ONCOLOGY

Lectin Binding to Mouse Blood Lymphocytes during Tumor Growth

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 10, pp. 446-449, October, 2005
Original article submitted September 8, 2004

Binding of FITC-labeled lectins to lymphocytes from intact mice and mice with transplanted Ehrlich carcinoma and CaO-1 ovarian carcinoma was studied by flow cytofluorometry. Specific binding of lectins by mannose and N-acetylgalactosamine was demonstrated. Lectin binding to lymphocytes from animals with tumors decreased by more than 50% in comparison with intact animals. Changed protein glycosylation during tumor growth is specific and differs for tumors of different origin.

Key Words: *experimental tumors; lymphocytes; lectins*

Detection of tumor-associated antigens is often used in oncology for the diagnosis and monitoring the disease course. On the other hand, there are virtually no data on tumor-specific changes in the body during tumor growth. The presence of these changes is demonstrated in reports indicating that serum and leukocytes of animals with transplanted tumors are characterized by a specific biological effect protecting tumor cells in the host [4,5,7]. We showed that Ehrlich carcinoma causes changes in mouse serum, specific of this tumor [2]. However, the nature of this phenomenon remains unclear, because no tumor-specific factor mediating the observed biological effects was detected. Presumably, new biological properties are determined by changed glycosylation of surface proteins on lymphocyte membranes. In order to verify this hypothesis, we studied changes in lectin binding to blood lymphocytes in mice during the growth of transplanted CaO-1 ovarian carcinoma and Ehrlich adenocarcinoma.

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MATERIALS AND METHODS

Male (CBA×C57Bl/6)F_1 hybrids and C57Bl/6 mice aged 2-3 months from Stolbovaya Breeding Center (Moscow region) were used in the study. Each group consisted of 10 animals. Ehrlich carcinoma and CaO-1 ovarian carcinoma cells were transplanted intramuscularly (10^6 cells in 0.2 ml RPMI-1640) [1]. Lymphocytes were isolated from the peripheral blood of mice on day 15 after tumor transplantation by the standard method in 1.093 density gradient [3]. Each experiment was repeated at least 3 times. The expression of markers on lymphocyte surface was evaluated using FITC-labeled lectins. The specificity of lectin binding to the cell surface was verified by inhibition of binding after addition of mannose and N-acetylgalactosaminesugars. The results were evaluated by flow cytofluorometry on a FACScan cytofluorometer (Becton Dickinson). Cell population gate was established on the basis of combination of frontal and lateral light scattering and cell size; 10,000 events per gate were evaluated.

The data were statistically processed using WINMDI 2.8 software.

0007-4888/05/1404-0445

RESULTS

Lymphocytes form a dense cloud of homogeneous cells (Fig. 1).

During incubation of normal mouse blood lymphocytes with FITC-labeled pea lectin (Fig 2, *a*) the histogram shifts to the right in comparison with the preincubation distribution of blood lymphocytes, *i.e.* cell fluorescence increases due to binding of labeled lectin. The percentage of bound cells reached 91.2%, the geometric mean of cell fluorescence is 58.04 arb. units. Incubation of blood lymphocytes from mice with transplanted CaO-1 ovarian carcinoma with FITC-labeled pea lectin (Fig. 3, *a*) also leads to histogram shift to the right in comparison with unstained lymphocytes (Fig. 3, *a*). Only 21.3% cells are present in the signal 323-600 registration, *vs.* 91.2% in the control. The mean geometrical of staining intensity is also appreciably lower (21.4 arb. units).

Lectin detects two lymphocyte populations: with low (peak 1) and high (peak 2) sugar content on the surface (Fig. 2, *b*). These peaks contain 43.7 and 56.4% cells, respectively. The geometric mean of cell fluorescence was 72.37 arb. units. The same regularity as for staining by pea lectin, was observed during incubation of blood lymphocytes of mice with transplanted CaO-1 ovarian carcinoma with FITC-labeled phaseolus lectin (Fig. 3, *b*). The intensity of peak 2 decreased to 31%, the geometric mean of cell fluorescence decreased to 61.35 arb. units, *i.e.* tumor growth was associated with a decrease in the amount of sugars available for lectins, which attests to a decrease in their content on lymphocyte surface.

