ONCOLOGY

Plasma Activity of Proteolytic Enzymes and Their Inhibitors in Mice with Neoplasms

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In BALB/c mice with syngeneic mastocytoma P-815, plasma activity of trypsin-like proteinases and the ratio of α_1-proteinase inhibitor activity to α_2-macroglobulin plasma level increased in comparison with the same parameters in noninbred mice with allogenic Ehrlich carcinoma. It is suggested that activation of the proteinase-inhibitor system is essential for *in vivo* tumor growth.

Key Words: *mastocytoma P-815; Ehrlich carcinoma; proteinases; α_1-proteinase inhibitor; α_2-macroglobulin*

Increased activity of proteolytic enzymes is the hallmark of tumor cell metabolism [4,10]. It is known that activation of collagenase and cysteine proteinases is critical for tumor dissemination and correlates with its malignancy. Activation of plasma proteinase is accompanied by an increase in plasma inhibitor activity [8]. However, the involvement of inhibitors in carcinogenesis is much broader than restriction of plasma proteinase activity *per se.*

It is established that α_1-proteinase inhibitor (PI) located near the tumor prevents the contact between tumor and immunocompetent cells and suppresses T killers [8]. α_2-Macroglobulin (MG) interacts with factors controlling different phases of the immune response and exhibits cytotoxicity against tumor cells [9].

We suggested that the use of syngeneic and allogenic transplantable tumors is a promising approach to studying the role of proteolysis in carcinogenesis.

This study was aimed at investigation of plasma activity of trypsin-like and elastase-like proteinases, α_1-PI, and α_2-MG in mice with transplanted mastocytoma P-815 or Ehrlich carcinoma.

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MATERIALS AND METHODS

Experiments were carried out on 90 male BALB/c and noninbred mice weighing 18-20 g. The animals were divided into 4 groups: group 1 comprised intact noninbred mice (n=10); group 2 consisted of noninbred mice with transplantable Ehrlich carcinoma (n=35); groups 3 and 4 comprised intact (n=10) and mastocytoma P-815-bearing BALB/c mice (n=35).

Mastocytoma P-815 cells (2×10^6) were inoculated to BALB/c mice and Ehrlich carcinoma cells (7×10^6) were injected intraperitoneally to non-inbred mice [7]. Mastocytoma P-815 is syngeneic for BALB/c mice by the H^{2D} histocompatibility locus; Ehrlich carcinoma is used as an allogenic tumor for noninbred mice. Carcinogenesis and the effect of antitumor drugs are usually studied on neoplastic cells at the stage of established tumor that corresponds to days 14 and 7 after transplantation of mastocytoma P-815 and Ehrlich carcinoma, respectively. At these time-points, the volume of ascitic fluid was 9 ml for both tumors. To evaluated the role of the proteinase-inhibitor system, the tumors were studied before they attained this stage (the volumes of ascitic fluid were also equal). Proteolysis in the plasma of BALB/c mice with mastocytoma P-815 or noninbred mice with Ehrlich carcinoma was assayed

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on days 7, 8, 10, and 14 and on days 1, 2, 5, and 8 after transplantation. respectively. At these time-points, the volume of ascitic fluid was 3, 5, 7, and 9 ml, respectively for both groups.

Activity of trypsin (EC 3.4.21.4) and elastase (EC 3.4.23.11) in mouse plasma was measured using N-benzoyl-L-arginine paranitroanilide and N-butoxycarbonyl-L-alanine nitrophenyl ester (BANE), respectively. To measure trypsin activity, 4 ml 0.02% N-benzoyl-L-arginine paranitroanilide in 0.2 M Tris-HCl (pH 7.8) was added to 0.5 ml citrate plasma diluted 2-fold with normal saline. The mixture was incubated at 37°C for 30 min, then 1 ml 30% acetic acid was added, and optical density was measured at 410 nm on an SF-410 spectrophotometer [2]. To measure elastase activity, 2.8 ml 0.05 M sodium phosphate buffer (pH 6.5) was added to 0.1 ml plasma containing 2 mg protein, incubated for 5 min, and 0.1 ml 0.01 M BANE acetonitrille was added. An increment in optical density at 347.5 nm was measured during 1 min [6]. Protein content was measured by the biuret micromethod.

Activity of plasma inhibitors was measured by the inhibition of hydrolysis of N-benzoyl-L-arginine ethyl ester [5]. To determine α_1-PI activity, two samples were prepared: the first contained 1.9 ml 0.05 M Tris-HCl (pH 8.0), 0.1 ml 0.01% trypsin in 1 mM HCl containing 10 mM $CaCl_2$; the second contained 1.8 ml 0.05 M Tris-HCl, 0.1 ml 0.01% trypsin, and 0.1 ml plasma diluted 50-fold with normal saline. The samples were incubated at 25°C for 5 min, then 1 ml 1.5 mM N-benzoyl-L-arginine ethyl ester was added, and increment of optical density (253 nm) per minute was measured for 5 min. Activity was calculated by the formula: $(V_1-V_2)\times 1365$=U/ml, where V_1 and V_2 are the increment in optical density per minute for the first and second samples, respectively. To determine α_2-MG activity, 0.05 ml 0.1% trypsin was added to 1.75 ml 0.05 M Tris-HCl (pH 8.0), the mixture was incubated at room temperature for 5 min, then 0.1 ml 0.3% soybean trypsin inhibitor was added. Five minutes later, 1 ml 1.5 mM N-benzoyl-L-arginine ethyl ester was added, and optical density at 253 nm was measured for 10 min. Activity was calculated by the formula $D_{253}\times 27.3$=U/ml, where D_{253} is 10-min increment in optical density. The data were processed statistically using Student's *t* test and nonparametric Mann—Whitney test.

