ONCOLOGY

Expression of Surface Lymphocyte Markers in Various Courses of Gastrointestinal Tumors

E. K. Oleinik, M. I. Shibaev, and V. M. Oleinik

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 4, pp. 437-440, April, 2004
Original article submitted June 27, 2003

Expression of activation antigens increased in patients with gastrointestinal tumors of various clinical courses. The count of $CD25^+$ and $CD71^+$ lymphocytes was high in patients with favorable course of non-progressive tumors. The increase in the expression of activation markers probably reflects adequate functional activity of the immune system in patients with tumors.

Key Words: *gastrointestinal tumors; lymphocytes; activation markers*

The relationship between the tumor and immune system is an urgent problem of clinical immunology. It is absolutely clear that the immune system reacts to tumor growth. However, most immunological assays started only from assumptions. There is no agreement regarding changes in immunological reactivity during tumor growth. Most likely the immune system reacts to tumor antigens. This reaction is most pronounced in the initial stage of tumor growth. The capacity of the immune system decreases with tumor progression, which is associated with survival and division of most malignant cells. These cells have specific mechanisms allowing them to escape from immunological recognition. Functional activity of the immune system in patients with tumors can be determined by activation of peripheral blood lymphocytes. It is interesting to evaluate the role of these changes in the course and outcome of tumors.

Tumors of the gastrointestinal tract (GIT) constitute the major part of oncological diseases [2]. Stomach cancer (SC) and colorectal cancer (CC) are most common malignant neoplasms in Russia. The rates of morbidity and mortality differ insignificantly in patients with SC and CC. The data illustrate unfavorable course and unsuccessful therapy of these tumors. The main criteria for favorable prognosis of the disease can be evaluated by studying functional activity of the immune system in patients with various courses of tumors.

Here we studied expression of surface lymphocyte markers in patients with various courses of GIT tumors and evaluated informative immunological criteria for favorable prognosis of the disease.

MATERIALS AND METHODS

We examined peripheral blood lymphocytes from 81 patients with GIT tumors (66 patients with SC and 15 patients with CC) and 38 healthy donors. Mononuclear cells were isolated from heparinized venous blood in a Ficoll-Verografin density gradient (1.077 g/ml). The blood was taken during primary examination before the start of therapy. Lymphocyte subpopulations were assayed by indirect immunofluorescence with monoclonal LT antibodies (Institute of Immunology, Russian Ministry of Health; Sorbent TM) against CD3, CD4, CD8, CD16, CD19, CD25, CD45, CD71, CD95, CD98, and HLA-DR [1]. The stage of the disease and the type of tumors were estimated after surgery and histological study of samples.

Patients with GIT tumors discharged from hospital by the end of treatment were examined at 3-month

Institute of Biology, Karelian Research Center, Russian Academy of Sciences, Petrozavodsk. ***Address for correspondence:*** mshib@karelia.ru. Shibaev M. I.

0007-4888/04/1374-0385

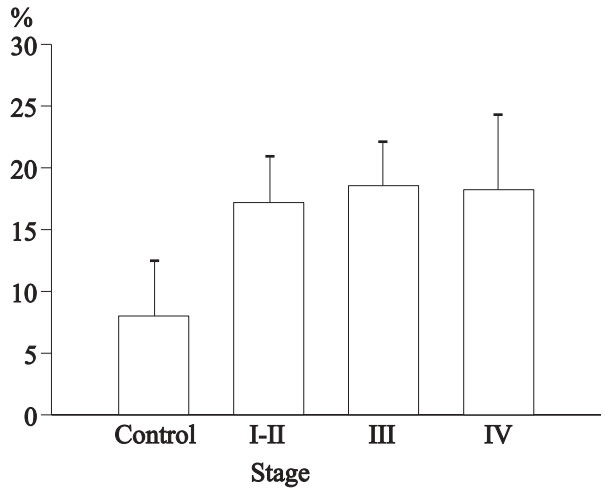

Fig. 1. CD95 expression in various stages of stomach cancer.

