ONCOLOGY

Cystatin C and Cysteine Proteinases during the Development and Therapy of Lewis Lung Adenocarcinoma in Mice

T. A. Usova, O. N. Poteryaeva, S. Ya. Zhanayeva, E. S. Yarygina, and T. A. Korolenko

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We measured plasma cystatin C concentration and activity of cathepsins B and L in tumor tissue as possible markers for the efficiency of antitumor therapy and prognostic criteria for Lewis lung adenocarcinoma in mice. Plasma cystatin C concentration markedly decreased in mice with tumors. During successive therapy the increase in plasma cystatin C concentration correlated with the degree of inhibition of tumor growth. Activities of cathepsins B and L in the liver increased in animals with tumors. In mice receiving successive antitumor therapy activities of cathepsins B and L increased in tumor tissue, but decreased in the liver (compared to untreated animals).

Key Words: *cystatin C; cathepsins B and L; Lewis lung adenocarcinoma*

Cystatin C an endogenous inhibitor of cysteine proteinases [9] synthesized in most cells of the body and present in high concentrations in biological fluids (blood, liquor, and semen) [2]. The imbalance between the contents of cystatin C and lysosomal cysteine proteinases (cathepsin B) accompanies various human diseases, including malignant neoplasms, multiple sclerosis, and AIDS [3]. Cystatin C concentration in human extracellular fluids can serve as a diagnostic and prognostic criterion. Changes in the content of this inhibitor during the therapy reflect its efficiency [4,9]. A positive correlation was found between high concentration of cysteine proteinase inhibitors in the plasma and severe course of human tumors, metastatic potential, risk of recurrences, and lifetime decrease (*e.g.*, for colorectal cancer [8] and melanoma [10]).

Our previous experiments showed that the development of mouse lymphosarcoma LS and hepatoma HA-1 is accompanied by a decrease in plasma cystatin C concentration and imbalance between the contents of cysteine proteinases and their inhibitors in tumor tissue [1,7].

Here we studied changes in plasma cystatin C concentration during the development of Lewis lung adenocarcinoma (LLA) and evaluated whether its content correlates with the efficiency of various schemes for treatment.

MATERIALS AND METHODS

Experiments were performed on male $(CBA \times C57Bl/6)F_1$ mice with LLA. Tumor cells were transplanted into right thigh muscles (1.6×10^6 cells). On day 10 after tumor transplantation some mice were intraperitoneally injected with cyclophosphamide (CP) in a single dose of 150 mg/kg (Konpo). Other mice received 150 mg/kg CP and one of the following glycans (25 mg/kg, Institute of Chemistry, Slovak Academy of Sciences, Bratislava): β-D-carboxymethylglucan (CMG), sulfoethylated β-D-glycan (SE-glycan), and sulfoethylated β-D-chitin-glycan (SE-chitin-glycan).

Institute of Physiology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

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On days 2, 7, and 10 after the therapy some mice were killed by cervical dislocation. We measured body weight and weight of the tumor. The blood, liver, spleen, and tumor tissue were sampled. Cathepsin B activity was measured using the fluorescence substrate Z-Arg-Arg-NMCA (Vektor) [1,7]. Cathepsin L activity was estimated with the substrate Z-Phe-Arg-NMCA (Vektor). The selective cathepsin B inhibitor CA-074 (Prof. Katunuma, Japan) was added to the incubation mixture. Cystatin C concentration in the plasma was determined by sandwich enzyme immunoassay using KRKA kit. The results were analyzed by Student's *t* test. The correlation coefficients were determined (*r*). The differences were significant at $p<0.05$.

TABLE 1. Tumor Weight and Plasma Cystatin C Concentration in Mice with LLA Receiving Various Schemes of Treatment on Day 17 after Tumor Transplantation (*M*±*m*)

Group (*n*=7)	Tumor weight, g	Tumor growth inhibition, %	Cystatin C, nmol/liter
Untreated	2.70±0.31	—	7.10±1.95
CP	1.70±0.29*	37	16.9±2.3*
CP+CMG	1.30±0.24*	52	18.60±2.69*
CP+SE-glycan	1.10±0.32*	59	19.40±2.48*
CP+SE-chitin-glycan	2.20±0.16*	19	11.90±3.21

Note. *$p<0.05$ compared to untreated mice.

