Establishment of Resistance to Growth of Ehrlich Ascites Carcinoma in C57 Black Mice.* (25328)

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The Ehrlich carcinoma, growing either as an ascites or solid cancer in mice, is known to kill nearly all its hosts. Our observation of the survival of about one C57BL mouse out of each 100 animals, following an initial and repeated injections of large doses of viable carcinoma cells, led us to consider the possibility of producing the resistance at will. We have been able to produce in C57BL mice a resistance to viable Ehrlich ascites carcinoma cells by repeated injection of x-irradiated Ehrlich cells. Likewise, Donaldson and Mitchell (1) have recently reported protection of mice against growth of Ehrlich ascites tumor employing x-irradiated cells. Revesz(2), working with a number of different cancer cells, including Ehrlich's carcinoma, showed that implantation of an inoculum of viable cells admixed with a larger amount of the cancer cells lethally damaged with x-rays, caused an increase in cancer growth in highly inbred mice strains, while there was a slowing of cell growth when injected into genetically incompatible animals.

Materials and methods. The mice used in this study were C57BL males and females produced in our vivarium by indiscriminate interbreeding of stock obtained from the Roscoe B. Jackson Memorial Labs., Bar Harbor, Maine, about 4 years ago. At start of experiments the mice were 8-16 weeks of age. The Ehrlich ascites carcinoma was obtained 7 years ago from the Massachusetts Gen. Hosp., Boston, and since then has been grown in our laboratory by weekly 0.1 ml intraperitoneal and less frequent subcutaneous passages through C57BL mice. Ascites carcinoma grown intraperitoneally for 7 days was employed for the studies. The liquid tumor was withdrawn from the peritoneal cavity under sterile conditions and employed untreated for injection or placed in special sterile flasks for rotation under the x-ray machine. Two to 3 ml of ascites tumor were placed in these 50 ml balloon flasks and rotated at a rate of 30 rpm. The tumor was irradiated with 2000, 4000 and 8000 r, at a rate of about 50 r per minute and a focal distance of 45 cm. Dose rate was calibrated in air for the 250 kv x-ray head having 0.21 mm of Cu, inherent. Additional filters were 1 mm of aluminum and 0.5 mm of Cu, parabolic. The half value layer was 2.1 mm of Cu. All tumor injections into the mice were made intraperitoneally with Ehrlich ascites carcinoma containing about 100,000 cancer cells per mm^3. All challenges were made with 0.05 ml of ascites carcinoma. Metabolic studies were carried out on the carcinoma cells following a 3-fold washing with cold isotonic 55 mM phosphate solution (3). Oxygen consumption measurements were performed as previously described(3) employing glucose as the substrate at 38°C. Glucose utilization determinations were made at intervals of 30 minutes on tumor cells incubated at 38°C employing the methods of Somogyi(4) and of Nelson(5).

Results. The data in Table I indicate that little resistance to growth of the cells in mice is produced until 5 or more injections of x-irradiated tumor were given. There appear to be only small differences in the results obtained employing 5 to 10 injections of x-irradiated tumor. As indicated in Table II, varying amounts of the 2000 r irradiated tumor (0.025 to 0.5 ml) were tested. There were no discernible differences in resistance produced when employing 8 injections of .025, .05 or .1 ml of x-irradiated tumor. One injection of .05 ml produced no effect, however at .2 and .5 ml there appeared to be some protection (Table II). The .5 ml dose is about the maximum amount tolerated, since the mice developed large quantities of tumor with grossly distended abdomens. However, the

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TABLE I. Production of Resistance in C57 Black Mice to Ehrlich Ascites Carcinoma Cells. (0.05 ml Ehrlich ascites carcinoma cells x-irradiated with 2000 r and injected intraperitoneally once each week.)*

No. of mice	No. of inj. x-irrad. cells	Animals surviving challenges† (No. of challenges, non-x-irradiated cells) 1	2	3	4	5	6	7	8	9	10
		(No. of animals)									
6	1	0									
4	2	0									
4	3	3	1	1	1	1	1	1	1		
2	4	0									
4	5	2	2	2	2	2	2	2	2		
4	6	4	4	4	4	4	4	4	4		
42	8	40	26(2)‡	20	19	19	19(4)‡	15	15(1)‡		
20	10	20	19	17	16	16	16	15(1)‡	14	14	13(1)‡

* Control groups are not indicated but each week 15-25 mice were inj. with 0.05 to 0.20 ml of Ehrlich ascites carcinoma and 99% of the animals died with avg time of death about 14 days.

