METABOLIC POOL OF PURINE COMPOUNDS IN MOUSE BLOOD ERYTHROCYTES DURING GROWTH OF IMPLANTED TUMORS

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Much attention has recently been paid to the study of purine (and also pyrunidine) metabolism in circulating erythrocytes of animals and man in various pathological states [7].

While performing the function of transport of purine metabolites in the body, erythrocytes constitute an effective system of communication between tissues requiring a supply of free nucleosides and nitrogenous bases for subsequent reutilization in kinase and phosphoribosyl transferase reactions [6].

The development of tumors in vivo is accompanied by significant changes in nuclear metabolism both in the tumor itself and in distant tissues [5]. For example, in immunocompetent cells, in which purine metabolism plays a key role in reactions of differentiation, maturation, and proliferation of lymphocytes [3], profound changes in activity of the enzymes of purine metabolism correspond to different stages of growth of a hepatoma [4].

Recognizing the transport functions of erythrocytes in relation to purine compounds and also various kinds of disturbance of purine metabolism in a tumor-bearing organism, it was considered important to study the character of changes in the pool of purine metabolites in circulating blood erythrocytes during growth of implanted tumors.

EXPERIMENTAL METHOD

Experiments were carried out on male C3HA/Kv and ICR mice. A syngeneic hepatoma 22 was transplanted subcutaneously in the dorsal region of C3HA mice in a dose of $(0.5\text{-}1.0) \cdot 10^6$ cells. An Ebrlich's ascites tumor was implanted intraperitoneally into intact ICR mice [8]. Blood was taken on the 5th, 11th, and 12th days after transplantation of the tumor. The acid-soluble fractions (ASF) of circulating erythrocytes of the animals were obtained with the aid of perchloric acid by the methods described previously [2].

Chromatographic analysis of the composition of the purine compounds of the erythrocytes was carried out by reverse--phase ion-pair high-performance liquid chromatography (HPLC) under gradient [9] and isocratic [11] conditions of elution.

EXPERIMENTAL RESULTS

During analysis of the material thus obtained, accumulation of phosphorylated forms of adenosine and guanosine was noted in the ASF of the circulating erythrocytes of animals both with Ehrlich' s ascites tumor (Table 1) and with solid hepatoma 22 (Table 2). An increase in the concentration of purine nucleotides was observed in both exponential (5th day) and stationary (11th and 12th days) stages of tumor growth. The dynamics of distributions of purine mono-, di-, and triphosphates in the erythrocytes of mice with hepatoma and, in particular, mice with Ehrlich's carcinoma, is evidence of activation of kinase reactions involved in their biosynthesis. For instance, on the 5th day of growth of Ehrlich's ascites tumor the AMP level rose

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TABLE 1. Concentrations of Purine Compounds in Erythrocytes of ICR Mice with Transplated Ehrlich's Ascites Tumor (nmoles/ml, $M \pm m$)

Compound	Control (n = 3)	Time after inoculation of tumor, days	
		5 (n=5)	12 (n=4)
ATP	1278,4±186,2	2017,0±163,8	2020,8±110,4
ADP	149,0±4,5	165,2±15,1	421,8±76,2
AMP	20,5±1,2	62,2±9,3	174,2±62,2
GTP	126,2±15,1	158,2±21,3	485,9±85,4
GDP	18,5±4,7	28,3±3,5	44,7±4,9
GMP+IMP	14,1±1,5	22,0±6,2	92,8±17,9
Ade	0,8±0,1	0,7±0,1	1,5±0,3
Ado	0,8±0,2	0,8±0,1	6,1±1,9
Ino	2,2±0,3	0,6±0,1	2,6±1,4

TABLE 2. Concentration of Purine Compounds in Erythrocytes of C3WA Mice with Transplanted Solid Hepatoma 22 (nmoles/ml; $M \pm m$)

