HETEROGENEOUS TRANSPLANTABILITY OF MOUSE SARCOMA CELLS DUE TO DIFFERENCES IN SENSITIVITY TO LYSIS BY MACROPHAGES

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In previous publications [1-4] the authors gave the characteristics of a collection of tumor cell lines obtained in order to study the mechanisms whereby tumor cells can overcome the immunologic reactions of the host. B6-4 cells of the C57Bl/6 genotype from this collection were rejected in 60% of syngeneic intact animals, but grew progressively in 100% of animals with suppressed immunity (irradiation in a dose of 4.5 Gy). This was evidence of the important role of the immune response in cell rejection. Assessment of the cytotoxic reaction of lymph node and spleen cells of syngeneic recipients against B6-4 showed that the immune response of these organs was much below the level at which rejection of a transplanted tumor usually takes place [4]. On account of the absence of the expected immune responses of the above-mentioned organs it was decided to study the effector component of immunity in this model. If the cell line mentioned proved to be heterogeneous for tumorigenicity, the question of the limiting factor could best be resolved on clones, which could be used to identify the feature whose variation correlates with the degree of transplantability.

In this paper we give data on the immunologic characteristics (sensitivity to effectors and immunogenicity) of nine clones (strain B6-4), differing in their degree of transplantability in syngeneic recipients.

High positive correlation was found between the degree of transplantability of the clones and their resistance to the lytic action of macrophages. In syngeneic mice, into which any of the above clones were transplanted, macrophages undertaking specific lysis of the transplanted cells appeared. These facts are evidence that macrophages are the chief effector component responsible for controlling growth of B6-4 cells.

TABLE 1. Transplantability and Rate of Growth of Clones in Intact, Immune, and Irradiated Syngeneic Animals, and also in nu/nu Mice

Clone No.	Recipient	Number of animals in experiment	Number of animals with tumors, days after transplantation of cells			Mean diameter of tumor on 30th day, mm
			5	14	30	
8	Intact	9	6	0	0	—
	Irradiated	10	8	9	4	4,0
	nu/nu	5	5	3	3	3,7
9	Intact	10	9	7	2	3,0
	Irradiated	10	9	10	10	14,7
	nu/nu	5	5	5	5	17,2
8	Intact	18	9	1	0	—
	Immunized (immunization with clone No. 8)	9	0	0	0	—
9	Intact	11	11	11	4	5,8
	Immunized (immunization with clone No. 9)	18	5	0	0	—
1	Intact	10	10	3	0	—
	Irradiated	9	9	9	4	8,0
	nu/nu	6	5	6	6	8,0
13	Intact	10	3	0	0	—
	Irradiated	8	5	8	2	1,0
	nu/nu	4	3	3	4	2,5

Legend. Cells transplanted subcutaneously into dorsal region in a dose of 10^6 per mouse.

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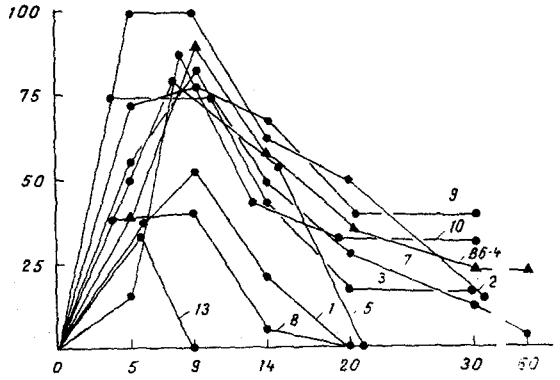

Fig. 1. Transplantability of cells of the B6-4 strain and its clones in syngeneic mice. Abscissa, days after transplantation of cells; ordinate, percentage of animals with tumors. Triangles indicate B6-4 cells, circles indicate clones.

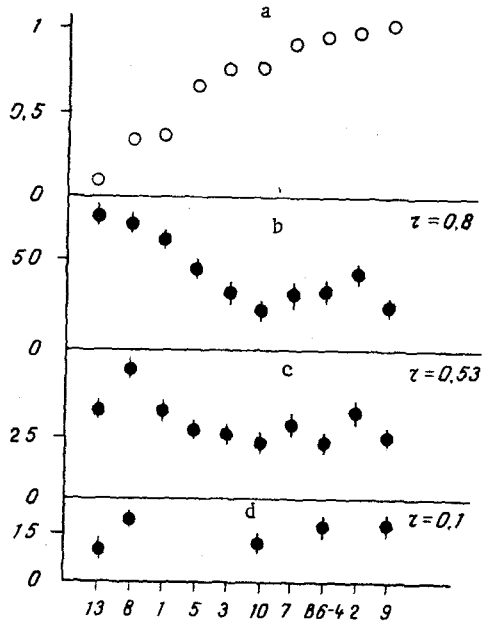

Fig. 2. Tumorigenicity (a), sensitivity to macrophages (b) and NK lymphocytes (c), and immunogenicity (d) of cells of the B6-4 strain and its clones. Abscissa, No. of clones; ordinate: a) degree of tumorigenicity of clones, estimated by integral of curves in Fig. 1; b, c, d) Fractions of lysed cells (in %), represented by mean of two values corresponding to two target–effector ratios: for b) 1:10 and 1:30; for c) 1:100 and 1:300; for d) 1:100 and 1:200. Immunogenicity represented by difference in activity of splenocytes from immunized and control animals. Splenocytes were isolated on 12th day after immunization.

