EFFECT OF THE GRAFT VERSUS HOST REACTION ON GROWTH OF A SYNGENEIC SARCOMA IN MICE

V. Ya. Shcherbakov and V. F. Semenkov

UDC 616-006.3.04-092.9-092:612.6.02.017.1

Inhibition of growth of a syngeneic tumor was observed in recipients both with marked and without manifestations of allogeneic disease if the tumor was grafted on the day after injection of DBA spleen cells. Subcutaneous injection of tumor cells mixed with sensitized spleen cells of C57BL/6 mice prevented growth of the tumor in most recipients, while intravenous injection of sensitized spleen cells of C57BL/6 mice stimulated growth of the tumor grafted subcutaneously.

An important problem in experimental oncology is the search for methods of inhibiting tumor growth. An interesting subject for study from this point of view is the effect of the reaction of immunocompetent cells to growth of a syngeneic tumor in the recipient, for under the conditions of the graft versus host reaction, rejection of syngeneic skin grafts is observed [2]. Growth of a syngeneic tumor is inhibited by allogeneic [6, 10] immunocompetent cells. The effect of inhibition of tumor growth was observed if the recipient of the syngeneic tumor was treated by injection of macrophages, or with lymph gland or spleen cells from allogeneic donors immunized with tumor cells [3, 7, 8].

The object of the investigation described below was to study the possibility of inhibiting growth of a syngeneic sarcoma with the aid of the "graft versus host" reaction induced in (CBA × C57BL/6)F_1 hybrids by injection of parental spleen cells.

EXPERIMENTAL METHOD

The recipients of the spleen and tumor cells were (CBA × C57BL/6)F_1 hybrids weighing 16-19 g. The recipients were inoculated with sarcoma A-3174 obtained in the authors' laboratory and giving growth in 100% of cases in (CBA × C57BL/6)F_1 hybrids. A tumor developed in one of the ten females after two doses of x-ray irradiation. As a rule the tumor was trypsinized and injected subcutaneously in doses of 10^4, 10^5, and 10^6 living cells. The viability of the tumor cells was determined by staining with 0.5% eosin solution. In the experiments of series I the "graft versus host" reaction was induced by spleen cells of intact C57BL/6 and CBA mice. To facilitate induction of the "graft versus host" reaction some recipients were irradiated in a dose of 400 R. A suspension of minced tumor in physiological saline (ratio 1:4) was injected into irradiated recipients in an equal dose of 0.4 ml to each animal, and trypsinized tumor cells in a dose of 10^6 were injected into unirradiated animals.

In the experiments of series II the graft versus host reaction was induced by means of spleen cells from C57BL/6 mice sensitized by eight injections of spleen cells from (CBA × C57BL/6)F_1 hybrids. In the experiments of series III mice of line C57BL/6 also were used as donors, but in this case they were immunized three times either with cells of sarcoma A-3174 or with spleen cells of (CBA × C57BL/6)F_1 hybrids. In all the experiments donors were immunized with spleen or tumor cells in a dose 30×10^6. The conditions of irradiation of the recipients were: RUM-11 apparatus, voltage 190 kV, current 15 mA, field

Department of Microbiology, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 70-73, February, 1973. Original article submitted April 10, 1972.

TABLE 1. Rejection of Syngeneic Sarcoma by (CBA × C57BL/6)F_1 Hybrids Receiving Injections of Spleen Cells from CBA and C57BL/6 Mice

Group of mice	Dose of irradiation (in R)	SC (×10^6)		Transplanation of tumor at various times after inject. of SC					
		CBA	C57BL/6	1-st day	$q \pm m$	5-th day	$q \pm m$	10-th day	$q \pm m$
1	400	50	—	4/4	100±50	—	—	—	—
2	400	100	—	6/6	100±40	—	—	—	—
3	—	50	—	4/4	100±50	—	—	—	—
4	—	100	—	2/2	100±66,6	0/3	0±57,1	0/5	0±44,4
5	—	—	50	1/3	33,3±33,3	0/3	0±57,1	—	—
6	—	—	100	0/6	0±40	1/3	33,3±33,3	0/7	0±36,4
7	400	—	—	0/15	0±21,1	0/15	0±21,1	0/15	0±21,1
8	—	—	—	0/20	0±16,7	0/20	0±16,7	0/20	0±16,7

Legend: SC) spleen cells of mice (SC of CBA mice injected intraperitoneally into irradiated hybrids; intravenously into unirradiated animals); numerator gives numbero f mice with inhibition of tumor growth, denominator gives total number of mice; q) index of inhibition of tumor growth (in percent).