(1st year) and 6-month intervals (2nd and 3rd year). The process was stabilized in 25 patients with SC and 7 patients with CC. The disease progressed in 37 patients with SC and 7 patients with CC.

The results were analyzed by Student's t test.

RESULTS

We estimated the relative number of lymphocytes belonging to various subpopulations. The count of peripheral blood $CD16^+$ cells in patients with GIT tumors surpassed the normal ($p<0.05$). The number of CD4 and CD8 cells remained unchanged. However, the ratio of $CD19^+$ lymphocytes in patients with tumors was much higher than in healthy donors (Table 1). Similar changes were observed in patients with other malignant tumors [5,7].

The count of lymphocytes carrying activation markers underwent pronounced changes in patients with tumors (Table 1). In these patients expression of CD25 and CD71 on peripheral blood cells markedly surpassed the control ($p<0.05$). In patients with SC the intensity of CD95 expression was higher than in patients with CC and healthy donors. Published data show that the development of other GIT tumors is accompanied by similar changes [4,6,9]. No inter-group differences were revealed in the expression of other activation markers (CD98 and HLA-DR).

TABLE 1. Main Subpopulations of Lymphocytes in Patients with GIT Tumors (%, *M*±*m*)

Surface markers	Control (*n*=38)	Patients with SC (*n*=66)	Patients with CC (*n*=15)
CD3	57.55±3.77	62.57±2.35	61.80±4.57
CD4	29.78±3.23	34.50±2.00	27.60±4.43
CD8	28.83±4.37	31.98±1.77	33.47±4.44
CD16	16.05±3.72	22.57±2.14*	23.60±3.88*
CD19	12.11±3.83	21.26±2.08*	20.60±5.49
CD25	9.80±0.90	19.81±2.30*	19.40±5.63*
CD71	8.55±4.06	18.64±1.93*	19.87±3.98*
CD95	8.00±4.60	18.22±2.10*	11.92±4.64
CD98	10.50±4.47	15.85±1.84	13.00±2.76
HLA-DR	15.83±2.71	19.12±1.74	18.67±3.44

Note. Here and in Tables 2 and 3: *$p<0.05$ compared to the control.

TABLE 2. Expression of Surface Lymphocyte Markers in Patients with Various Tumors Stages I-II (%, *M*±*m*)

Surface markers	Control (*n*=38)	Patients with SC (*n*=18)	Patients with CC (*n*=7)
CD3	57.55±3.77	62.81±4.80	64.00±6.26
CD4	29.78±3.23	34.50±4.49	30.00±7.03
CD8	28.83±4.37	30.87±4.02	35.17±7.23
CD16	16.05±3.72	17.87±3.88	23.50±7.02
CD19	12.11±3.83	16.37±3.07	21.00±10.50
CD25	9.80±0.90	16.31±4.11*	17.60±5.38*
CD71	8.55±4.06	15.87±3.26*	19.33±5.70*
CD95	8.00±4.60	17.19±3.78*	11.67±8.31
CD98	10.50±4.47	14.15±2.00	13.00±5.60
HLA-DR	15.83±2.71	16.37±3.51	19.33±8.65

