REPRODUCTION OF SPECIFIC ANTITUMOR IMMUNITY IN AN ISOLOGOUS SYSTEM

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L. A. Zil'ber, O. M. Lezhneva, and B. D. Brondz

Division of Immunology and Oncology (Head, Active Member AMN SSSR Professor L. A. Zil'ber), N. F. Gamaleya Institute of Epidemiology and Microbiology (Director, Professor P. A. Vershilova), Moscow

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The study of the antigenic difference between the tissues of the host and of an isologous, or even an autologous tumor by the method of iso- and autoimmunization [1, 2, 4-13] has been the subject of many investigations conducted on highly inbred mice, characterized by the complete genetic (and, hence, antigenic) identity of the individuals within a single line. It has been shown that the artificial absorption or operative removal of an isologous tumor, like the treatment of mice with tumor cells irradiated with x-rays or killed with alcohol, produces resistance of the mice to subsequent inoculation of living cells of the same tumor. This immunity cannot be explained by the residual heterogeneity of the line or by mutation changes in the antigens of the tumors in the process of transplantation; the investigations cited were carried out on first generations or primary tumors arising in mice of lines whose genetic homogeneity was confirmed by the 100% survival of isologous skin grafts. Although the immunity induced in this manner is weak in intensity and can be overcome by large doses of cells of an isologous tumor, the presence of an antigenic difference between the host and the isologous tumor, and in some cases of an autologous tumor, is in no doubt.

Studies were made of tumors of different histological structure, induced by various methods. These tumors were sarcoma [1, 4, 7-10, 12], adenoacanthoma [11], glioma [13], induced by carcinogens; tumors induced by a cellophane film [6], by the virus of Gross [5] and by polyoma virus [2, 15], and also spontaneous adenocarcinomas [7]. In most cases immunity to the isologous tumor could be produced. The intensity of immunity was maximal following immunization with tumors induced with methylcholanthrene, and minimal following immunization with spontaneous adenocarcinomas. No cross immunity was observed between the different tumors induced by one carcinogen in mice of the same line [1, 4, 8]. On the contrary, different tumors induced by the same virus possessed the ability to immunize each other, i.e., they had a common specific tissue antigen [2, 5]. The state of resistance may be transmitted passively to another isologous host by immunologically competent cells [3, 8, 16]. Leukemias induced by Gross's virus also form humoral antibodies, which may be detected by the cytotoxic reaction in vitro [16].

The findings described in this paper represent one stage of the research being carried out in our laboratory on the study of specific antitumor immunity in highly inbred mice to tumors induced within the same inbred line.

METHOD

The investigation was carried out on mice of 7 highly inbred lines: C57BL/10Sn, B10, D2, C3H/Sn, C3H, NB, CC57W, CC57BR, and A. The breeding of these inbred mice was undertaken by I. K. Egorov in the nursery of the division of immunology and oncology of the N. F. Gamaleya Institute of Epidemiology and Microbiology. Tumors MC-1, MC-2, MC-3, MC-4, MC-5, MC-7, and MC-8 were induced with methylcholanthrene and tumors BZA-2, BZA-SS with 9,10-dimethyl-1,2-benzanthracene. The carcinogens were injected subcutaneously in a dose of 0.2-0.5 mg per mouse in olive oil. Tumors appeared after 2-4 months. Histologically they were all polymorphocellular or spindle-cell sarcomas. Besides those listed above, one adenocarcinoma of the mammary gland arising spontaneously in a female of line A was also studied.

Transplantation of skin. To verify the immunological compatibility of donor and recipients, a piece of skin from the tail was transplanted from a mouse with a primary induced tumor to the dorsal region of recipient mice, i.e., to the mice taken in the experiment for immunization.

Results of Experiments to Reproduce Specific Antitumor Immunity in an Isologous System

