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# MIGRATING ABILITY OF TUMOR CELLS TREATED WITH IMMUNE SERA

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UDC 616-006.6-092.9-018.15-02:615.373-092.4

The action of antitumor sera (ATS) on the adhesive properties of L cells and of Ehrlich's ascites carcinoma cells was studied. The ability of the tumor cells to adhere to plastic and glass and to form rosettes with sheep's red blood cells was reduced after treatment with immune serum and, when injected into mice, fewer of these cells were held up in the lungs, spleen, and liver. The results show that antibodies may play an essential role in the metastasization of tumor cells.

KEY WORDS: adhesion; migration of cells; tumor cells; metastasization of tumors; antibodies

The action of antibodies on the proliferation and viability of tumor cells is well known [7]. However, the problem of whether antibodies can alter the migration of tumor cells in vivo remains unsolved. However, such an effect is possible, for antibodies are known to be able to affect the distribution of lymphocytes in the body [4]. The importance of the solution to this problem is determined by the fact that metastasization of tumors is an activity which largely depends on the migrating ability of the neoplastic cells [1].

In the investigation described below the distribution of tumor cells treated with antitumor sera (ATS) and changes in their adhesive properties in an in vitro system were studied.

## EXPERIMENTAL METHOD

In the experiments L cells and Ehrlich's ascites carcinoma cells were used. The ATS were obtained by immunizing rabbits with these cells [7]. The cytotoxic titer of the sera for L cells was 1 : 512 and for Ehrlich's carcinoma cells 1 : 256. The cells were incubated for 30 min at 37°C in Hanks' solution without Ca and Mg ions (to reduce aggregation of the cells), and with 20% ATS or with normal rabbit serum, after which they were washed or diluted with medium 199 to a certain concentration. The distribution of $^{51}Cr$-labeled tumor cells in the body was studied by the method described previously [3, 6]. CBA mice and (CBA × C57BL)$F_1$ hybrids were used. The reaction of spontaneous rosette formation of the cells with sheep's red blood cells was carried out by the method described previously [5]. To obtain adhesion, tumor cells in medium 199 with the addition of 20% bovine serum were placed in sterile plastic dishes (obtained from Corning) and in tubes with glass wool and incubated for 90 min at 37°C in an atmosphere of air with 5% $CO_2$ [2].

## EXPERIMENTAL RESULTS

In the experiments of series I the distribution of tumor cells in the mice was studied (Table 1). As a result of treatment of these cells with ATS their distribution among the organs was disturbed, as reflected in an increase in radioactivity in the blood and a decrease in the lungs, spleen, and liver, and with no significant

Research Center, N. I. Pirogov Second Moscow Medical Institute. N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 12, pp. 712-714, December, 1977. Original article submitted May 31, 1977.

  

TABLE 1. Radioactivity in Organs and Blood of Mice at Different Periods after Intravenous Injection of Tumor Cells Incubated with Rabbit Antitumor (e) and Normal (c) Serum (P ± m)

| Type of cells | Number of cells injected, × 10⁶ | Time after injection of cells | Method of incubation | Radioactivity in organs, % | | | | | | Ratio of radioactivity in organ to radioactivity of blood, % | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | lungs | blood | spleen | liver | bone marrow | kidneys | spleen/blood | liver/blood | bone marrow/blood |
| Ehrlich's carcinoma cells | 20 | 30 min | c | 77,5±4,1 | 4,2±0,1 | 1,7±0,04 | 14,2±0,8 | 0,7±0,1 | 1,7±0,1 | 40±6 | 335±34 | 18±4 |
| | | | e | 69,5±3,4 | 11,2±0,3 | 0,3±0,05 | 9,6±0,7 | 3,1±0,5 | 6,3±0,4 | 2±0,5 | 85±10 | 20±6 |
| | 3 | 30 min | c* | 91,4±2,2 | 3,4±0,5 | 0,2±0,03 | 3,7±0,2 | 0,4±0,1 | 0,9±0,1 | 5±0,7 | 110±11 | 11±0,3 |
| | | | e* | 51,1±3,8 | 25,3±5,1 | 0,6±0,1 | 7,4±0,5 | 2,8±0,3 | 12,7±1,6 | 2±0,3 | 29±2 | 11±0,2 |
| | 3 | 30 min | c | 84,2±4,9 | 4,2±0,2 | 0,6±0,06 | 8,5±0,3 | 0,9±0,1 | 1,4±0,1 | 13±0,9 | 192±18 | 20±1,5 |
| | | | e | 89,1±3,2 | 3,7±0,3 | 0,2±0,01 | 5,0±0,4 | 0,7±0,1 | 1,2±0,1 | 5±0,4 | 135±13 | 20±2 |
| L cells | 3 | 5 min | c | 88,5±4,5 | 4,1±0,6 | 0,3±0,03 | 5,6±0,2 | 0,5±0,0 | 1,0±0,2 | 7±1 | 136±9 | 13±1 |
| | | | e | 89,0±2,8 | 4,9±0,5 | 0,1±0,01 | 4,4±0,3 | 0,6±0,1 | 1,0±0,1 | 3±0,3 | 91±6 | 12±3 |
| | 3 | 1 h | c | 81,4±2,1 | 2,4±0,2 | 1,4±0,1 | 12,7±0,7 | 1,3±0,3 | 1,0±0,2 | 62±6 | 555±40 | 56±6 |
| | | | e | 81,6±3,7 | 3,9±0,3 | 0,5±0,1 | 11,2±0,5 | 1,4±0,1 | 1,3±0,1 | 13±1,5 | 293±20 | 38±6 |
| | | 14 h | c | 12,9±3,2 | 2,6±0,1 | 13,9±0,5 | 61,6±2,3 | 4,2±0,6 | 5,0±0,3 | 538±31 | 2386±95 | 161±11 |
| | | | e | 23,8±6,2 | 3,1±0,2 | 6,7±0,7 | 55,5±2,6 | 4,3±0,3 | 6,6±0,4 | 204±12 | 1786±87 | 136±11 |

