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Acceptance of Walker 256 Carcinosarcoma by Neonatally Thymectomized C57 BL/6 Mice. (30806)

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The significance of the thymus in the development of immunologic competence in many animals is well documented(1). The thymus also plays a role in the growth of certain tumors in rodents. Some experimental leukemias in mice are dependent on the presence of the thymus(2). Conversely, after neonatal thymectomy, the incidence of tumor formation in rats inoculated with the polyoma virus is increased(3,4), and the natural resistance of C57 BL mice to the oncogenic effects of this virus is decreased(5). Thymectomy performed in mice 3 days of age is followed by an increased incidence of skin tumors induced by benzopyrene(6). Neonatally thymectomized Z (C_3 H) mice accept allogeneic grafts of a mammary tumor from A strain mice despite histo-incompatibility between these strains at the H-2 locus(7).

The Jensen sarcoma, transplanted allogeneically to neonatally thymectomized Sprague-Dawley rats, "takes" more often and grows more rapidly than in control rats(8). Rejection of xenografts of the mouse sarcoma 1 ascites tumor by neonatally thymectomized inbred Lewis rats is delayed(9). On the other hand, neonatal thymectomy in Sprague-Dawley and Long-Evans rats does not alter the incidence, rate of growth, or development of metastases of transplanted Walker 256 tumor, a neoplasm that grows well in this species in any case(10).

Extracts of thymus have been shown to contain both tumor-promoting and tumor-inhibiting substances, the latter, at least, being not specific for thymic tissue(11).

The present work was undertaken in search of a combination of tumor and host that would delineate sharply and reasonably rapidly the role of the thymus in mice with

respect to growth of a transplanted tumor.

Methods. A colony of C57 BL/6 mice* was established, fed a commercial mouse chow† *ad libitum,* and maintained in an air-conditioned room at an environmental temperature of 70 to 72°F with free access to water. Mice were weaned at 4 weeks of age.

Thymectomies were performed by the technique of Sjodin and associates(12); hypothermia was used for anesthesia on a group of mice operated on within 24 hours of birth and ether on another group operated on at 35 to 40 days of age. At the conclusion of the experiments, all mice except the few that had been eaten by cagemates were examined grossly; if necessary, specimens were studied microscopically to ascertain whether thymectomy had been complete. Control series consisted of other newborn mice that were subjected to sham operations in which the thymuses were exposed but not removed, and of unoperated mice.

The Walker 256 carcinosarcoma tumor‡ was maintained by serial passages in Sprague-Dawley rats. For this purpose and for transplantation to mice, each rat bearing the tumor was anesthetized with ether, the fur in the region of the tumor was clipped, the skin was prepared with 70% ethanol and Merthiolate, and the tumor was dissected from the subcutaneous tissues, minced, ground in 2.5 to 4.0 ml of Tyrode's solution by means of a Ten Broeck hand homogenizer, and decanted into a test tube; sterile technique was used throughout. Aliquots of the suspension of tumor cells were diluted with 0.5% acetic acid in leukocyte pipettes and counted in a hemocytometer. Accurate counting was made difficult by clumping of cells, and estimates ranged from 50,000 to 100,000/mm^3. The suspension was injected into recipient animals subcutaneously in the region of the back in volumes of 0.05 to 0.2 ml adjusted to give an estimated dose of 5×10^6 tumor cells. Although this figure must be regarded only as an estimate, it was reasonably constant from experiment to experiment. Samples of each tumor from the rats were fixed in 10% neutral formalin, blocked in paraffin wax, sectioned at 7 to 8 μ, stained with hematoxylin and eosin, and examined by light microscopy for histologic confirmation of the presence of the Walker tumor. A portion of the cell suspension was cultured in each case, and only data from experiments in which the bacterial cultures were negative were accepted. In each experiment, rats were given the same dose of tumor cells given to the mice, and in each case large tumors resulted.

Mice were examined at least 3 times weekly for 4 to 5 weeks or until death. The presence or absence of tumors was noted, and their sizes were measured with a graduated caliper. Some mice were killed to permit histologic assessment of their tumors, and sections were made of tumors of others found dead before autolysis or cannibalism made such examination impossible.

Results. Because preliminary review of the data showed no differences with respect to sex, results from males and females have been combined.

Findings in unoperated control mice. Since it was possible that age when tumor was injected might be a factor in the results, and in order to have controls of the same ages as those of 2 groups of thymectomized mice, the controls were divided into 4 groups according to their ages at the time of injection of tumor cells.

1. Tumor injected within 24 hours of birth. Four of 14 mice died within 2 days. Palpable subcutaneous nodules developed in 5 of the remaining 10, reached an average maximal size of 4 mm, and disappeared within 14 days in 2 animals in which this observation could be made (Table I). Nodules 2 to 3 mm in diameter removed from 3 mice for histologic study after 7 days consisted of inflammatory tissue in 2 instances and of Walker tumor in the third.

2. Tumor injected 4 to 6 days after birth. Three of 28 mice died within 4 days. Palpable subcutaneous nodules developed in 17 of the remaining 25 and reached an average maximal size of 7 mm. Nodules disappeared

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TABLE I. Walker 256 Carcinosarcoma Cells Injected Subcutaneously into C57 BL/6 Mice.