Line of mice	Name of tumor, method of induction, sex of donor	Sex of recipients	No. of mice	State of skin grafts after 6-10 months	Method of immunization: With irradiated cells 1st	With irradiated cells 2nd	With irradiated cells 3rd	With formalinized cells 1st	With formalinized cells 2nd	With formalinized cells 3rd	With formalinized cells 4th	Injection of living tumor into ears and tail 1st	Injection of living tumor into ears and tail 2nd	Injection of living tumor into ears and tail 3rd	Ligation of tumor 1st	Ligation of tumor 2nd	Testing of immunity with living cells (No. of cells per mouse)	No. of mice without tumor* (total No. of mice in parentheses)	P
C57 BL/ 10Sn	MC-1, sarcoma, Induced with methylcholanthrene ♂	♂	5	Alive	Primary	1st gen.	2nd gen.										1×10^6 4th gen.	5(5)	< 0.01
			11	×	Not immunized													2(11)	
		♂ and ♀	13	×				1st gen.	1st gen.	3rd gen.	4th gen.						3×10^6 6th gen.	4(13)	0.063
			11	×				Not immunized										0(11)	
B10. D2	MC-2, sarcoma Induced with methylcholanthrene ♂	♂ and ♀	16	Alive								1st gen.	1st gen.	2nd gen.			1×10^5 4th gen.	9(16)	< 0.01
			11	×								Not immunized						0(11)	
B10. D2	MC-3, sarcoma, Induced with methylcholanthrene ♀	♂ and ♀	6	Alive											1st gen.		3×10^6 4th gen.	5(6)	< 0.01
			9	×											Not immunized			0(9)	
C3H/ Di Sn	MC-4, sarcoma, Induced with methylcholanthrene ♀	♂ and ♀	9	Alive							Primary	1st gen.	1st gen.				6×10^5 4th gen.	7(9)	< 0.01
			11	×							Not immunized							1(11)	–
C57 BL/ 10Sn	MC-5, sarcoma, Induced with methylcholanthrene ♀	♂ and ♀	33	Alive	Primary	1st gen.	2nd gen.										5×10^4 and 10^5 3rd gen.	0(33)	
			25	×	Not immunized													0(25)	
CC57 BR	MC-7, sarcoma, Induced with methylcholanthrene ♂	♂	10	Alive											1st gen.	2nd gen.	1×10^4 3rd gen.	6(10)	0.073
		♂ and ♀	10	×														2(10)	
CC57 W	MC-8, sarcoma, Induced with methylcholanthrene ♂	♂ and ♀	9	Alive											1st gen.		5×10^4 3rd gen.	5(9)	0.013
			21	×											Not immunized			2(21)	
C3H. NB	BZA-2, sarcoma Induced with 9,10-dimethyl-1,2-benzanthracene ♂	♂	5	Alive	Primary	1st gen.	3rd gen.										5×10^4 5th gen.	0(5)	–
			6	×	Not immunized													0(6)	
C3H. NB	BZA-2 ♂	♂ and ♀	11	×				1st gen.	2nd gen.	2nd gen.	3rd gen.						5×10^4 5th gen.	0(11)	–
			6	×				Not immunized										0(6)	
C57 CBR	BZA-CC, sarcoma Induced with 9,10-dimethyl-1,2-benzanthracene ♂	♂	9	Alive								Primary	1st gen.	1st gen.			5×10^4 5th gen.	4(9)	
			8	×											4th gen.	4th gen.		0(8)	
			24	×								Not immunized						6(24)	
A	CMG, carcinoma of mammary gland, spontaneous	♂ and ♀	10	×								Primary			1st gen.		1×10^6 2nd gen.	0(10)	–
			6	×								Not immunized						0(6)	

Legend: gen.—generation of tumor; primary—primary induced tumors. ×—mice in which tumors either did not develop or developed and were absorbed.

Skin taken from the tail was cut into pieces measuring 3×4 mm. These were placed in physiological saline with penicillin (10,000 units/ml). The recipient mice were given an intraperitoneal injection of Nembutal in a dose of 0.08 mg/g body weight. With this dose, the mice went to sleep after 3-5 min and slept for 2-3 h. The anesthetized mouse was fixed, the hair on the dorsum was shaved, and the skin was painted with Rivanol or 70° alcohol. Next, with curved, pointed scissors, the epidermis was excised, and in this way a "bed" was prepared for the graft. This bed should preferably be the same size as the prepared skin grafts. It must be emphasized that only the epidermis must be excised, and care must be taken not to injure the blood vessels situated in the panniculus carnosus. A drop of penicillin solution (50,000 units/ml) was placed in the "bed" and the skin graft was then applied with forceps and covered with a small, sterile gauze dressing. The graft was fixed with a strip of adhesive tape not more than 2 cm wide. This had to anchor the graft securely, but at the same time it had not to be so tight that it prevented the mouse from breathing freely. The dressing was removed on the 6th-7th day after transplantation.

Preparation of the cell suspension. The tumors were minced with scissors, and pieces about 1.5 mm in diameter were washed in Ringer's solution, treated with 0.3% trypsin solution, and allowed to stand overnight at 4°. Next day a cell suspension was prepared by trypsinization on a magnetic blender for 10 min. The cells were washed three times with Ringer's or Earl's solution, suspended in the same solution, and the number of living (not stained with eosin) cells in 1 ml was counted by Schrek's method [14].

Immunization of mice. Method 1. The primary induced tumor was removed in aseptic conditions. Part of it was taken for histological examination, and another small part was used to inoculate 4-5 fresh isologous mice to maintain the tumor strain, while the cell suspension was prepared from the remainder of the tumor. Ringer's solution was added to the residue of washed cells in a proportion of 1:2. The suspension was irradiated with x-rays (300-400 R/min) with a focus distance of 33.5 cm and in a total dose of 15,000 R. After irradiation, the suspension was used to inoculate isologous mice subcutaneously in a dose of 0.1 ml each. Immunization was repeated twice by a similar method, at intervals of between 2 and 5 weeks. For this purpose the 1st, 2nd, or 3rd generation of the same tumor was used.