*Animals with ligated lobe of lung.

TABLE 2. Effect of Immune (e) and Normal (c) Rabbit Serum on Adhesive Properties of Tumor Cells

| Type of cells | Method of incubation | Index | | | | | |
|---|---|---|---|---|---|---|---|
| | | RFC (%) | | number of cells adherent to plastic (per unit area) | | retention of cells in column with glass wool, % | |
| | | a | b | a | b | a | b |
| L cells | c | 44 (60) | 42 (57) | 46,3 | 40,8 | — | — |
| | e | 16 (10) | 25 (20) | 0 | 9,0 | — | — |
| Ehrlich's carcinoma cells | c | 38 (40) | 31 (46) | 38,2 | 29,1 | 52,3 | 40,6 |
| | e | 3 (0) | 12 (15) | 15,1 | 7,2 | 36,0 | 20,7 |

Legend. a) Cells not washed, b) washed to remove serum. RFC) Rosette-forming cell, meaning a cell to which more than 3 red blood cells were adherent. Percentage of RFC with more than 5 adherent red blood cells given in parentheses.

change in the level of radioactivity in the bone marrow and kidneys. However, these changes varied substantially under different experimental conditions.

A decrease in the number of cells treated with ATS was observed in the lungs only after injection of 20 million cells into the animal or after injection of 3 million cells into animals in which one lobe of the lung had been ligated, causing a severe disturbance of the hemodynamics of the organ. After injection of a small number of cells into animals without the operation a tendency was observed actually for more cells treated with ATS to be held up in the lungs. These differences are evidently attributable to mechanical retention of aggregates, which are constantly formed after treatment of cells with antibodies, in the lung capillaries.

Most of the radioactivity after injection of L cells and Ehrlich's carcinoma cells into the animals was found 5 min after injection in the lungs, but this hold up was largely mechanical, for later a redistribution of the radioactivity took place. In most cases radioactivity was increased in the blood of animals receiving injections of cells treated with ATS. Since the level of adhesion of the cells in the organs depends essentially on the concentration of cells passing through the organs in the blood stream, to determine the true adhesive power of the cells in the organs it was necessary to equalize the radioactivity in the blood of the control and experimental animals, for which purpose the ratio of radioactivity in the organs was determined relative to that in the blood. This index shows that in every case the deposition of tumor cells treated with ATS in the spleen and liver of the mice was significantly lower than in the control animals. However, no significant differences could be found for adhesion of the cells in the bone marrow.

The change in migration of cells treated with ATS in vivo was evidently not connected with the more rapid death of the tumor cells, for the effect could be detected as early as 5 min after injection of these cells into the mice, when it was too early to expect death of the cells to have begun under the influence of complement.

It might be expected that the effect observed was due to disturbance of the adhesive power of the surface of the tumor cells treated with antibodies. This was tested in the experiments of series II, in which the ability of tumor cells treated with ATS to form rosettes with sheep's red blood cells, to adhere to the surface of plastic, and to be retained in glass wool, was studied (Table 2). The considerable inhibition of the ability of the cells to form rosettes by ATS indicates that the antibodies block the surface receptors of the cells responsible for fixation of red blood cells. The ATS partially or completely inhibited the ability of the tumor cells to adhere to the surface of plastic and to be retained in glass wool. After washing to remove ATS, the L cells placed in glass flasks became spread out over the glass after 24 h and began to multiply.

These results indicate that antibodies not only block definite receptors on the surface of tumor cells, but they also reduce the total adhesive power of these cells, so causing a change in their migration in vivo. There is thus reason to suppose that antibodies play an essential role in the metastasization of tumors. This process is evidently complex and includes not only a change in the distribution of malignant cells in the body, but also a disturbance of migration of the cells from the primary tumor.

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