† All challenges were made with 0.05 ml of ascites carcinoma containing about 100,000 cancer cells/mm³.

‡ No. in parentheses are No. of animals sacrificed for complement fixation studies.

animals were able to control their tumor growth. Table III indicates that between 2000 and 4000 r is the optimum amount of x-irradiation under the conditions employed. If less than 2000 r were employed there was too little alteration of the cells and the mice died of cancer. Various procedures were employed in unsuccessful attempts to produce altered cells which would be effective in producing resistance in mice. These attempts included freezing and thawing, grinding with a Ten-Broeck tissue grinder, ultra-sonic vibration and lyophilizing. Either there was inadequate alteration as in the first 3 conditions and the cancer cells overwhelmed the mice or the cells were completely destroyed and the mice showed no resistance, as was observed with lyophylizing.

TABLE II. Influence of Size and Number of Injections on Production of Resistance in C57 Black Mice to Ehrlich Ascites Carcinoma Cells. (Ehrlich ascites carcinoma cells were x-irradiated with 2000 r and inj. intraper. twice each week.)

No. of mice	No. of inj. x-irrad. cells	Size of dose (ml)	Animals surviving challenges (No. of challenges, viable cells) 0	1	2	3
			(No. of mice)			
5	8	.025	5	5	2	2
5	8	.05	5	5	2	2
5	8	.10	5	5	2	2
26	1	.20	26 (22)*	1	0	
6	1	.50	6 (4)*	1	0	

* Sacrificed to determine tumor volume.

Before first challenge of those receiving the 0.20 ml dose 3 mice died, and of those receiving the 0.50 ml dose 1 mouse died. These deaths were due to inadequate x-irradiation of the tumor.

TABLE III. Influence of Varying X-ray Dosage on Production of Resistance in C57 Black Mice to Ehrlich Ascites Carcinoma Cells. (0.05 ml of Ehrlich ascites carcinoma cells x-irradiated with 2000 r and inj. intraper. twice each week.)

No. of mice	No. of inj. x-irrad. cells	Amt of x-irrad. (r)	Animals surviving challenges (No. of challenges, viable cells) 1	2
6	8	2000	6	4(1)*
6	8	4000	6	2(2)*
6	8	8000	0	

* Animals sacrificed for complement fixation studies.

Table IV shows the slow rate of growth and eventual control of the injected irradiated tumor, compared to the rapid rate of cell growth with non-treated cells. Average survival time of the latter was about 14 days. It was determined that the x-irradiated tumor growing in mice for 2 to 6 days and being controlled by the host would, when injected into non-resistant mice, kill within 18 to 23 days. This shows a remarkable recovery of viability of x-irradiated cells as well as a production of resistance in the first host.

Table V indicates some alteration of metabolism of the cancer cells produced with various amounts of x-irradiation. It is interesting that there was inhibition of oxygen con-

TABLE IV. Growth of X-Irradiated and Non-Irradiated Ehrlich Ascites Carcinoma Cells. (Single 0.2 ml intraper. inj. of ascites tumor, 0.05 ml cells.)

Treatment of tumor, r	Vol of tumor cells (ml) Days after inj. 2	4	5	6	7	8	9
None	.20	.50	.66	1.06	1.15	1.25	1.90
2000	.20	.21*	.22*	.23*	.13	.13	.12

* Tumor from these 3 animals which had received the 2000 r tumor, when inj. into non-resistant mice killed in 19, 23 and 18 days, respectively. A second serial passage of the regressing tumor killed the mice in 14 to 16 days. Avg survival time of mice given non-treated and viable tumor survived an avg of 14 days.

sumption when the cells were treated with 500 r, and stimulation when greater amounts of irradiation were employed. Glucose consumption on the other hand was stimulated by irradiation particularly at 2000 r. Microscopic examination of the irradiated cells indicated the nuclei to be altered markedly. More detailed enzymatic studies are being carried out to evaluate more completely the cellular changes effected by x-irradiation.

