

Spontaneous Mammary Adenocarcinoma in Mice: Influence of Thymectomy and Reconstitution with Thymus Grafts or Spleen Cells¹

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SUMMARY

Neonatal thymectomized C3H MTV⁺ (mammary tumor virus) female mice which were grafted with syngeneic thymus from either MTV⁺ or MTV⁻ animals developed spontaneous mammary tumors as frequently as did sham-operated controls.

Neonatal thymectomized C3H MTV⁺ females which were injected with spleen cells obtained from syngeneic MTV⁺ mice had a significant reduction in mammary tumor incidence and a prolonged latent period of tumor development.

Neonatal thymectomized C3H MTV⁺ females which were injected with spleen cells obtained from syngeneic MTV⁻ mice had a higher mammary tumor incidence and shorter latent period of tumor development than the neonatal thymectomized group treated with spleen cells obtained from syngeneic MTV⁺ mice.

These results suggest that, during early life, the thymus is critical for the normal development of spontaneous mammary carcinoma. We propose three possible mechanisms to explain the role of the thymus in spontaneous mammary carcinoma in mice: (a) The thymus may provide a necessary site for MTV latency and/or replication. (b) It could be that there are either endocrine functions of the thymus or endocrine interactions between the thymus and other organs which are not yet known. (c) The intact thymus is necessary for a "balanced" virus-host tumor cell immunologic interaction in a process of negative adaptation (tolerance) to the virus and/or virus-induced antigen(s). In this case, development of cancer may be a result of the tolerant state. Alternatively, cancer may develop as a result of breakdown of tolerance to the virus and/or virus-induced antigen(s). Our experiments seem to favor the latter explanation since our data suggest that host immune response (enhancing antibodies) or other immunologic reaction may contribute to the development and establishment of the cancer cells.

INTRODUCTION

In a previous paper (17), it was reported that thymectomy at 6 days of age in mammary tumor virus (MTV)-infected female

C3H mice reduced the incidence and increased the latent period required for development of spontaneous mammary adenocarcinomas. These results have recently been confirmed (14). Removal of the thymus produces similar effects in mice infected with the Gross leukemia virus, in that there is a decreased incidence of leukemia and a prolongation of the latent period necessary for development of detectable leukemia. Both of the agents (MTV and the Gross leukemia virus) are transmitted vertically after birth, and tolerance to each virus or viral-induced antigen may occur (18, 21, 22). Removal of the thymus in the neonatal period might prolong the latent period and reduce the incidence of malignancy. By contrast, it is known that neonatal thymectomy in mice decreases the latent period and increases tumor incidence in malignancies induced by the polyoma virus (10, 15, 16, 20), SV40 virus (1), adenovirus 12 (13), and some chemical carcinogens (3, 10, 19, 24). Some of these malignancies are highly antigenic, and the effect of thymectomy may bear direct relationship to host immune response.

To further investigate the importance of the thymus in mammary carcinogenesis in mice, experiments were performed in C3H MTV⁺ female mice thymectomized at birth. Wasting disease was prevented by thymus grafting or by the injection of syngenic spleen cells. It was found that neonatal thymectomized female C3H MTV⁺ mice, grafted with syngeneic thymus from animals either having or lacking MTV, developed mammary tumors as frequently as did sham-operated controls. By contrast, neonatal thymectomized mice treated with spleen cells obtained from C3H MTV⁺ mice had a reduced incidence of tumors and a prolonged latent period of tumor development. Neonatal thymectomized mice treated with spleen cells obtained from C3H MTV⁻ mice showed a higher incidence of tumors than the group treated with spleen cells obtained from C3H MTV⁺ mice. Our results are interpreted as indicating that the early removal of the thymus delays the development of the malignancy either by delaying the breakdown of immunologic tolerance or by interfering with induction of immunologic tolerance to the virus or viral-induced antigens.

MATERIALS AND METHODS

MTV⁺ and MTV⁻ mice of the C3H strain originally maintained in the colony of the late J. J. Bittner were used. Details of the origins of these strains have been reported elsewhere (6, 9).

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Female mice of the C3H MTV⁺ strain were thymectomized within 12 hours after birth by the technic routinely employed in this laboratory (1). One week after surgery, the thymectomized mice were divided into four groups and subjected to the following treatments: *Group I.* Mice in this group were given a single intraperitoneal injection of 50×10^6 spleen cells obtained from adult syngeneic C3H MTV⁺ donors. *Group II.* Mice in this group were treated as in Group I except that spleen cells from C3H MTV⁻ donors were used. *Group III.* Neonatally thymectomized mice were each given a subcutaneous transplant of one thymus from 2-week-old C3H MTV⁺ donors. *Group IV.* Animals in this group received a subcutaneous thymus transplant obtained from 2-week-old C3H MTV⁻ donors. Another group (*Group V*) was composed of sham-operated mice given 5×10^7 C3H MTV⁻ spleen cells intraperitoneally.

All mice were returned to their own cages and raised by their own MTV⁺ mothers. Following weaning at 30 days of age, they were housed in plastic cages in groups of 4–5 females per cage. One normal adult male of the C3H MTV⁻ strain was introduced into each cage for breeding. The females were separated when pregnant and their litters were weaned when they were 30 days of age. Breeding behavior was measured by the number of litters produced from each mother. The mice were provided Purina Laboratory Chow and tap water *ad libitum*. The incidence of spontaneous mammary adenocarcinoma in all groups was determined by weekly inspection of each individual mouse. At the time a tumor appeared, the age of the mouse was recorded. When tumors showed progressive growth and attained a size greater than 2 cm in the longer diameter, mice were sacrificed and the tumor prepared for histologic study. In all thymectomized mice, careful postmortem examination of the mediastinal region was carried out under a dissecting microscope, and any suspicious tissue of the area was studied microscopically in paraffin sections stained with hematoxylin and eosin. Those animals with thymus remnants, as well as mice dying within one month after the operation, were discarded and not included in the final analysis of the data.