TABLE 2. Growth of Syngeneic Sarcoma in (CBA × C57BL/6)F_1 Hybrids Receiving Injections of Sensitized Spleen Cells from C57BL/6 Mice

Group of mice	Meth. of injection of s/c	Dose		No. of mice	P_1	Mean time (M ± m) of survival (in days)	P_2	Mean time (M ± m) of appearance of tumor (in days)	P_3
		SC ×10^6	TC						
1	—	—	10^4	2/10	—	27±0,67	—	80,7±5,61	—
2	—	—	10^5	10/10	—	16,2±0,22	—	31,7±1,45	—
3	s/c	40	—	0/10	—	—	—	90	—
4	i/v	40	—	0/7	—	—	—	90	—
5	s/c	40	10^4	0/10	>0,05	—	—	90	>0,05
6	s/c	40	10^5	3/20	<0,05	36,7±2,24	<0,01	61±4,04	<0,01
7	i/v	40	10^4	9/9	<0,05	20,4±0,67	<0,01	45,11±2,69	<0,01
8	i/v	40	10^5	8/8	—	14±0,45	<0,05	32,25±2,91	>0,05

Legend (here and in Table 3): s/c) spleen cells, TC) tumor cells; numerator gives number of mice with tumor, denominator gives number of mice in group; s/c) subcutaneous; i/v) intravenous injection; P_1) index of significance of death of mice from tumor; P_2) index of significance of time of appearance of tumor; P_3) index of significance of survival time.

TABLE 3. Growth of Syngeneic Sarcoma in (CBA × C57BL/6)F_1 Hybrids after Injection of Sensitized C57BL/6) Spleen Cells

Group of mice	Meth. of injection	Dose of cells		Number of mice	P_1	Mean time (M ± m) of appearance of tumor (in days)	P_2	Mean time (M ± m) of survival (in days)	P_3
		SC ×10^6	TC ×10^5						
1	—	—	1	10/10	—	13,22±1,34	—	33,9±4,91	—
2	s/c	40*	1	2/10	<0,05	22,5±0,34	<0,01	81,2±5,16	<1,01
3	i/v	40*	1	10/10	—	10,11±0,56	<0,05	25,9±2,07	>0,05
4	s/c	40†	1	0/10	<0,05	—	—	90*	<0,01
5	i/v	40†	1	10/10	—	9,7±0,55	<0,05	31,0±4,27	>0,05
6	s/c	40	1	10/10	—	18,2±0,96	<0,05	41,3±4,95	>0,05

*Spleen cells sensitized with normal hybrid tissue.
†Spleen cells sensitized with hybrid tumor.

20 × 20 cm, focus distance 40 cm, filters 0.5 mm Cu and 1 mm Al, dose 400 R. The mouse spleens were minced in a hand-operated glass homogenizer with the addition of cold Hanks's solution. The resulting cell suspension was filtered through fine silk gauze, kept on ice, and injected into the recipients 2 h after irradiation.

EXPERIMENTAL RESULTS

In the experiments of series I (Table 1) the sublethally irradiated hybrids developed a marked form of allogeneic disease and definite delay of growth of the sarcoma was observed after intravenous injection of 50 and 100 million spleen cells from intact CBA mice ($P < 0.05$). The sarcoma appeared 7-8 days later in the experimental hybrids (groups 1 and 2) than in the controls (group 7) into which no spleen cells were injected. Some recipients died from allogeneic disease 2 weeks after irradiation and injection of the spleen cells without the appearance of a tumor. Intravenous injection of spleen cells from CBA and C57BL/6 mice in doses of 50 and 100 million into the unirradiated animals as a rule was not followed by any clinically apparent form of the disease. Delay of growth of the tumor by 4-5 days compared with the control was observed if the tumor cells were inoculated on the day after injection of spleen cells from CBA mice (groups 3, 4, and 8). When the tumor cells were injected on the 5th-10th day after injection of spleen cells from CBA and C57BL/6 mice, in most experimental animals the tumor appeared 6 days sooner than in the control mice (groups 5, 6, and 8).

