Effect of Rat Tumor Allografts on the Blood Level of Passively Transferred Alloantibodies[1] (36328)

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(Introduced by W. F. Bale)

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There is evidence suggesting that most tumors may be antigenic even in the autochthonous host, and might be expected to elicit a humoral antibody response. In studies with animals, including cases where the tumor is an allograft, antibodies have often not been detected by serological means until after surgical removal or the time of graft rejection (1–4), or the titer measured increased significantly following tumor removal (5). Similar data has been reported for patients with some types of cancer (6, 7).

Among possible explanations for the absence of detectable antibodies are that tumors can induce immunological tolerance or paralysis preventing this type of immune response, or that antibody production does occur, but that antibodies are removed after complexing with tumor antigens in the circulation or by deposition on tumor cells *in situ*. The latter possibility is suggested by evidence that some tumors in the autochthonous host have antibodies bound to them (8, 9). Other explanations for the absence of detectable antibodies have been suggested (7).

This paper reports the results of experiments in which high titer antibodies to Fischer-344 transplantation antigens are produced in Buffalo strain rats and passively transferred to recipient animals. The relative level of circulating antibody is measured in two types of recpient animals: (a) immunosuppressed Buffalo rats bearing transplanted tumors carrying Fischer transplantation antigens; and (b) Buffalo rats similarly treated with immunosuppressive agents, but without tumors. It is shown that these transplantation antibodies disappear more rapidly from the circulation of the tumor-bearing animals.

Materials and Methods. Animals. Inbred female Buffalo (BUF) and Fischer-344 (F344) strain rats, which differ at the major histocompatibility locus (10), were purchased from Microbiological Associates.

Tumor. A chemically induced sarcoma (in the 6 to 20) transplant generations was used in these experiments. The primary tumor was obtained by the sc injection of 0.4 ml of 3-methylcholanthrene (MC) in olive oil (10 mg/ml) into the flank of a F344 rat. This tumor was transplanted from the original host 21 weeks after injection of MC and has been maintained in F344 rats by serial sc trocar implantations.

Antisera. BUF anti-F344 alloantisera were produced by immunizing untreated adult BUF rats with the MC(F344) tumor by a total of 3 or 4 sc trocar implantations of viable tumor performed at biweekly and weekly intervals. The rats were bled 7 to 10 days following the last immunization and the individual sera were stored at $-20°$. In rats major transplantation antigens are expressed on the erythrocyte; sera having hemagglutination titers for F344 red blood cells in the range 1000 to 65,000 were pooled and gamma globulin was prepared.

Gamma globulin preparation. Globulins of immune rat sera were prepared by Na_2SO_4 precipitation as described elsewhere (11, 12). The final precipitate was dissolved with 0.2 *M* phosphate buffer and brought to a final volume of 15% of the starting serum volume. Two preparations of immune BUF gamma

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TABLE I. Titers of Serum from Treated BUF Rats with and without Tumors that Received Preparation 1 of Immune Gamma Globulin.

Rat no.	X-ray dose (R)	Cortisone (mg/injection)	Rat wt (g)	Tumor growth after γ-globulin injection[a] (wt on day of bleeding)	Titer[b]	Day bled after γ-globulin injection
5	400	3	79	Growing (1.2 g)	64	4
6	400	3	67	Growing (2.0 g)	≤16	4
7	400	3	70	Growing (1.2 g)	≤16	4
8	400	3	72	Regressing (0.2 g)	128	4
11	400	3	46	Growing (4.2 g)	≤16	4
12	400	3	75	Control	128	4
13	400	3	70	Control	128	4
14	400	3	65	Control	128	4
15	400	3	63	Control	128	4
16	600	3	68	Growing slowly (0.8 g)	≤16	4
17	600	3	42	Growing (1.7 g)	≤16	4
18	600	3	60	Control	128	4
19	600	3	60	Control	128	4
20	600	0	57	Growing slowly (0.6 g)	32	3
21	600	0	70	Growing (2.6 g)	64	3
22	600	0	78	Growing (1.5 g)	64	3
23	600	0	57	Growing (1.2 g)	32	3
24	600	0	46	Control	128	3
25	600	0	57	Control	64	3

[a] Immune BUF γ-globulin with a titer of 16,000 injected iv 7 days after MC (F344) tumor transplantation.

[b] Reciprocal of highest dilution of serum showing agglutination of F344 red blood cells.

globulin were used in these experiments. They each had a hemagglutination titer of 16,000 when titered against F344 red blood cells.