Age (days) when tumor was injected	Palpable "tumors" Incidence: No. tumors /No. mice	Incidence: %	Maximal size (mm), mean and range	Regressed within 28 days
Controls				
1	5/10	50 } 63	4 (2- 8)	Yes
4- 6	17/25	68 }	7 (3-16)	"
11-27	13/27	48	2 (1- 5)	"
40	6/18	33	2 (1- 5)	"
Sham operation neonatally*				
13-27	7/15	47	4 (3- 7)	"
Thymectomy:				
Operation at age 35-40 days; tumor injected 5-7 days later	11/19	58	4 (2-10)	"
Thymectomy neonatally:				
Operation at age 0-24 hr; tumor injected at age 13-27 days	28/32	88	26 (15-40)	No

* Within 24 hr of birth.

within 28 days in 14 of 15 surviving mice and within 14 days in 7 of these. The 5-mm nodule in the remaining animal, dissected on the 29th day, was liquefied completely and could not be sectioned for histologic study.

3. Tumor injected 11 to 27 days after birth. Subcutaneous nodules developed in 13 of 27 mice and were palpable within 7 days in 11 of the animals. Their average maximal size was 2 mm. The nodules disappeared within 14 days in 8 of 9 mice in which this observation could be made and after 21 days in the ninth animal. Three nodules, 1-2 mm in size, examined histologically between 7 and 14 days after injection showed necrotic Walker tumor.

4. Tumor injected 40 days after birth. Subcutaneous nodules developed in 6 of 18 mice within 7 days of injection of tumor cells and averaged 2 mm in greatest size. These nodules disappeared within 14 days in the 5 animals in which this observation could be made. In the remaining mouse, histologic sections of the nodule made on the 13th day showed only inflammatory tissue.

Sham-operated control mice. Tumor cells were injected into 15 mice aged 13 to 27 days that had undergone the sham operation within 24 hours of birth. Subcutaneous nodules developed in 7 of the 15 animals, were palpable in all but one of these by the seventh day, and averaged 4 mm in greatest size. The nodules disappeared by the 14th day in 4 of 5 mice in which this observation could be made. Nodules removed from 3 mice for histologic study consisted only of inflammatory tissue.

Thymectomized mice. 1. Mice thymectomized 35 to 40 days after birth. Walker tumor cells were injected into 23 mice 5 to 7 days after thymectomy was carried out 35 to 40 days after birth. Four mice subsequently proved to have residual thymic tissue and were excluded from the series. Palpable subcutaneous nodules developed in 11 of the remaining 19 animals, averaged only 4 mm in greatest size, and disappeared in all but 1 case by the 14th day. None of the nodules was studied histologically. Thus these mice responded to injections of the Walker tumor as did control mice.

2. Mice thymectomized within 24 hours of birth. Walker tumor cells were injected into 36 mice aged 13 to 27 days, 2 of which were excluded later because residual thymic tissue was found and 2 because they died within 5 days after injection. Palpable subcutaneous masses developed in 28 of the remaining 32 mice and were evident within 7 days in all but 2; none disappeared within the 28 days of the experiment. The tumors that developed in the neonatally thymectomized mice were much larger than those seen in controls, reaching sizes of 40 mm in many

instances and averaging 26 mm in diameter 14 days after injection of tumor cells. After 14 days, the tumors tended to remain static in size, although some became smaller when ulceration was followed by escape of liquefied debris. The mortality after inoculation with tumor cells was much greater in neonatally thymectomized mice than in controls. Only 2 of 30 mice in which events were allowed to run their course survived more than 28 days, and 23 mice died within 3 weeks. Tissue examined histologically 13 to 37 days after inoculation showed Walker tumor in 8 of 9 cases, the exception being an instance in which a mass of partially liquefied debris examined on the 37th day showed only necrotic material with some inflammatory tissue. No distant metastases were found in any of the animals.

Comment and conclusions. Although palpable nodules, shown in some cases to contain tumor cells, developed in some but not all control C57 BL/6 mice injected with the Walker 256 tumor of rats, these nodules regressed and finally disappeared within 2 to 4 weeks. Thus, the Walker tumor grows briefly in C57 BL/6 mice, but under the conditions of these experiments it was soon rejected by the host.

It would not have been surprising had the tumor been rejected more slowly when given to normal mice less than 1 day old or even 4 to 6 days old than when given to older animals, because such young mice are relatively incompetent immunologically at this age. Indeed, if circumstances were right, development of acquired immunologic tolerance might be possible(13). In the present experiments, palpable nodules developed in a higher percentage of mice less than a week old than was the case in older control animals, and the nodules usually became larger in the young mice before regression occurred. In our experiments, the absence of evidence of acquired tolerance developing in the very young mice might be related to the dosage of tumor cells, but to explore this relationship further studies would be needed in which larger doses of tumor cells were used.

Clearly, thymectomy performed within 24 hours of birth, but not at 35 to 40 days of age, permitted Walker 256 tumor cells to grow in C57 BL/6 mice and usually to kill the host within 28 days.

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