphate (triacylglycerol precursor) into dihydroxyacetone phosphate, which enters into glycolysis. Therefore, changes in enzyme activity reflect the relationship between lipid and energy metabolism. G-3-PDH serves as the enzyme of the glycerophosphate shuttle. NADPH formed in the cytoplasm cannot enter the mitochondria. The glycerophosphate shuttle transfers hydrogen from cytoplasmic NADPH to the flavin coenzyme CoQ, which transfer electrons accepted together with hydrogen into the respiratory chain [1,5].

The contribution of glycerol-3-phosphate into cell energy metabolism increased on days 7-13, which was probably accompanied by the increase in the efficiency of energy metabolism. Increased G-3-PDH activity contributes to activation of the glycerophosphate shuttle. Proton efflux from the cytoplasm to mitochondria increases under these conditions. It confirms the increase in the intensity of cell energy metabolism. G-3-PDH activity significantly decreased at the terminal stage of tumor growth (day 15).

On day 5 G-6-PDH activity in lymphocytes from treated mice was much lower compared to intact ani-

mals. Enzyme activity increased on day 9, but progressively decreased to the 15th day.

G-6-PDH is an initiating enzyme of the pentose phosphate pathway, which plays a role in the redistribution of glucose from energy metabolism to plastic processes. Enzymes of the pentose phosphate pathway catalyze the formation of considerable amounts of NADPH, which contributes to regeneration of reduced glutathione (major endogenous antioxidant) [11,12]. Ribose-5-phosphate is a product of the pentose phosphate pathway. The inhibition of this pathway probably suppresses nucleotide synthesis in cells, but promotes the increase in the concentration of glucose required for energy metabolism.

Our study shows that metabolic changes in lymphocytes depend on the stage of tumor growth, which is manifested during transition from one phase to another. The observed intracellular changes contribute to activation of the reactions of energy production. Activity of enzymes sharply decreases 15 days after tumor cell inoculation (terminal stage), which probably reflects exhaustion of adaptive reserves in immunocompetent cells.

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