ONCOLOGY

Tumor-Specific Changes in Mouse Serum during Ehrlich Carcinoma Growth

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Using the method of flow cytofluorometry we found that proteinase activity eliminating antigenic determinants from the surface of tumor cells disappeared from the serum of mice with Ehrlich carcinoma. This activity towards Ehrlich carcinoma cells is present in the sera of mice without tumors and in mice with other transplanted tumors. The serum from mice of one strain with Ehrlich carcinoma showed no protease activity against Ehrlich carcinoma cells in mice of other strain. Hence, Ehrlich carcinoma growth is associated with tumor-specific changes in the serum resulting in disappearance of specific protease activity of the serum against tumor cells.

Key Words: *experimental tumors; serum factors; blocking factors*

Recent research of the mechanisms of tumor growth is mainly focused on changes in tumor cells allowing tumor growth in the body. In particular, disappearance of main histocompatibility complex molecules from the surface of tumor cells, the release of substances suppressing immunity, and activation of oncogenes in tumor cells were demonstrated [5]. On the other hand, little is known on tumor-specific changes in the body during tumor growth. These changes are responsible for relapses of residual tumor after its elimination in the absence of changes in the immune status of patients. In 1950-80s many reports were devoted to the effects of serum factors from cancer patients and animals with transplanted tumors on the functional and biological activity of blood leukocytes and tumor cells. Based on the available data we conclude that blood serum proteins undergo changes promoting tumor growth. Some scientists called these factors blocking. Biological effects of blocking factors were reproduced and confirmed in many laboratories, but their nature remained unknown [1,2]. Presumably, investigations of changes in blood serum proteins during tumor growth were discontinued because of low technological level of those studies. Here we studied the effects of sera from tumor-bearing mice of different strains on tumor cells. We used standard methods for the test of inhibition of leukocyte adhesion with evaluation of the effect on a flow cytofluorometer [4].

MATERIALS AND METHODS

Female BALB/c, C57Bl, and DBA/2 mice aged 2-3 months were used in the study. Ehrlich ascitic carcinoma (from Tumor Strain Bank of Cancer Research Center) was transplanted to BALB/c and DBA/2 mice intraperitoneally (1×10^6 cells in 0.2 ml medium 199). P388 leukemia cells (from the same Bank) were transplanted to DBA/2 mice intraperitoneally (1×10^6 cells in 0.2 ml medium 199). The blood from mice with and without tumors was collected after decapitation. The serum was heated at 56°C for 60 min directly before

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the experiment (complement inactivation). Ehrlich carcinoma cells were isolated from ascitic fluid on day 7 after tumor transplantation, washed by centrifugation in phosphate buffered saline (PBS; pH 7.4), and incubated with serum diluted 1:3 as described previously [4]. Changes in cell characteristics were evaluated on a Calibur flow cytofluorometer. For evaluation of serum enzyme activity we used FITC-labeled Ehrlich carcinoma cells (incubation with FITC in PBS, pH 7.4, 4°C, 60 min). Unbound label was removed by 3-fold centrifugation at 800*g* for 5 min directly before adding the labeled cells to the serum. The percentage of viable tumor cells was evaluated by flow cytofluorometry (propidium iodide absorption) and by cell capacity to induce tumor growth *in vivo*. The molecular weight of FITC-labeled molecules eliminated from cell surface was evaluated by gel filtration on Sephadex G 200 with subsequent analysis of the samples on a Hitachi spectrofluorometer.