Compound	Control (n = 3)	Time after inoculation of tumor, days	
		5 (n=3)	12 (n=4)
ATP	632,6±87,4	686,8±92,8	773,9±81,4
ADP	105,9±11,4	90,2±10,4	105,9±11,4
AMP	15,5±2,2	19,5±2,6	25,6±5,1
GTP	37,6±5,4	62,2±8,8	43,6±6,2
GDP	17,0±2,9	22,3±4,4	19,3±3,1
GMP	3,8±0,5	2,7±0,4	2,9±0,3
Ade	1,6±0,2	—	2,7±0,3
Ado	1,0±0,3	0,2±0,01	—
Hyp	2,8±0,4	—	2,1±0,4
Ino	2,6±0,4	0,4±0,05	2,2±0,5

threefold, and on the 12th day it rose eightfold. The ATP concentration under these circumstances was increased 1.5-fold (Table 1). GMP and IMP levels rose by 1.5 and 5 times respectively, whereas GTP level was 3.8 times higher on the 12th day.

A similar tendency was noted in blood erythrocytes of animals with a solid hepatoma (Table 2). In the exponential phase of growth of the hepatoma, for instance, the concentrations of adenylate, GTP and GDP were raised, but the GMP level was lowered. By the 11th day, the relative concentrations of purine nucleotides in the erythrocytes remained the same.

The concentration of purine nucleotides in reticulocytes is known to be significantly higher than in mature blood cells [12]. According to our data, during development of Ehrlich's ascites tumor a reticulocytosis develops (Fig. 1), but its quantitative characteristics cannot explain the significant rise in the concentrations of adenine and guanine nucleotides in the erythrocytes fraction of the blood in the tumor-bearing animal.

The following changes were found in the metabolic pool of purine nucleotides and nitrogenous bases. The exponential phase of growth of both tumors is accompanied by a fall of the purine level in circulating erythrocytes of the animals, which was particularly marked for solid hepatoma 22 (Tables 1 and 2). For instance, the bases hypoxanthine and adenine disappeared from the composition of ASF of the erythrocytes by the 5th day, whereas the concentration of adenosine and inosine nucleosides was reduced fivefold (Table 2). In the stationary phase of growth, levels of purine bases and nucleosides in the erythrocytes were restored. This time course can be explained by active reutilization of these compounds from circulating erythrocytes by proliferating tumor cells, for we know that activity of the "salvage" enzymes of purine nucleotide synthesis rises sharply in rapidly growing hepatomas [10].

This investigation thus revealed accumulation of purine nucleotides in circulating erythrocytes of a tumor-bearing animal at different stages of development of Ehrlich's ascites carcinoma and of solid hepatoma 22. This may be connected with an increase in the unique "buffer capacity" of the erythrocytes or purine compounds during progression of a neoplasm. Protective mechanisms for "retention" of purine compounds in erythrocytes are triggered by accumulation of phosphorylated forms of nucleosides, which are known not to pass through the plasma membrane. This hypothesis is supported by exhaustion of the nucleosides and nitrogenous bases of the erythrocytes (particularly in mice with solid hepatoma 22) in the exponential phase of

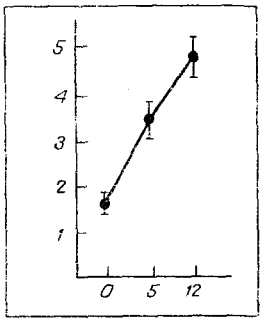

Fig. 1. Reticulocyte count (in percent, ordinate) in blood of ICR mice at different times of growth (in days, abscissa) of Ehrlich's ascites tumor.

tumor growth, possibly due to acite scavenging of precursors of nucleic acids from the blood erythrocytes of the animals not only by tumor cells, but also by other cells of the body, including immunocompetent cells, in which abrupt changes of purine metabolism take place in the early stages of growth of a hepatoma [1].

Consequently, the idea of the transport role of erythrocytes, supplying purine compounds to various organs and tissues of the body, demands more accurate investigations aimed at studying the mechanisms of this phenomenon, especially under experimental and clinical pathological conditions.

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