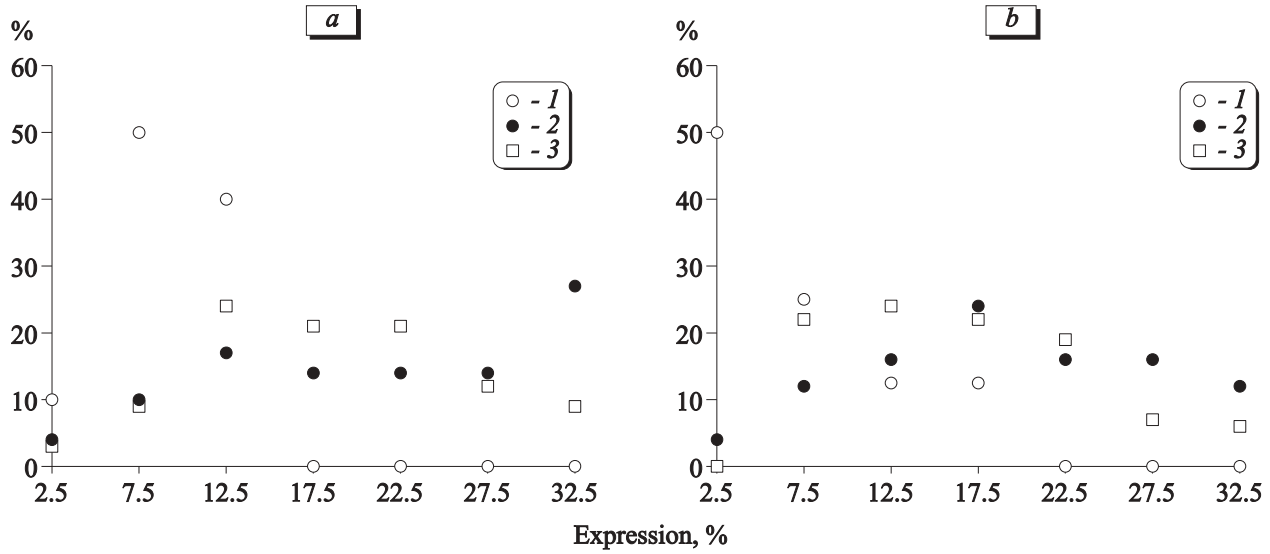

Fig. 2. Number of patients with stomach cancer characterized by different expression of CD25 (*a*) and CD71 on lymphocytes (*b*) during stabilization (*2*) or progression of tumor growth (*3*). Healthy donors (*1*).

Of particular interest is the expression of differentiation and activation antigens on peripheral blood lymphocytes during the initial stage of the disease. Radical treatment can be successful in this period (Table 2).

We did not observe changes in the main lymphocyte populations in patients with tumors of stages I-II. However, the ratio of $CD25^+$ and $CD71^+$ lymphocytes in the peripheral blood from these patients was higher than in the control ($p<0.05$). Moreover, the intensity of CD95 expression on blood cells from patients with stage I-II SC surpassed that in healthy donors ($p<0.05$).

The late stage of SC and CC was characterized by high expression of activation markers CD25 (22.43±5.68 and 23.00±9.44%, respectively) and CD71 on peripheral blood lymphocytes (21.14±7.78 and 16.44±3.63%, respectively). The count of $HLA\text{-}DR^+$ cells did not depend on the stage of the disease. It should be emphasized that the number of peripheral blood $CD95^+$ lymphocytes increased in various stages of SC ($p<0.05$, Fig. 1).

Changes in the expression of activation markers in patients with tumors do not give an estimate of variations in immunological reactivity. We evaluated the relationship between expression of activation antigens and clinical course of the pathological process. The dependence of the outcome of the disease on expression of surface lymphocyte markers was estimated 3 years after the start of examination. Expression of differentiation antigens CD8, CD16, and CD19 was

TABLE 3. Expression of Surface Markers Depending on the Outcome of the Disease (%, *M*±*m*)

Surface markers	CC		SC	
	favorable outcome (*n*=7)	unfavorable outcome (*n*=7)	favorable outcome (*n*=25)	unfavorable outcome (*n*=37)
CD3	64.00±8.77	60.86±6.16	63.96±3.99	61.46±3.34
CD4	28.71±8.59	26.28±6.75	36.44±3.82	32.94±2.58
CD8	36.57±8.03	31.71±5.87	33.12±2.47	31.46±2.56
CD16	27.28±5.11*	18.86±5.13	23.56±4.11	22.16±2.65
CD19	25.43±10.10	16.00±6.65	21.44±3.74*	21.46±2.90*
CD25	22.50±2.84*	16.60±4.30*	23.32±3.40*+	17.41±2.18
CD71	25.43±5.69*+	14.43±3.02	21.52±3.31*+	16.83±2.22*
CD95	14.50±9.22	9.71±4.01	18.28±3.86*	16.43±2.47*
CD98	14.75±3.47	11.60±3.74	16.20±2.31	15.36±2.58
HLA-DR	22.71±5.29	14.71±3.95	18.72±2.89	19.62±2.47