EXPERIMENTAL METHOD

Male and female C57Bl/6 mice and nude nu/nu mice of BALB/c genotype, aged 2-3 months and bred at the Experimental Animals Laboratory, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, were used. Cell lines B6-4 and BC-5 were obtained from tumors induced by 20-methylcholanthrene in C57Bl/6 and BALB/c mice respectively [1].

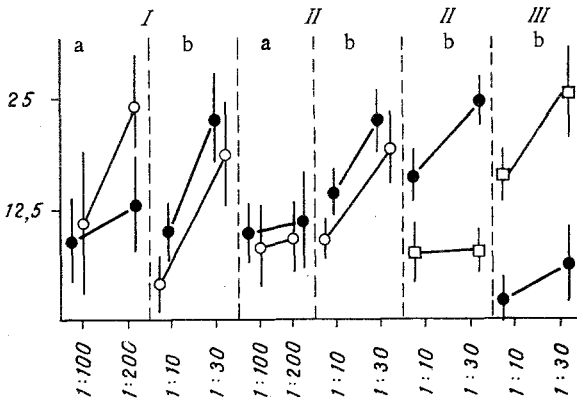

Fig. 3. Immune response of lymphocytes (a) of regional lymph nodes and macrophages (b) from peritoneal cavity of C57Bl/6 mice with transplanted cells of clones Nos. 8 (I) and 9 (II) and with BC-5 cells (III).

Nine clones of strain B6-4 were obtained by double recloning in 96-well plates (Flow Laboratories, England) by the limiting dilutions method. All the cell cultures were maintained by passage in vitro on Eagle's medium with 10% bovine serum. They were free from mycoplasmas, judging by incorporation of tritiated uridine and uracil [12].

Macrophages were obtained from the peritoneal cavity of control and immunized mice on the 3rd day after intraperitoneal injection of starch [2]. To improve the resolution of the difference in the degree of lysis of the target cells, NK-effector cells (NK denotes normal killer cells) were isolated from lymph nodes of mice 3 days after rat splenocytes were introduced into their regional lymph nodes. The possibility of obtaining this increased resolution in similar reactions has been demonstrated in principle more than once [5, 6, 9]. To evaluate the specific immune response we used lymphocytes from the spleen and regional lymph nodes of mice receiving a subcutaneous injection of 10^6 of the corresponding cells into each limb [3]. The degree of lysis of the target cells was assessed by the outflow of radioactive label from the disintegrated cells [10]. In the cytotoxic test with macrophages the target cells were labeled with ^{3}H-thymidine, whereas in the test with lymphocytes, ^{3}H-uridine was used. The tests were carried out in 96-well plates from Flow Laboratories.

EXPERIMENTAL RESULTS

Data on transplantability of the cells of the nine clones obtained and cells of the mother culture in syngeneic intact recipients are given in Fig. 1. Cells of clones Nos. 1, 5, 8, and 13, which formed tumor nodules in 30-75% of animals after 5-9 days, were completely rejected by the 9th-20th day. The remaining clones, which displayed great differences in their transplantability, were similar with respect to this property to the mother culture, the cells of which formed tumor nodules in 90% of animals which grew progressively in one-quarter of the recipients.

Data on the degree of lysis of all the above-mentioned cells by macrophages and by NK lymphocytes are given in Fig. 2. Cells of the least tumorigenic clones Nos. 1, 5, 8, and 13 were lysed most intensively by macrophages, whereas the remaining clones did not differ so appreciably from one another. Nevertheless, the coefficient of correlation between the degree of tumorigenicity of the cells and the degree of their lysis by these effectors, calculated for all clones, was high ($r = 0.8$). The degree of lysis of the above-mentioned cells by NK lymphocytes showed smaller differences, and this was reflected in a lower value of the coefficient of correlation with tumorigenicity ($r = 0.53$).

The study of the specific immune response of the host was limited to the mother culture and four clones, and under these circumstances this reaction was particularly carefully weighted in two clones, with the greatest contrast for transplantability. The results given in Figs. 2 and 3 are evidence that the degree of the recipients' immunologic response to cells of clones differing in transplantability was absolutely identical, and under these circumstances the clones exhibited immunologic cross-reactivity. In the other cell line the

immune response to the clones was specific even as regards macrophages (Fig. 3). The possibility that macrophages may have such specific antitumor activity is confirmed by data in the literature [8, 11]. The development of the specific immune response recorded in vitro is in good agreement with data on stimulation of cell growth in immunodepressed animals and total rejection of the cells in recipients immunized beforehand (Table 1). In all the experiments described above in vivo, cells of clones differing in tumorigenicity behaved qualitatively identically, although in all cases they preserved the original quantitative difference in their tumorigenicity. This difference also was preserved on transplantation of clones Nos. 8 and 9 into immunodepressed animals and into nu/nu mice (Table 1). All this is evidence that the specific immune response cannot dictate a difference in the degree of tumorigenicity of the above-mentioned clones. The difference is evidently due to the only feature by which these clones differ, namely their sensitivity to the lytic action of macrophages. These cells are probably the main effectors, restraining growth of clones of this tumor which have low tumorigenicity.

Preparation of the cell lines whose growth in vivo is entirely limited by a certain immunologic factor is extremely essential for correct weighting of this feature in tumor growth. This also applies to macrophages, whose important role in the control of tumor growth has frequently been emphasized in the literature [7, 13, 14]. From this point of view the cell clones highly sensitive to macrophages, which we obtained, may prove to be useful material.

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