RESULTS

The distribution of Ehrlich carcinoma cells from BALB/c mice after their incubation with the serum from BALB/c mice with this tumor (Fig. 1, *a*) did not differ from that after their incubation in a serum-free phosphate buffer (pH 7.4, Fig. 1, *b*). Ascitic fluid contained many large tumor cells; 70-80% cells fell into the right upper quadrant. Incubation of the same amount of cells with the serum from BALB/c mice without tumor shifted the Dotplot distribution to the left and down (Fig. 2, *a*), which probably attested to altered interactions between normal serum proteins and tumor cells in comparison with serum proteins from animals with tumors. The number of cells in the right upper quadrant decreased to 23%. The distribution of large Ehrlich ascitic carcinoma cells isolated from BALB/c mice after interaction with the serum from DBA/2 mice with transplanted Ehrlich carcinoma in the Dotplot (Fig. 2, *b*) did not differ from Dotplot after their incubation with the serum from BALB/c mice with Ehrlich carcinoma and with serum-free medium (>70% large cells, Fig. 1). This probably suggests that growth of Ehrlich carcinoma in mice of different strains is associated with similar changes in the sera resulting in similar distribution of these cells in Dotplot. On the other hand, incubation of Ehrlich carcinoma cells isolated from BALB/c mice with the serum from DBA/2 mice with P388 leukemia (Fig. 2, *c*) and without tumor (the data are identical) shifted Dotplot cell distribution to the left and down in comparison with the initial cells (Fig. 1). This suggests that P388 leukemia and Ehrlich carcinoma induce different changes in mouse serum. The number of cells in the right upper quadrant was 30% *vs.* 70-80% before incubation. The percentage of dead tumor cells was the same after incubation with the sera from mice with and without tumors (<5%).

Presumably, changes in Dotplot distribution of Ehrlich carcinoma cells after their incubation with the serum from mice without tumors are determined by the effect of serum protein on tumor cell membrane. Incubation of tumor cells with the serum from animals with tumors produced no such changes, because tumor cells were not foreign for these animals, while the system was alien when the sera from animals without tumors interacted with tumor cells. For better understanding of alien interaction we introduced an *a priori* foreign antigen (FITC) on the surface of tumor cells. FITC-labeled cells gave a clear-cut solitary peak (Fig. 3, *a*). After incubation of these cells with the sera from

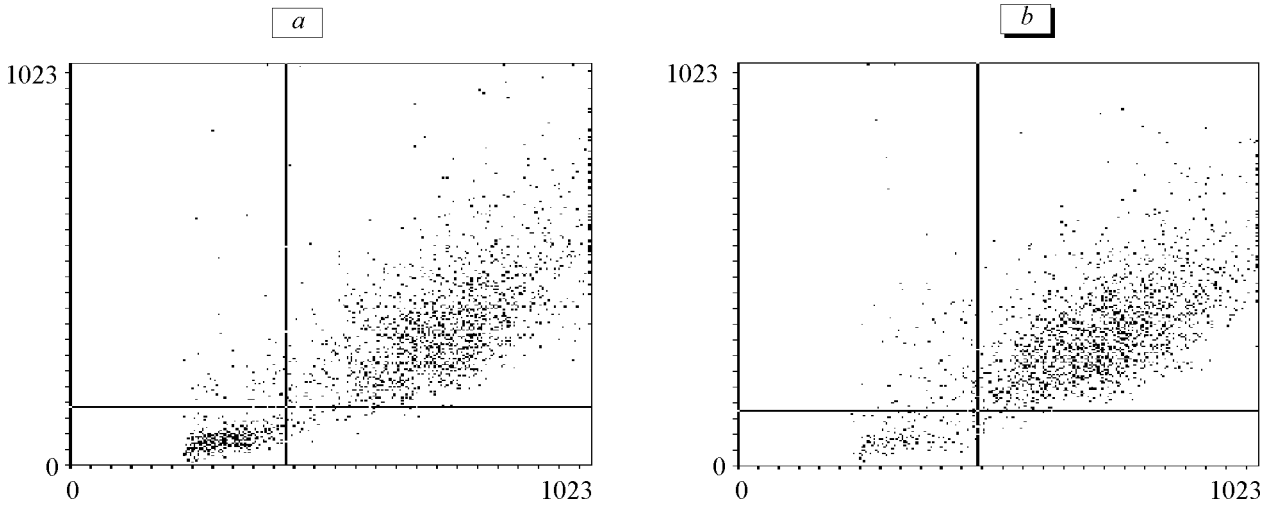

Fig. 1. Dotplot distribution of Ehrlich ascitic carcinoma cells from BALB/c mice after their incubation with serum from BALB/c mice with Ehrlich carcinoma (*a*) and in serum-free medium (*b*). Here and in Fig. 2: abscissa: FSC-H frontal light scattering; ordinate: SSC-H lateral light scattering.