Hemagglutinin titration. The hemagglutination technique employed was a modification of the method used by Stimpfling (13). The diluent was 0.5% polyvinylpyrrolidone (PVP-360; av mol wt, 360,000; Sigma Chemical Co.) in phosphate buffered saline (7.65 g of NaCl, 0.725 g of Na_2HPO_4, 0.212 g of KH_2PO_4, and 1 liter of distilled water) at pH 7.1. The final diluent contained 1% heat-inactivated (56°, 30 min) normal F344 serum, which eliminated the occurrence of nonspecific agglutination. One-tenth milliliter of BUF antiserum or gamma globulin was added to the first tube of a series of tubes containing 0.1 ml of diluent; and doubling dilutions were made. One-twentieth milliliter of a 2.5% suspension of washed F344 red blood cells in 0.9% saline was added to the tubes containing 0.1 ml of diluted antiserum or gamma globulin; and the tubes were gently shaken. The tubes were incubated for 2 hr at room temperature, then centrifuged for 90 sec at 100*g*. Five-tenths milliliter of saline was added to each tube and they were read by gently flushing the pellet with a pasteur pipet. The hemagglutinin titers are expressed as the reciprocals of the last dilution exhibiting positive agglutination.

In one series of studies sera from BUF tumor and control rats, which had all been treated with cortisone and X-irradiation but not injected with gamma globulin, were added to 0.5% PVP containing 1% heat-inactivated normal F344 serum. The concentrations of these sera in the PVP were 6.25 and 3.125%. Each of these 4 preparations of serum in PVP were used as the diluent in the titration of a BUF anti-F344 alloantiserum.

Immunosuppression. X-Irradiation and cortisone treatment were used to obtain growth of the MC tumor in BUF rats (14). Control and experimental BUF rats, approximately 4

TABLE II. Titers of Serum from Treated BUF Rats with and without Tumors that Received Preparation 2 of Immune Gamma Globulin.

Rat no.[a]	Rat wt (g)	Tumor growth after γ-globulin injection[b] (wt on day of bleeding)	Titer[c]	Time bled after γ-globulin injection
1	69	Growing (3.8 g)	≦16	4 days
3	65	Growing (2.9 g)	≦16	4 days
18	59	Growing (1.0 g)	≦16	4 days
19	91	Growing (3.2 g)	≦16	4 days
5	74	Control	64	4 days
6	66	Control	64	4 days
22	68	Control	32	4 days
15	94	Control	64	4 days
2	57	Growing slowly	128	6 hr
			≦16	4 days
7	48	Growing slowly	256	6 hr
			≦16	4 days
14	53	Static	256	6 hr
			64	4 days
17	46	Growing	128	6 hr
			≦16	4 days
24	52	Growing	256	6 hr
			≦16	4 days
23	50	Control	256	6 hr
			64	4 days
5	62	Control	128	6 hr
			64	4 days
19	50	Control	128	6 hr
			64	4 days

[a] BUF rats treated with a total of 550 R X-irradiation and 3 mg of cortisone/injection.

[b] Immune BUF γ-globulin with a titer of 16,000 injected iv 7 days after MC (F344) tumor transplantation.

[c] Reciprocal of highest dilution of serum showing agglutination of F344 red blood cells.

weeks old, received a total of 400–600 R whole-body X-irradiation in two equal doses on consecutive days from a General Electric Maxitron unit with irradiation factors: 300 kV; 20 mA; HVL, 2.0 mm Cu; TSD, 88 cm; and exposure rate, 29 R/min. Experimental animals were given about 50 mg of the MC tumor subcutaneously in the flank on the day following the last treatment with radiation. Immediately after transplantation, most rats were injected subcutaneously in the back of the neck with 3 mg of cortisone acetate (Upjohn). This was done again on alternate days for a total of 4 injections. Control rats were treated similarly, but did not receive a tumor transplant. The tumors grew to a palpable size 4 days after transplantation and were measured every 2 or 3 days with calipers.

Transfer studies. Immune BUF gamma globulin with a titer of 16,000 was injected iv into BUF rats (0.25 ml/80 g) 7 days after MC (F344) tumor transplantation and into matched control BUF rats treated with the same dose of X-irradiation and cortisone. Animals were bled by heart puncture 3 or 4 days after injection and their sera were tested for hemagglutination of F344 red blood cells. In some instances, 0.1 ml blood samples were taken from the tail 6 hr after injection.

Results. Table I presents the results obtained with one gamma globulin preparation which was injected into experimental and control BUF rats 1 week after tumor trans-

plantation. These animals were bled 3 or 4 days later; and their sera were tested for hemagglutination of F344 red blood cells. The results obtained with a second preparation of gamma globulin injected into the two groups of BUF rats are presented in Table II. Some of these animals were, in addition, bled 6 hr after injection.