In the experiments of series II (Table 2) subcutaneous injection of tumor cells mixed with sensitized spleen cells of C57BL/6 mice prevented the development of the tumor in most recipients (group 6; $P < 0.05$), while in animals which developed a tumor the day of its appearance was considerably delayed ($P < 0.05$) and the survival time of the animals was increased ($P < 0.01$). Intravenous injection of sensitized spleen cells into the hybrids followed by subcutaneous injection of tumor target cells led to stimulation of growth of the tumor, which appeared in all the mice receiving 10^4 tumor cells (group 7, $P < 0.05$) and had a shorter incubation period for groups 7 and 8, for which $P < 0.01$ and $P < 0.05$ respectively, as well as a shorter survival period in group 7 ($P < 0.01$).

In the experiments of series III (Table 3) spleen cells of donors sensitized with tumor tissue induced complete suppression of growth of the tumor (group 3). Spleen cells of donors immunized with normal tissue had the same action. These cells prevented growth of the tumor in most animals (group 1), although in two mice a later development of the tumor was observed ($P < 0.01$). After intravenous injection of spleen cells of donors sensitized with both tumor and normal cells, the appearance of the tumor was delayed ($P < 0.05$).

The graft versus host reaction thus inhibits growth of a syngeneic sarcoma transplanted on the day after injection of spleen cells of CBA mice, whether clinical features of allogeneic disease are present or not. It was shown as long ago as in 1936 that spleen tissue can inhibit tumor growth [1]. In the present experiments marked inhibition of tumor growth was observed after combined subcutaneous injection of sensitized spleen cells of C57BL/6 mice and tumor cells. This indicates that in order to obtain inhibition of tumor growth under the conditions of the graft versus host reaction contact between the sensitized spleen cells and the tumor target cells is essential. Such conditions are probably created by transplantation of the tumor on the 1st day after injection of the spleen cells. Later the immunocompetent cells evidently produce humoral antibodies which block the tumor cells and prevent contact between the immunocompetent cells and the target cells, with the result that growth of the tumor is stimulated. This hypothesis is confirmed by the fact that sensitized spleen cells of C57BL/6 mice, if injected intravenously, led to intensified growth although the tumor was inoculated simultaneously with the injection of spleen cells. Since sensitization of donors with tumor tissue was slightly more effective than immunization with normal tissue it can be postulated that the antitumor action of sensitized lymphocytes observed in these experiments is not confined to the reaction against the host's transplantation antigens. The reaction against tumor-specific antigens evidently also plays an important role.

LITERATURE CITED

1. L. M. Shabad and F. M. Khaletskaya, Arkh. Biol. Nauk, 43, No. 1, 75 (1936).
2. A. S. Shevelev and V. F. Semenkov, Dokl. Akad. Nauk SSSR, 187, No. 6, 1408 (1969).
3. B. Bennet, J. Immunol., 95, 656 (1965).
4. W. Hans, in: Swedish Cancer Society Yearbook, Stockholm (1963), p. 309.
5. J. Medzihradsky, Neoplasma (Bratislava), 9, 387 (1962).

6. A. M. Nassan and A. E. Stuart, Lancet, 2, 496 (1963).
7. G. A. M. Rook and D. B. Cater, Brit. J. Exp. Path., 49, 241 (1968).
8. M. F. A. Woodruff and J. L. Boak, Brit. J. Cancer, 19, 411 (1965).
9. M. F. A. Woodruff and M. O. Symes, Brit. J. Cancer, 16, 707 (1962).
10. M. F. A. Woodruff, M. O. Symes, and A. E. Stuart, Brit. J. Cancer, 17, 320 (1963).