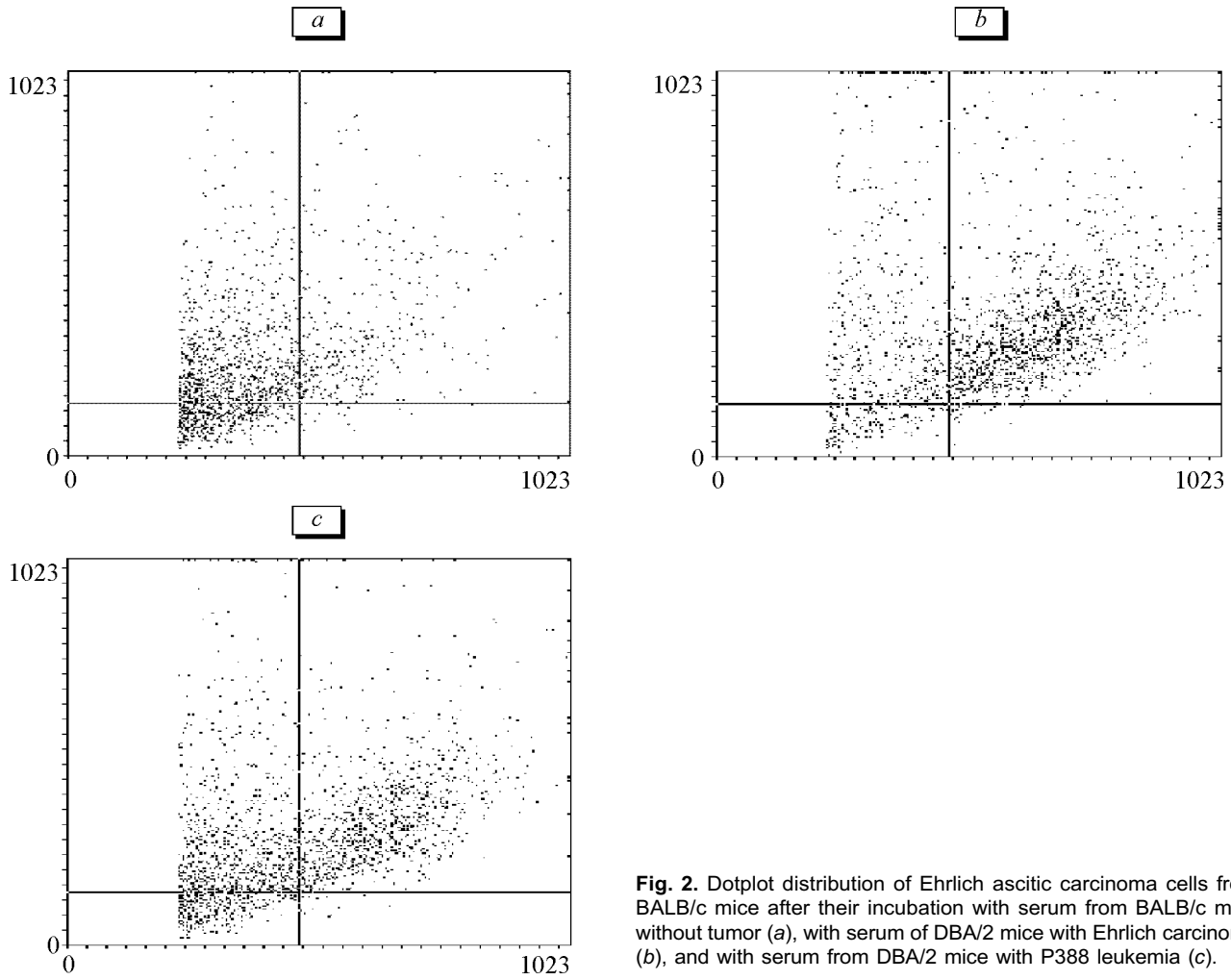

Fig. 2. Dotplot distribution of Ehrlich ascitic carcinoma cells from BALB/c mice after their incubation with serum from BALB/c mice without tumor (*a*), with serum of DBA/2 mice with Ehrlich carcinoma (*b*), and with serum from DBA/2 mice with P388 leukemia (*c*).