RESULTS

In BALB/c mice, elastase activity was increased in comparison with noninbred mice (Table 1). It is known that increased activity of the coagulation system and plasma kallikrein is also characteristic of this mouse strain [1].

TABLE 1. Plasma Activity of Trypsin, Elastase, and Inhibitors in Intact BALB/c and Noninbred Mice (*M±m*, *n*=10)

Parameters	Mice	
	noninbred	BALB/c
Trypsin, mU/ml	2.6±0.3	2.5±0.3
Elastase, pmol BANE/min/mg protein	608.0±42.0*	1113.0±69.0*
α_1-PI, U/ml	23.8±5.6	22.8±5.5
α_2-MG, U/ml	3.2±0.4	2.7±0.4

Note. *$p<0.05$ compared with noninbred mice.

In inbred mice, activity of elastase-like enzymes increased; in BALB/c mice with mastocytoma P-815 a considerable activation of proteolysis was noted (Table 2). In the plasma of tumor-bearing mice, trypsin-like proteinase activity increased and on days 8 and 10 it 1.9-fold surpassed the control. No significant changes in plasma activity of trypsin- and elastase-like enzymes accompanied the growth of allogenic Ehrlich carcinoma in noninbred mice.

The role of proteolysis in the tumor growth is primarily associated with the involvement of proteinases in tumor invasion and dissemination. Considerable attention is drawn to the interaction between proteinases and extracellular matrix, secretion of hydrolytic enzymes by cells (or induction of this secretion), and local degradation of the matrix. Local lysis of the matrix occurs at the site closely adjacent to the tumor cell, where the concentration of active enzyme surpasses the content of inhibitors [10,11].

Increased plasma activity of elastase-like enzymes in intact mice and trypsin-like enzymes during the development of mastocytoma P-815 can be attributed to significant activation of proteolysis and metastatic potential of P-815 mastocytoma compared to Ehrlich carcinoma; it is obviously a major determinant in the development of irreversible catabolic reactions in the body caused by the tumor.

When evaluating proteolytic activity, it is important to consider inhibitors. In BALB/c mice with mastocytoma P-815, increased activity of α_1-PI and inhibition of α_2-MG were observed in parallel with the tumor growth. In noninbred mice, α_1-PI activity was also increased by day 7 of allogenic Ehrlich tumor growth, but α_2-MG activity remained practically unchanged. The α_1-PI/α_2-MG activity ratio in BALB/c mice with mastocytoma P-815 and noninbred mice with Ehrlich carcinoma at the sage of established tumor increased 3- and 1.3-fold, respectively, in comparison with the control (Table 2). Increased α_1-PI activity is probably a response to activation of plasma proteolysis typical of metastasizing tumors. Low activity of α_2-MG

TABLE 2. Plasma Activity of Trypsin and Elastase and α_1-PI/α_2-MG Ratio in BALB/c and Noninbred Mice ($M\pm m$)

Mice		Trypsin, mU/ml	Elastase, pmol BANE/min/mg protein	α_1-PI/α_2-MG
BALB/c				
	control	2.5±0.3	111.3±6.9	8.4±0.9
	day 7 (n=9)	3.2±0.4*	78.5±7.5*	13.0±0.8*
	day 8 (n=9)	4.7±0.2**	115.7±0.8	13.2±0.9*
	day 10 (n=9)	4.8±0.3**	120.0±3.6	19.7±1.2**
	day 14 (n=8)	2.2±0.2	118.2±7.8	25.4±1.3**
Noninbred				
	control	2.6±0.3	60.8±4.2	7.5±0.5
	day 1 (n=9)	2.2±0.1*	60.5±4.2	9.1±0.7
	day 2 (n=8)	2.7±0.3	63.0±2.2	9.3±0.8
	day 5 (n=9)	2.7±0.3	71.3±1.7	9.5±0.9*
	day 7 (n=9)	1.9±0.3*	72.3±1.7*	8.6±0.5

Note. *p<0.05, **p<0.001 compared with the control.

possessing a cytotoxic activity promoted tumor growth and contributes to its malignancy. A high the α_1-PI/α_2-MG ratio in the plasma of BALB/c mice with mastocytoma P-815 attests to more profound alterations in the proteolysis system in comparison with noninbred mice, which promote tumor growth in recipients.

Thus, it can be suggested that proteolysis plays an important role in the tumor growth. Increased elastase-like proteinase activity in BALB/c in comparison with noninbred mice, significant activation of trypsin-like proteinases, and increased α_1-PI/α_2-MG ratio in the plasma of tumor-bearing mice can promote tumor growth *in vivo*. Our findings suggest that in evaluating the state of proteolysis, it is important to determine activity of both enzymes (in allogenic growth they remain practically unchanged) and PI. The complex assay of proteinase and PI activity can be used for evaluating both the state of proteolysis in tumor development and the effect of anticancer drugs. These data should be considered in selecting experimental animals for biomedical experiments.

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