Note. $^+p<0.05$ compared to unfavorable outcome.

high in patients with favorable outcome of the disease. However, the number of $CD3^+$ and $CD4^+$ lymphocytes practically did not differ in patients with various outcomes of the disease (Table 3).

Most significant differences were revealed in the expression of activation markers on peripheral blood lymphocytes. In patients with SC and further stabilization of the process, expression of CD25 and CD71 during primary examination was higher than in patients with the progressive disease (Fig. 2). Expression of CD95 in patients of both groups was higher than in the control (16.20±2.31, 15.36±2.58, and 8.00±4.60%, respectively).

Similar results were obtained during examination of patients with CC. Expression of CD25 and CD71 was high in patients with favorable prognosis of CC. However, primary examination did not reveal differences in the expression of these markers in patients with progressive disease and healthy donors. No intergroup differences were observed in the number of lymphocytes carrying other activation markers (CD95, CD98, and HLA-DR).

Our results indicate that expression of activation markers on peripheral blood lymphocytes increases in patients with various tumors of GIT. Expression of CD25, CD71, and CD95 underwent most significant changes ($p<0.05$). Similar results were obtained during examination of patients with lymphoproliferative diseases [8] and malignant tumors of different localization [3]. It should be emphasized that expression of activation markers increases in the early stage of GIT tumors. The ratio of $CD25^+$ and $CD71^+$ lymphocytes in patients with favorable outcome of the disease is much higher than in patients with progressive tumors.

These data characterize the state of patients during active tumor growth. The increased expression of activation antigens probably represents a reaction of the immune system to tumor growth. This systemic activation manifested in increased expression of CD25 and CD71 on blood lymphocytes reflects adequate functional activity of the immune system in patients with tumors, which probably determines the course and outcome of the disease. Further studies are required to determine surface markers of lymphocytes that can be used as reliable prognostic criteria for tumors.

REFERENCES

1. *Standardization of Methods for Immunophenotyping of Cells from the Blood and Bone Marrow (Methodical Recommendations), Med. Immunol.*, 1, No. 5, 21-43 (1999).
2. J. D. Ahlgren, *Prim. Care*, **28**, No. 3, 647-660 (2001).
3. A. Y. Baryshnikov, E. R. Polosukhina, T. N. Zabotina, *et al.*, *Russ. J. Immunol.*, **2**, No. 2, 115-120 (1997).
4. S. Donina, L. Engele, I. Jaunalksne, and G. Zakenfelds, *Ann. Oncol.*, **11**, No. 4, 47 (2000).
5. S. Koyama, T. Ebihara, K. Fukao, and T. Osuga, *Gan No Rinsho*, **34**, No. 4, 435-441 (1988).
6. B. Melichar, M. Touskova, D. Solichova, *et al.*, *Scand. J. Clin. Lab. Invest.*, **61**, No. 5, 363-370 (2001).
7. E. F. Murta, J. M. de Andrade, R. P. Falcao, and S. Bighetti, *Tumori*, **86**, No. 5, 403-407 (2000).
8. L. E. Van de Hove, P. Vandenberghe, S. W. Van Gool, *et al.*, *Leuk. Res.*, **22**, No. 2, 175-184 (1998).
9. X. Wang, S. Xu, G. Lu, *et al.*, *Chin. Med. J.*, **114**, No. 12, 1320-1322 (2001).