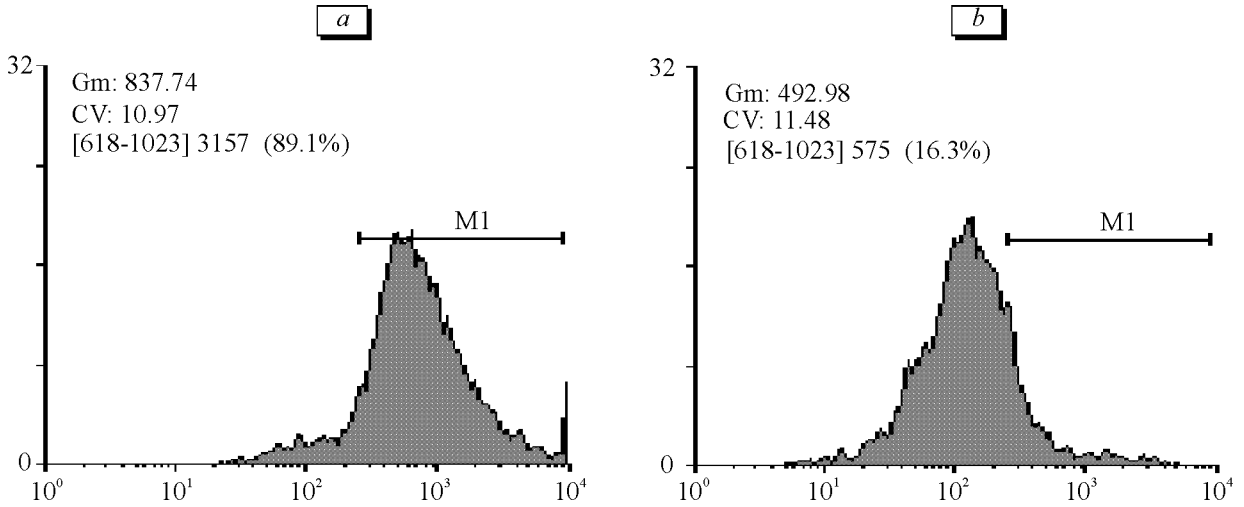

Fig. 3. Fluorescence of FITC-labeled Ehrlich carcinoma cells after their incubation in serum-free medium (*a*) and with serum from mice without tumor (*b*). Abscissa: FL1-H intensity of cell fluorescence; ordinate: number of events.

animals with or without tumors the intensity of this peak decreased and the percentage of stained cells decreased from 89.1 to 16.3% (Fig. 3, *b*). The approximate weight of FITC-containing molecules released from the cell surface into the serum after incubation with tumor cells was evaluated by gel filtration on Sephadex G 200. FITC was covalently bound to molecules with a molecular weight of <40 kD. Hence, incubation of Ehrlich carcinoma cells containing a foreign antigen with the serum from mice with or without

tumor leads to elimination of not only the antigen, but also a fragment of the molecule to which it was attached.

Thus, we found that the growth of Ehrlich carcinoma in mice of different strains is associated with some changes in the serum resulting in disappearance of its lythic activity towards tumor cells. This means that not only the tumor cells adapt to the organism, but host proteins are also modified during tumor growth. Let us note at least one crucial difference between the complement system and the described lythic activity of the serum: thermostability. Presumably, this activity is aimed at elimination of modified biomacromolecules from the body. If so, this system distinguishes own molecules from foreign or modified. Interestingly, Ehrlich carcinoma causes similar changes in the sera of mice of different strains, and they are specific for this tumor.

We believe that tumor growth in the body and development of a relapse of residual tumor after its elimination, the most prevalent and severe complication in oncology, become possible because of disappearance of serum lythic activity towards tumor cells in animals with tumors [3]. Further research in this field are required.

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