RESULTS

The weight of tumors increased to 1.70±0.08, 2.70±0.31, and 6.10±0.24 g on days 12, 17, and 21, respectively (Table 1). On day 12 after transplantation of LLA the concentration of plasma cystatin C in untreated mice was much lower than in intact animals (Table 2). This

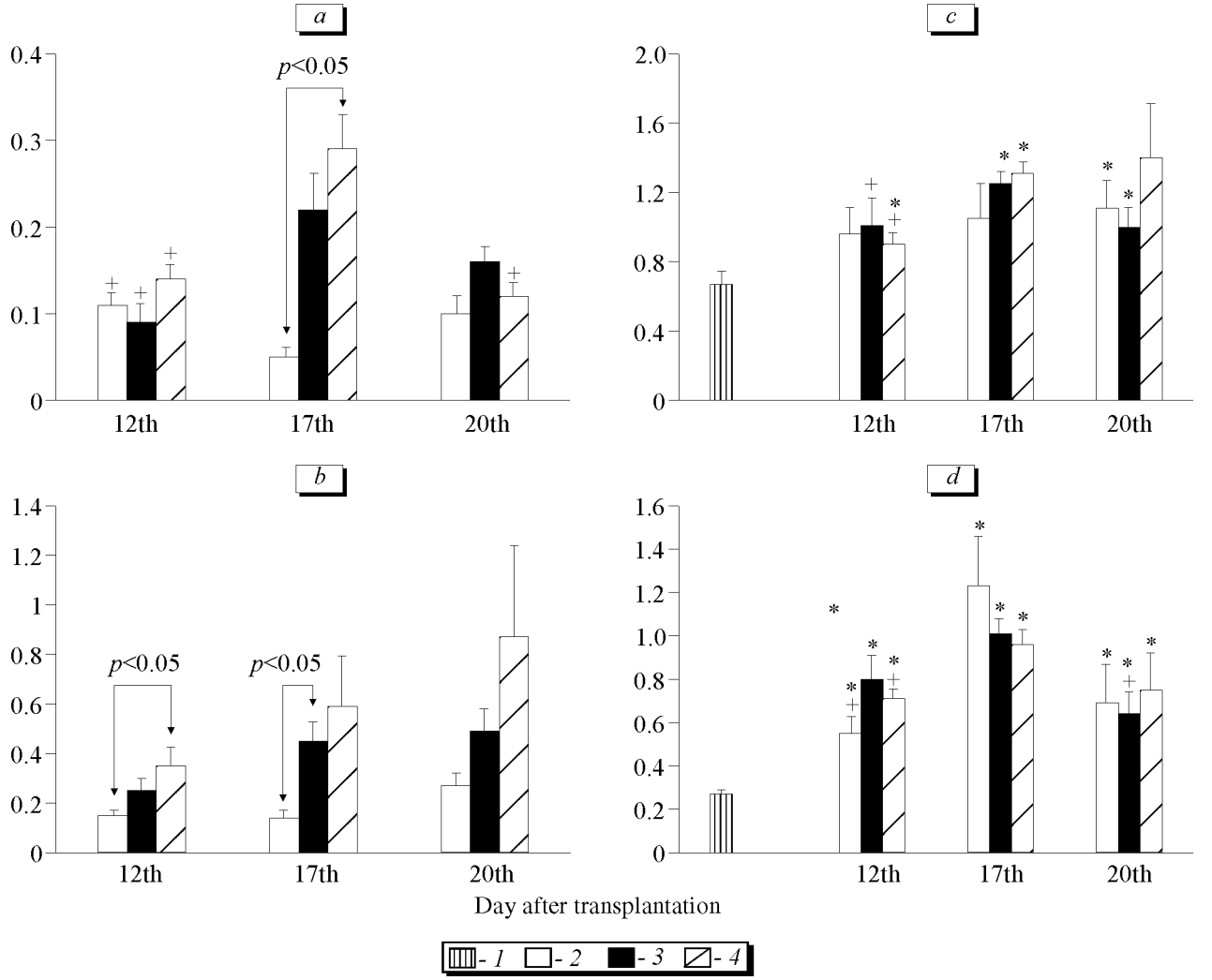

Fig. 1. Activities of cathepsins B (*a*, *c*) and L (*b*, *d*) in tumor tissue (*a*, *b*) and liver (*c*, *d*) from mice with Lewis lung adenocarcinoma. Ordinate: nmol monoclonal antibodies/mg protein/min. Here and on Fig 2: intact mice (*1*), untreated animals (*2*), and mice receiving cyclophosphamide alone (*3*) or in combination with β-D-carboxymethylglycan (*4*). *$p<0.05$ compared to intact mice; $^{+}p<0.05$ compared to day 17.