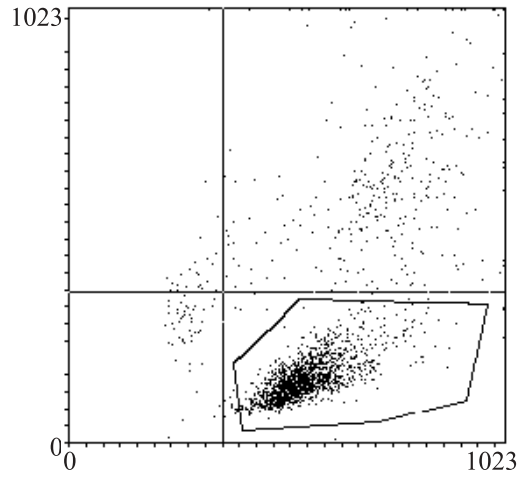

Fig. 1. Distribution of intact mouse blood lymphocytes. Abscissa: frontal light scattering; ordinate: lateral light scattering.

The histogram of peripheral blood lymphocytes from mice with transplanted Ehrlich carcinoma differed from the histogram of cells from intact mice or mice with transplanted CaO-1 ovarian carcinoma (Fig. 3, *c*); peak 2 was virtually absent, but the trend to a decrease of sugar availability for lectins during tumor growth was preserved. The percentage of cells stained with certain intensity in the 384-600 channels decreased from 56.4 (control; Fig. 2, *b*) to 14.1%. The geometric mean of cell fluorescence was 45.27 arb units.

Similar results were obtained with other strains of murine transplanted tumors.

Hence, tumor growth was associated with a decrease in the intensity of staining of peripheral blood

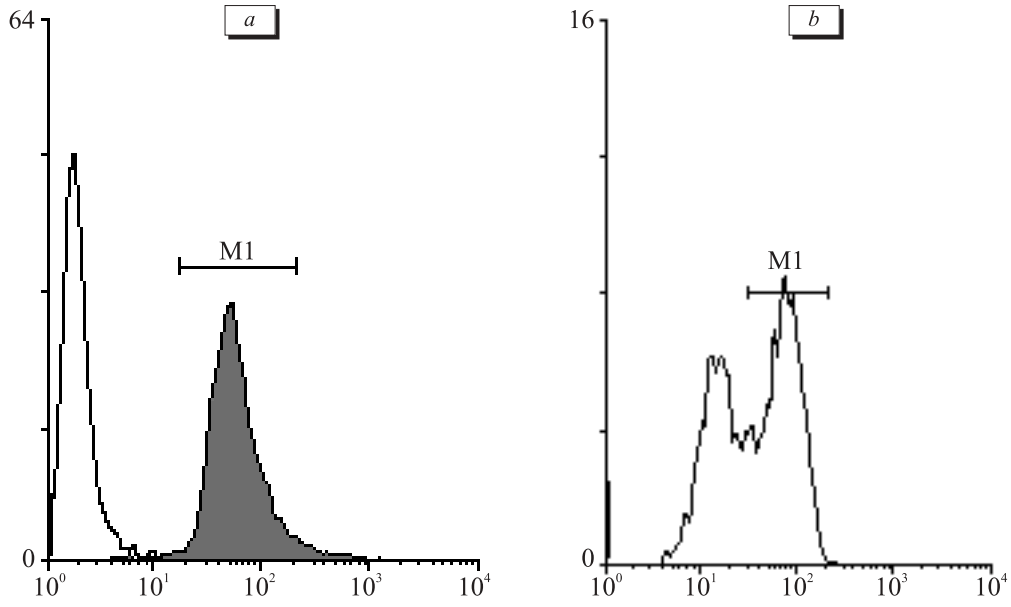

Fig. 2. Histograms of peripheral blood lymphocytes from intact mice after incubation with FITC-labeled pea (*a*) and phaseolus (*b*) lectin. Here and in Fig. 3: abscissa: fluorescence intensity (arb. units); ordinate: number of events.

