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Experimental Studies of Factors Influencing Hepatic Metastases

XV. Effect of Neonatal Thymectomy[1]

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SUMMARY

As a result of accumulating evidence which indicates that the thymus plays a vital role in the development of immunologic competence in mammals, and that this gland may be of importance in tumor growth, it was deemed worthwhile to evaluate the effect of neonatal thymectomy upon the development of experimentally induced hepatic metastases. In 2 strains of rats at various ages which exhibit variable susceptibility to transplantation of Walker carcinoma, it was observed that in no instance did thymectomy at birth result in subsequent augmentation of the incidence or growth of tumor. Rather, a significant reduction in metastases from that observed in intact controls resulted. This effect was not related to wasting, runting, or differences in the nutrition of animals. Since neonatal thymectomy in this species has been demonstrated to suppress the transplantation reaction, it is suggested, contrary to the general contention, that immunologic problems of organ and tumor transplantation may be different. A possible mechanism involving the pituitary gland to account for the metastatic suppression observed has been considered.

With increasing appreciation of the role of the thymus gland in the development of normal immunologic competence (2, 19, 20), it naturally followed that consideration should be given this organ relative to its influence on tumor growth. The results of a few such studies directed toward investigations of thymic function on various parameters of neoplasia have been reported. Perri *et al.* (23) noted that implanted Jensen sarcomas became measurable in intact and neonatally thymectomized Sprague-Dawley rats at the same time following inoculation, but that subsequent tumor growth was more rapid in the latter. Martinez *et al.* (18) transplanted an A strain mammary adenocarcinoma in Z (C3H) mice thymectomized shortly after birth. The incidence of "takes" was greater in such mice, and they died more rapidly of tumor than did control animals. The longer the interim between thymectomy and grafting, the less frequently tumor occurred in both thymectomized and control mice.

The observations that rats thymectomized at birth were more sensitive to the oncogenic activity of the polyoma virus than were normal animals (17, 22, 25) and that skin tumors arise earlier and regress less readily in such animals treated with 3,4-benzopyrene (21) suggest a relationship of the thymus through immune mechanisms to carcinogenesis. It has been noted by Maisin (16) that less tumors occurred in mice painted with 20-methylcholanthrene when they were the recipients of thymic grafts or homogenates. On the other hand, Fumarola and Giordano (12) observed that thymectomy inhibited and thymic extracts augmented sarcomatous growth following s.c. injection of benzopyrene.

Other reports (3, 4, 24) and the role of the thymus in experimental leukemia (13, 14) further implicate this gland with tumor growth. None, however, has related the thymus to the phenomenon of metastasis. Because of our interest for almost a decade in evaluating the influence of a variety of host factors upon the development of experimental metastases (7, 10), it seemed worthwhile to determine the effect of neonatal thymectomy upon this facet of the neoplastic process.

MATERIALS AND METHODS

Pregnant female Sprague-Dawley rats were obtained from 2 different suppliers (Holtzman, Madison, Wisc.; Simonsen, Gilroy, Calif.) and will be referred to as Sprague-Dawley/H or Sprague-Dawley/S animals. They were housed in individual cages and fed an *ad libitum* diet of rat chow supplemented with canned dog meat, cabbage, and apples. Within 12 hr after birth the newborn rats were randomized so that ½ of the members of each litter were subjected to thymectomy and the others served as unoperated controls. Three to 4 weeks following birth they were weaned from the mothers, placed in individual cages and fed the adult diet.

After attempting a variety of technics for thymectomy,

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a standardized procedure was developed. A longitudinal incision was made over the sternum, and a small right angle hemostat was inserted behind it at the sternoclavicular junction. The sternum was divided so as to expose the thymus. A small loop of fine wire on the end of a hemostat acting as a snare was introduced so that the thymus was encompassed by it. As the snare was withdrawn the thymus was removed. Slight bleeding was readily controlled, and the skin was closed with a continuous 6-0 black silk suture. The surgical mortality was minimal. Cannibalism was the major cause of death, and in spite of a variety of measures, it could not be totally controlled.

At approximately 1 and 2 months after birth animals were inoculated intraportally with cells from Walker carcinomas. This tumor has been propagated in this laboratory for the past 7 years. The method of preparation of cell suspensions and the technic of injection have been previously described in detail (8).

Fourteen days following inoculation all animals were sacrificed and livers were inspected for the presence of tumor. Complete autopsies were performed. Careful examination for a thymic remnant was made, and animals demonstrating incomplete thymectomy were catagorized as a separate group. Questionable thymus was confirmed by histologic study. Pituitary, adrenal, thyroid, gonad, and spleen weights were obtained, and these will form the basis of a subsequent report.

RESULTS

Intact, control Sprague-Dawley/H rats (Group A) inoculated intraportally with 5000 tumor cells demonstrated a comparable incidence of liver metastases when they were injected either at 30 or 60 days of age (55% in the former; 45% in the latter) and examined 14 days later (Table 1). Animals (Group B) which had been thymectomized at birth and inoculated with tumor at similar ages to those in Group A, but which at sacrifice were found to

TABLE 1

EFFECT OF THYMECTOMY ON THE PRODUCTION OF HEPATIC METASTASES IN SPRAGUE-DAWLEY/H RATS

Group	At Tumor Cell Injection		Liver Metastases			
	Age (days)	Body wt. (gm)	No. of rats used	No positive	No negative	% positive
5,000 tumor cells injected intraportally						
A. Normal control	34 ± 3[a]	93 ± 24	90	50	40	55
	62 ± 3	199 ± 47	113	51	62	45
B. Partial thymectomy	34 ± 5	86 ± 20	39	23	16	59
	65 ± 4	192 ± 47	52	28	24	54
C. Total thymectomy	35 ± 3	89 ± 24	46	22	24	48
	64 ± 5	188 ± 55	52	6	46	12

[a] Mean ± standard deviation.