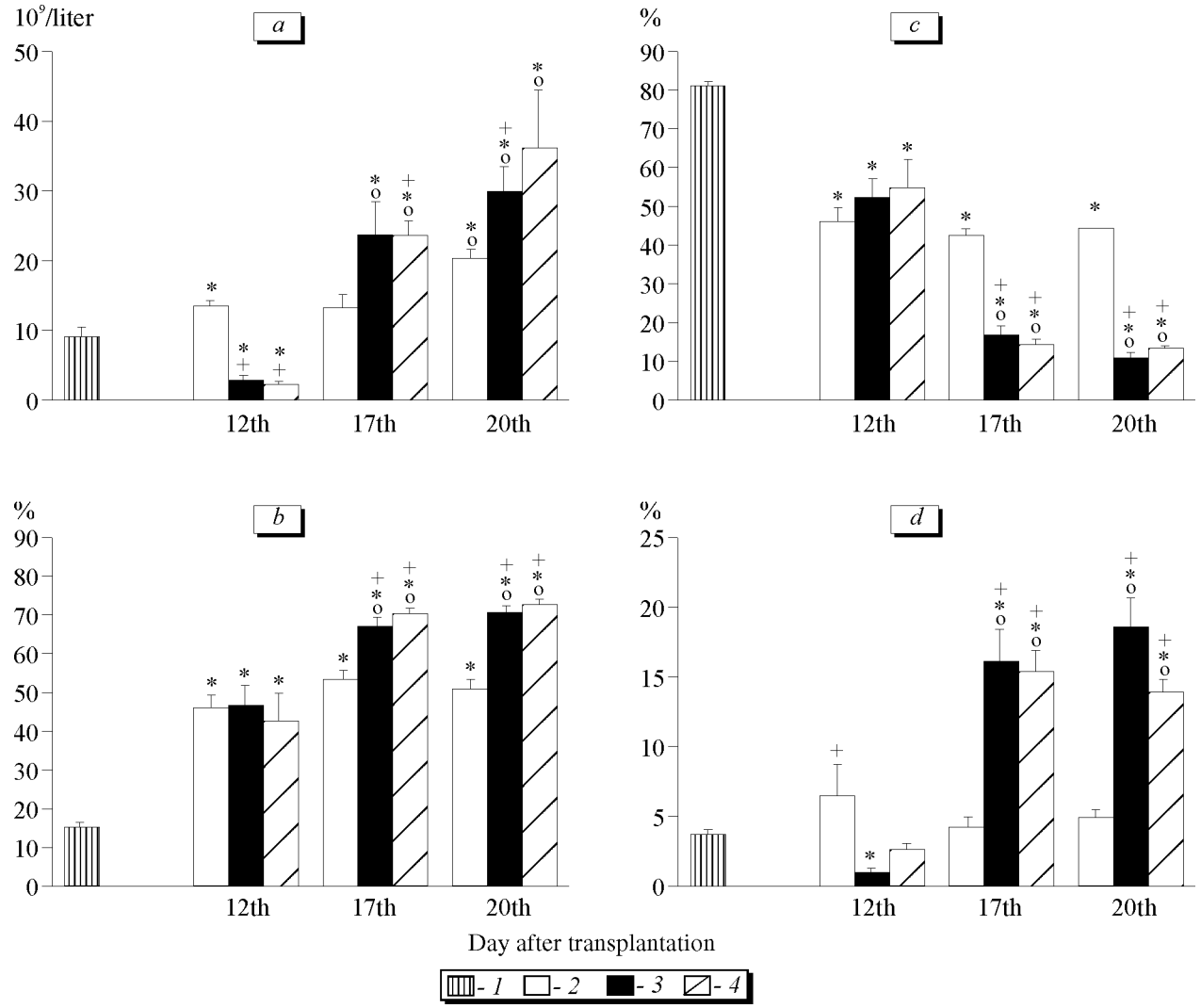

Fig. 2. Leukocyte count (*a*) and ratios of polymorphonuclear leukocytes (*b*), lymphocytes (*c*), and monocytes (*d*) in the blood from mice with Lewis lung adenocarcinoma. **p*<0.05 compared to intact mice; +*p*<0.05 compared to untreated animals; °*p*<0.05 compared to day 12.