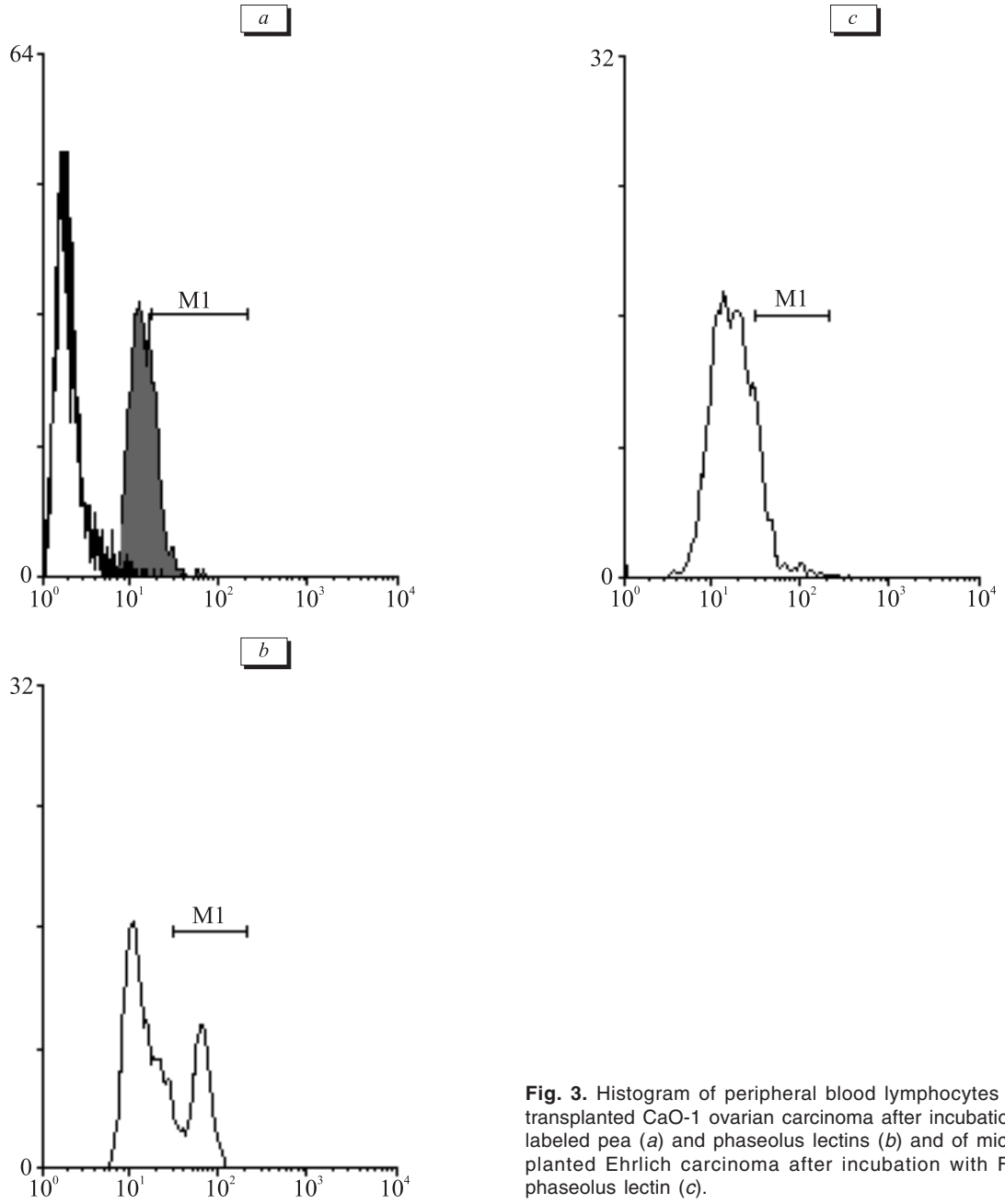

Fig. 3. Histogram of peripheral blood lymphocytes of mice with transplanted CaO-1 ovarian carcinoma after incubation with FITC-labeled pea (*a*) and phaseolus lectins (*b*) and of mice with transplanted Ehrlich carcinoma after incubation with FITC-labeled phaseolus lectin (*c*).

leukocytes with lectins. The patterns of histograms after incubation of lymphocytes isolated from mice with tumors were different for tumors of different origin. Presumably, changes in protein glycosylation during tumor growth are specific and differ for tumors of different genesis. This modification can be compensatory. The surface of tumor cell contains antigenic determinants (atoms with certain charge and spatial structure), which are recognized by other charged structures of host molecules. Tumor growth is associated with accumulation of surface structures promoting aggregation of molecules. Protein glycosylation is a mechanism "masking" surface structures responsible for pathological aggregation. Block of aggregation between lymphocytes and tumor cell surface can lead to immunological paralysis and promote tumor growth [6].

The absence of defense immune reactions to tumor growth remains a phenomenon still unexplained. Our data suggest that modulation of protein glycosylation is the most possible basis for the blockade of the immune reactions; this modulation can lead to drastic antigenic restructuring of the protein molecule. We analyzed significant quantitative changes in the glycoside compartments of the molecule and the specificity of changes in lymphocyte glycosylation during

the growth of tumors of different genesis, which can be used for the diagnosis of tumor diseases.

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