TABLE 2

EFFECT OF THYMECTOMY ON THE PRODUCTION OF HEPATIC METASTASES IN IN SPRAGUE-DAWLEY/S RATS

Group	At Tumor Cell Injection		Liver Metastases			
	Age (days)	Body wt. (gm)	No. of rats used	No positive	No negative	% positive
5000 tumor cells injected intraportally						
A. Normal control	36 ± 3[a]	106 ± 21	55	9	46	16
	75 ± 1	212 ± 17	47	2	45	4
B. Partial thymectomy	36 ± 4	105 ± 37	44	7	37	16
	71 ± 12	197 ± 44	19	2	17	11
C. Total thymectomy	35 ± 4	97 ± 36	31	0	31	0
	72 ± 13	208 ± 59	33	0	33	0
100,000 tumor cells injected intraportally						
A. Normal control	58.6 ± 6	201 ± 41	37	5	32	14
B. Partial thymectomy	65.6 ± 7	221 ± 60	26	6	20	23
C. Total thymectomy	68.5 ± 9	211 ± 44	34	1	33	3

[a] Mean ± standard deviation.

have thymic remnants, demonstrated a similar incidence of hepatic metastases to the unoperated control animals. Rats (Group C) treated in similar fashion to the other 2 groups, but found at sacrifice to be totally thymectomized, demonstrated a significant difference in metastases relative to their age at tumor cell injection. Of animals inoculated at approximately 30 days of age, 48% demonstrated tumor, whereas only 12% of those injected at approximately 60 days had neoplasms.

No significant difference was observed in body weights of animals of comparable age in the 3 groups at the time of tumor cell injection.

When intact control Sprague-Dawley/S rats (Group A, Table 2) were inoculated with 5000 tumor cells intraportally, the incidence of liver metastases was markedly less than in Sprague-Dawley/H animals. Also, the incidence of tumors was less in older animals (16% at approximately 30 days *versus* 4% at 75 days). Essentially the same findings were noted in thymectomized animals found to have a thymic remnant at sacrifice (Group B).

Not one of 64 totally thymectomized animals (Group C) injected with tumor at about 35 or 73 days was found to have metastases at sacrifice.

Results were similar when 100,000 tumor cells were injected. Only 1 (3%) of 34 totally thymectomized rats grew tumor, whereas 14% of normal controls and 23% of partially thymectomized rats had metastases. As with Sprague-Dawley/H animals, Sprague-Dawley/S animals demonstrated no difference in body weight between the control and thymectomized groups.

DISCUSSION

Two strains of animals were employed in this study because of the differences noted in their susceptibility to transplantation of the Walker tumor. It was contemplated that by their use the effects of thymectomy might become more apparent. Normal Sprague-Dawley/H rats have repetitively and predictably developed hepatic metastases in approximately 50% of animals inoculated intraportally with 5000 tumor cells, and the age of animals has not influenced this incidence. Sprague-Dawley/S animals, on the other hand, have grown tumors less readily, and older members of this strain exhibited even less incidence of growth. This experience is similar to that of Martinez *et al.* (18), who also observed a decreased susceptibility to transplantation of a mammary carcinoma of A strain mice to Z strain recipients with advancing age.

While differences in metastatic growth were observed in this study to be related to strain and age of the animals, in no instance was there evidence that neonatal thymectomy augmented this phenomenon. In fact, all thymectomized tumor-inoculated animals, with the exception of 1-month Sprague-Dawley/H rats, demonstrated a significant reduction in the occurrence of hepatic metastases. Explanation for results in younger Sprague-Dawley/H animals is not apparent, but it is possible that the mechanism(s) responsible for metastatic suppression in thymectomized animals had not as yet become operative. The failure of thymectomy to augment metastases supports other reported observations by us (11) relative to the growth of s.c. transplants of Walker tumor in neonatal thymectomized rats of a variety of strains. In those studies no difference in incidence, early growth, or histologic appearance of tumor was evident in thymectomized and control rats of any strain. Since increased or prolonged skin homograft survival did occur in such animals, it was concluded that, contrary to the general contention, the immunologic problems of organ and tumor transplantation are separable.

Since it has been previously demonstrated by us (5, 9) that, in the experimental model employed, nutrition and state of health may influence tumor growth, it is emphasized that the findings are not related to these factors. Both control and thymectomized animals were of comparable weight, consumed approximately the same amount of food, and were in good health. Our experience has been similar to that of others (1, 15) with this species in that only thymectomized rats which are in poor health—with wound or lung infections—demonstrate "runting".

That these results are divergent from those of others suggests that if the thymus does exert a protective influence on tumor growth via immunologic mechanisms, as has been proposed, some degree of tumor, as well as species specificity for this function must exist. Moreover, the influence of the thymus on the phenomenon of metastases may be mediated indirectly by an as yet undisclosed mechanism rather than through its relationship to immunologic competence. While it is popularly held that the phenomenon of metastasis may be related to host immunologic mechanisms, no evidence to support this hypothesis is, to our knowledge, available.

The suppression of metastases in thymectomized animals is reminiscent of similar findings observed in hypophysectomized animals (6). Preliminary observations that the pituitary glands in thymectomized rats may weigh less than in controls suggests the possibility that the decreased tumor incidence might be the result of an as yet undisclosed thymic-pituitary interrelationship. Definitive studies directed toward the elucidation of such a mechanism are in progress.

No correlation could be ascertained between the amount of thymic remnant present and the occurrence of metastases.

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