Method 2. The tumor was finely minced with scissors and two parts by weight of Ringer's solution containing 0.2% formalin was added. The suspension was incubated in formalin for 23 days at 4°, and then inoculated subcutaneously into the experimental animals. The course of immunization consisted of 4 injections: 2 of 0.1 ml and 2 of 0.15 ml at intervals of between 1 and 5 weeks. Tumors of the 1st-4th generations were used for immunization.

Method 3. The cell suspension obtained by trypsinization of the primary induced tumor was inoculated intradermally into isologous mice. Each mouse received an injection of 10^6 living cells in a volume of 0.02 ml. On the 8th-10th day after inoculation, when the diameter of the tumor was roughly 0.5-0.7 mm, a ligature was tied around its base, as a result of which necrosis of the tumor took place.

Method 4. Finely minced tumor tissue was injected successively into the right and left ear and the tail at intervals of between 10 and 20 days. Each of these parts of the body was removed 10-20 days after injection of the tumor, regardless of whether or not a tumor had developed there. In one experiment the last two methods were combined.

Testing of immunity. After 3-4 weeks the immunized and control (fresh) mice of the same highly inbred line were inoculated with a previously titrated dose of living tumor cells (from 1×10^4 to 1×10^6, see table), which led to the development of a tumor in not less than 4 of the 5 fresh isologous mice. The mice were inspected weekly and the development of the tumor was noted. After 3 months the mice which had not developed a tumor, in both the experimental and control series, were reinoculated with the same or a larger dose of tumor cells. Tumors of the 2nd-6th generation were used to test immunity.

RESULTS

The results of 12 experiments on the immunization of mice of highly inbred lines by the methods described above are given in the table. Altogether 7 tumors induced with methylcholanthrene, 2 tumors induced with 9,10-dimethyl-1,2-benzanthracene, and 1 spontaneous adenocarcinoma were studied.

Six tumors induced with methylcholanthrene immunized isologous mice; consequently, these tumors possessed an antigen (or antigens) absent from the isologous host. The total number of mice in the immunization experiments with these 6 sarcomas, induced with methylcholanthrene, was 68, of which 41 mice (60.3%) were resistant to inoculation of living tumor cells. The mice were immune to an inoculum containing 10^4-10^6 living tumor cells. Of the

84 control mice, 7 were "resistant" (8.3%). The methods of immunization that were used were unable to reveal any antigenic differences between the spontaneous adenocarcinoma and the sarcomas induced with dibenzanthracene, on the one hand, and the isologous host, on the other. It should be noted that the results given in the table were analyzed by the goodness-of-fit test (the statistical significance was determined by values of P less than 0.05).

The results described in the present paper were obtained in an isologous system, i.e., in a system composed of highly inbred animals and a tumor arising spontaneously or induced in individuals of this highly inbred line. The state of immunity, expressed by resorption of the cells of the isologous tumor in immunized animals, is a manifestation of reactions of incompatibility between the tumor and host, caused by tissue antigens and specific tumor antigens. Objections relating to residual heterogeneity of the inbred mice can be disregarded, for skin grafts from the mouse donating the tumor to the mice undergoing immunization remained viable for 8-10 months. This is evidence of the practically absolute antigenic identity of the mice within the limits of each of the lines used.

We used primary induced tumors and their first generations for immunization. This factor may mean that primary induced tumors possess specific tissue antigens absent from the tissues of the host. The presence of these antigens in at least 25-30 generations of tumor [8] and the absence of any form of cross immunity between tumors induced by the same carcinogen are evidence against the view that the carcinogen itself enters the determinant group of the specific antigens. The results of the present investigation are in agreement with those obtained by other workers.

LITERATURE CITED

1. M. Feldman, A. Globerson, and D. Yaffe, Proceedings of the 8th International Cancer Congress [in Russian], 3, Moscow-Leningrad (1963), p. 207.
2. K. Habel, J. exp. Med., 115 (1962), p. 181.
3. E. Klein and H. O. Sjögren, Cancer Res., 20 (1960), p. 452.
4. G. Klein, H. O. Sjögren, E. Klein, et al., Ibid., p. 1561.
5. G. Klein, H. O. Sjögren, and E. Klein, Ibid., 22 (1962), p. 955.
6. Idem. Ibid., 23 (1963), p. 84.
7. P. Koldovskii, Folia biol., 7, Praha (1961), p. 170.
8. L. J. Old, E. A. Boyse, D. A. Clarke, et al., Ann. New York Acad. Sci., 101, Art. 1 (1962), p. 80.
9. R. T. Prehn and J. M. Main, J. nat. Cancer Inst., 18 (1957), p. 769.
10. R. T. Prehn, Cancer Res., 20 (1960), p. 1614.
11. Idem, Ann. New York Acad. Sci., 101, Art. 1 (1962), p. 107.
12. L. Revesz, Cancer Res., 20 (1960), p. 443.
13. L. C. Scheinberg, M. C. Levine, K. Suzuki, et al., Ibid., 22 (1962), p. 67.
14. R. Schrek, Am. J. Cancer, 28 (1936), p. 389.
15. H. O. Sjögren, Virology, 15 (1961), p. 214.
16. B. Slettenmark and E. Klein, Cancer Res., 22 (1962), p. 947.