Furthermore, preliminary studies show that 8 injections of x-irradiated Ehrlich ascites carcinoma cells also prevent growth of Ehrlich cancer cells injected subcutaneously in C57 black mice.

Discussion. Other technics have been applied with less success for production of immunity in the host animal carrying a tumor. Fink, Smith and Rothlauf(6) were able to show production of antibody in BALB/C mice following injection of lyophilized tumor S 621 in Freund's adjuvant. Hirsch, Bittner, Cole and Iversen(7) have reported that inbred strain C Bagg albino mice can be partially immunized against its own tumor, C 10040, employing the technic of Martinez(8). Also, Lund(9) has been able to vaccinate rats against Yoshida ascites sarcoma by injecting the viable carcinoma cells intracaecally; and Bradner, Clarke and Stock(10) stimulated the defenses of the female Swiss albino mouse to sarcoma 180 by injecting zymosan; Prince, Fardon, Nutini and Sperti(11) produced immunization with a transplantable mouse tumor. It is our belief that x-irradiation of the Ehrlich ascites carcinoma temporarily modifies the cells in such fashion as to alter and slow their growth and make them more antigenic. There is stimulation of formation of antibodies, which increases with repeated injections of the altered tumor. This is strongly indicated by the control of growth of the x-irradiated Ehrlich cells in the original mouse, and subsequent killing of the recipient mouse into which the regressing tumor is transferred. Further evidence for immunization of the C57BL mouse to the Ehrlich ascites carcinoma cell are our preliminary complement fixation analyses showing high titres of antibodies in the blood of the mice to the ascites carcinoma cells.

TABLE V. Influence of X-Irradiation on Oxygen Consumption and Glucose Utilization of Ehrlich Ascites Carcinoma Cells.

Treatment of cells (r)	Glucose (μM/0.04 ml cells/hr)	Oxygen (μM/0.04 ml cells/hr)
0	4.1	3.1
500	4.3	2.6
1000		3.0
2000	6.8	3.4

Summary. It has been possible to immunize C57BL mice against Ehrlich carcinoma by giving 5 to 10 injections of Ehrlich carcinoma x-irradiated with 2000 to 4000 r. Of 70 mice so immunized 66 survived the first challenge with viable cells; and all 35 of the mice receiving 8 challenges survived. It appears that the carcinoma cells are altered by x-irradiation and thus stimulate production of resistance to both x-irradiated and completely viable Ehrlich carcinoma cells.

1. Donaldson, D. M., Mitchell, J. R., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 564.

2. Revesz, L., *J. Nat. Cancer Inst.,* 1958, v20, 1157.

3. McKee, R. W., Lonberg-Holm, K., Jehl, J., *Cancer Research,* 1953, v13, 537.

4. Somogyi, M., *J. Biol. Chem.,* 1945, v160, 61.

5. Nelson, N., *ibid.,* 1944, v153, 375.

6. Fink, M. A., Smith, P., Rothlauf, M. V., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 590.

7. Hirsch, E. M., Bittner, J. J., Cole, H., Iversen, I., *Cancer Research,* 1958, v18, 344.

8. Martinez, C., Aust, J. B., Bittner, J. J., Good, R. A., *ibid.,* 1957, v17, 205.

9. Lund, H. J. C., *Brit. J. Cancer,* 1950, v9, 475.

10. Bradner, W. T., Clarke, D. A., Stock, C., *Cancer Research,* 1958, v18, 347.

11. Prince, J. E., Fardon, J. C., Nutini, L. G., Sperti, G. S., *ibid.,* 1957, v17, 312.

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