was probably associated with exhaustion of reserves of the endogenous inhibitor due to intensive secretion of proteinases by developing tumors. On day 17 plasma cystatin C concentration slightly increased, but remained below the normal. The content of this substance decreased 20 days after LLA transplantation (Table 2). Previous studies showed that the development of some malignant tumors in humans is accompanied by an increase in plasma cystatin C concentration [5]. On days 12, 17, and 20 cathepsin B activity in tumor tissue markedly decreased compared to that in the liver of intact animals (Fig. 1, *a*, *c*). The decrease in cathepsin L activity was less pronounced (Fig. 1, *b*, *d*). Activities of cathepsins B and L in the liver increased in animals with LLA (Fig. 1, *c*, *d*). Cathepsin L activity in tumor tissue tended to increase at various stages of the disease (Fig. 1, *b*). In the blood of mice with tumors the total leukocyte count (Fig. 2, *a*) and the percentage of polymorphonuclear leukocytes (Fig. 2, *b*) and monocytes increased (Fig. 2, *d*), while the percentage of lymphocytes decreased compared to intact animals (Fig. 2, *c*).

In mice receiving CP monotherapy the weight of tumors decreased by 37% on day 17 after transplantation (Table 1). CMG potentiated the effect of CP and suppressed tumor growth by 52%. Combination treatment with CP and SE-glycan produced a most pronounced effect and decreased tumor weight by 59%. However, SE-chitin-glycan attenuated the effect of CP and decreased tumor weight only by 19% (Table 1).

CP increased plasma cystatin C level (particularly on day 12, Table 2). Combination treatment with CP and CMG produced a more significant increase in plasma cystatin C concentration. The concentration of this inhibitor increased most significantly in mice receiving CP and SE-glycan (Table 1). SE-chitin-glycan had no positive effect and only slightly increased plasma cystatin C concentration (Table 1). A positive

TABLE 2. Plasma Cystatin C Concentration in Mice during the Development and Therapy of Tumors (*M*±*m*)

Group (*n*=7), day after transplantation		Cystatin C, nmol/liter
Intact		23.70±0.92
Untreated	12	7.10±1.95*
	17	12.20±0.82*++
	20	7.40±0.48*++
CP	12	21.70±4.85+
	17	15.6±1.8*
	20	15.60±3.16+
CP+CMG	17	22.3±5.9

Note. *$p<0.05$ compared to intact mice; +$p<0.05$ compared to untreated animals at the same term; ++$p<0.05$ compared to the previous term.

correlation was found between the increase in plasma cystatin C concentration and degree of tumor growth inhibition in animals treated by various schemes (r=0.97).

Cathepsin B activity in tumor tissue tended to increase in mice receiving CP. Combination treatment with CP and CMG considerably increased cathepsin B activity on day 17 (Fig. 1, *a*). Cathepsin L activity tended to increase in tumor tissue. In mice receiving CP alone and in combination with CMG cathepsin L activity significantly increased on days 17 and 12, respectively, compared to untreated animals (Fig. 1, *b*).

CP sharply decreased leukocyte count (Fig. 2, *a*) and ratio of monocytes (Fig. 2, *d*) on day 12 after tumor transplantation. In mice receiving CP the count of leukocytes (Fig. 2, *a*) and ratios of polymorphonuclear leukocytes (Fig. 2, *b*) and monocytes increased (Fig. 2, *d*), while the percentage of lymphocytes decreased on days 17 and 20 (Fig. 2, *c*, compared to intact and untreated animals).

Our results indicate that plasma concentration of cysteine proteinase inhibitor cystatin C and, to a lesser extent, activities of cathepsins B and L in tumor tissue can be used as prognostic criteria for tumor growth and markers for the efficiency of antitumor treatment. Macrophage activators glycans produce a strong therapeutic effect on mice with LLA and potentiate the influence of CP, which depends on the type and structure of these compounds.

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