Control studies showed that the presence of serum from immunosuppressed BUF rats bearing MC tumors, in amounts equal to or greater than 4% of the 0.1 ml diluent volume in agglutination tubes, sometimes reduced the apparent hemagglutination titer by a factor of 2 compared with similar amounts of serum from immunosuppressed BUF rats without tumors. Corrections for this inhibitory effect have been made in Tables I and II as follows: in all cases where the tubes with tumor serum dilutions of one-half and greater were negative the antibody titer has been recorded as equal to or less than 16. In any instance where the antibody titer from tumor sera was read as 16 or 32, it has been increased to 32 or 64, respectively. Corrections are not made for sera with higher titers because the concentrations of the diluted BUF sera plus 1% F344 serum in the end-point tubes are less than 4%. The correction factor used is probably in most instances an overcorrection for any specific inhibitory effect of serum from tumor-bearing animals.

Discussion. With a few exceptions, serum taken from rats bearing sc tumors had titers lower by a factor of 4 or more than serum from control rats without tumors 3 or 4 days after gamma globulin injection. Serum samples taken from tumor and control rats 6 hr after injection appeared to have the same antibody titers. These results indicate that, in the experimental system studied, the presence of tumor leads, in most instances, to the more rapid disappearance of passively administered transplantation antibodies. A likely corollary is that the antigens furnished by tumor are, in some way, available to react with the circulating antibodies to an extent that the proportion of antibody removed can be large during a 3 to 4 day period.

Several investigations have been concerned with the question of whether there is a more rapid removal of antibodies reacting with tumor from the circulation of tumor animals. Amos (3) found that free allogeneic tumor cells injected iv into mice just after the ip injection of alloantiserum reduced the titer of their sera by one-eighth to one-sixteenth compared to those of controls without the tumor injection. He also reported, without presenting detailed data, that animals with growing solid allogeneic tumors receiving alloantiserum had about the same titers as controls. Takasugi and Hildemann (4) reported, without much detail, that animals that received passive antibody and a sc inoculum of tumor cells on the same day had slightly lower titers than controls not receiving the tumor inoculum. Gorer (15) found that the titers of antibodies produced following an allogeneic tumor transplant were transiently depressed following a second or third solid tumor transplant.

The fate of this transferred antibody in tumor-bearing animals was studied (16) by the use of purified radiolabeled antibodies isolated from the pool of immune serum used in this study. It was shown that there is a very substantial localization of this antibody in tumor tissue.

Summary. High titers of transplantation antibodies, as measured by a hemagglutination test, were induced in Buffalo strain rats by repeated inoculations of a carcinogen-induced tumor maintained in Fischer-344 rats. This tumor grew in Buffalo rats immunosuppressed by X-irradiation and cortisone. When a gamma globulin fraction from pooled immune serum was injected intravenously into such tumor-bearing rats the antibody titer in blood declined more rapidly than in similar immunosuppressed rats without tumor transplants.

1. Pilch, Y. H., and Riggins, R. S., Cancer Res. **26**, 871 (1966).

2. McKann, C. F., and Harder, F. H., *in* "Proceedings of the First International Congress of the Transplantation Society" (J. Dausset, J. Hamburger, and G. Mathé, eds.), p. 495. Paris (1967).

3. Amos, D. B., Brit. J. Cancer **9**, 216 (1955).

4. Takasugi, M., and Hildemann, W. H., J. Nat. Cancer Inst. **43**, 857 (1969).

5. Feldman, M., and Sachs, L., J. Nat. Cancer Inst. **18**, 529 (1957).

6. Gold, P., Cancer **20**, 1663 (1967).

7. Lewis, M. G., Phillips, T. M., Cook, K. B., and Blake, J., Nature (London) **232**, 52 (1971).

8. Witz, I. P., Isr. J. Med. Sci. **7**, 230 (1971).

9. Ran, M., and Witz, I. P., Int. J. Cancer **6**, 361 (1970).

10. Palm, J., and Black, G., Transplantation **11**, 184 (1971).

11. Witz, I., Klein, G., and Pressman, D., Proc. Soc. Exp. Biol. Med. **130**, 1102 (1969).

12. Witz, I., Yagi, Y., and Pressman, D., J. Immunol. **101**, 217 (1968).

13. Stimpfling, J. H., Transplant. Bull. **9**, 109 (1961).

14. Toolan, H. W., Cancer Res. **13**, 389 (1953).

15. Gorer, P. A., Cancer Res. **7**, 634 (1947).

16. Izzo, M. J., Buchsbaum, D. J., and Bale, W. F., Proc. Soc. Exp. Biol. Med. **139**, 1185 (1972).

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