RESULTS

Crude Tumor Incidence and Tumor Age in C3H MTV⁺ Female Mice. The crude data of these experiments is summarized in Table 1. The group of sham-thymectomized and both groups of neonatally thymectomized mice treated with either MTV⁺ or MTV⁻ thymus grafts had a similar tumor incidence (81, 83, and 79%) and a similar average mean tumor age (298, 337, and 321 days). The groups of thymectomized mice treated with spleen cells show lower incidences of spontaneous mammary tumors. This incidence was 35% in the neonatally thymectomized mice treated with MTV⁺ spleen cells and 60% in the group treated with MTV⁻ spleen cells obtained from syngeneic C3H mice. However, the only group with a grossly different cancer age (419 days) is the group of thymectomized mice treated with MTV⁺ spleen cells. Table 1 also shows that in all groups of mice, the breeding behavior as judged by the number of litters in each mouse group is comparable.

The results in Table 1 afford rather convincing evidence of decreased tumor incidence for the groups given spleen cells

and an increased tumor age for the group given the MTV⁺ spleen cells. However, a more detailed actuarial analysis was carried out, to separate the risks of cancer development from the risks of normal death from other causes³. The results are shown in Charts 1 and 2.

Chart 1 demonstrates clearly the retardation of tumor development in the thymectomized animals receiving MTV⁺ spleen cells. The thymectomized animals receiving MTV⁻ spleen cells show some suggestion of slight retardation of tumor development.

Chart 2 shows the normal death risks, with tumor risk removed. The five groups are clearly comparable in normal death experience, an important further assurance that the various operations themselves are not affecting natural mortality and thus indirectly and artificially affecting crude tumor incidence rates.

DISCUSSION

It is clear from these experiments that neonatally thymectomized C3H mice, grafted with syngeneic thymus obtained from donors which either possessed or lacked the MTV, developed spontaneous mammary carcinomas at a rate and age similar to that at which malignancy appeared in controls. These results demonstrate that early thymectomy in susceptible mice reduces the incidence of spontaneous mammary carcinoma and causes a prolongation of the latent period required for cancer development.

In the intact mouse, the most important factors influencing the development of spontaneous mammary carcinoma are inherited susceptibility, proper hormonal stimulation, and the presence of the mammary tumor virus (5). The results of the present experiments suggest that the presence of the thymus during the early life of the animal is also a contributing factor. The inherited susceptibility for tumor development is genetically controlled and presumably does not change after thymectomy. It could be that the mechanism of prevention of mammary adenocarcinoma by thymectomy is related to alteration of hormonal functions yet unknown. In the experiments reported herein, the breeding behavior taken as an expression of gonadal function was not different in thymectomized and non-thymectomized groups. Other reflections of hormonal differences were not investigated.

The removal of the thymus in the neonatal period decreases the incidence and increases the latent period of mammary cancer development as is the case with mouse leukemia (18). It is possible that the thymus is essential during early life for latency and/or replication of the mammary tumor virus, either during the passage from the gastrointestinal tract to the hemopoietic cells or in the passage from those cells to the mammary tissue (23). A somewhat similar interpretation has been made

³The actuarial analysis was carried out by dividing the total age axis into small (20-day) intervals and estimating probabilities of survival for the small intervals. The model used in each interval was exponential with competing risks of tumor development, normal death, and withdrawal from the experiment. See Berkson and Elveback (4) and Chiang (7) for further discussion of model and analysis.

Table 1

Group	Number of animals starting experiment	Number of animals excluded from analysis ^a	Crude tumor incidence (mean ± S.E.)	Animals developing tumors			Animals dying without tumors		
				Number	Survival in days (mean ± S.E.)	Average no. of litters	Number	Survival in days (mean ± S.E.)	Average no. of litters
1. Neonatal thymectomy treated with spleen cells C3H MTV ⁻	59	1	0.60 ± 0.06	35	326 ± 10 (193, 438) ^b	4.5	23	381 ± 33 (52, 669) ^b	3.8
2. Neonatal thymectomy treated with spleen cells C3H MTV ⁺	54	5	0.35 ± 0.07	17	419 ± 19 (285, 531)	4.5	32	406 ± 21 (119, 579)	4.2
3. Neonatal thymectomy treated with thymus graft C3H MTV ⁻	63	3	0.83 ± 0.05	50	337 ± 11 (188, 514)	4.5	10	308 ± 42 (61, 457)	4.4
4. Neonatal thymectomy treated with thymus graft C3H MTV ⁺	50	3	0.79 ± 0.06	37	321 ± 12 (206, 490)	4.2	10	385 ± 43 (43, 567)	4.0
5. Sham thymectomy treated with spleen cells C3H MTV ⁻	52	4	0.81 ± 0.06	39	298 ± 10 (174, 417)	4.4	9	384 ± 40 (175, 536)	4.4

Tumor incidence, tumor age, and breeding behavior in C3H MTV⁺ mice.

^aThese animals were excluded due to death within one month of initial operation or discovery of remnant thymus in the thymectomized animals.

^bMinimum, maximum are presented in parentheses.

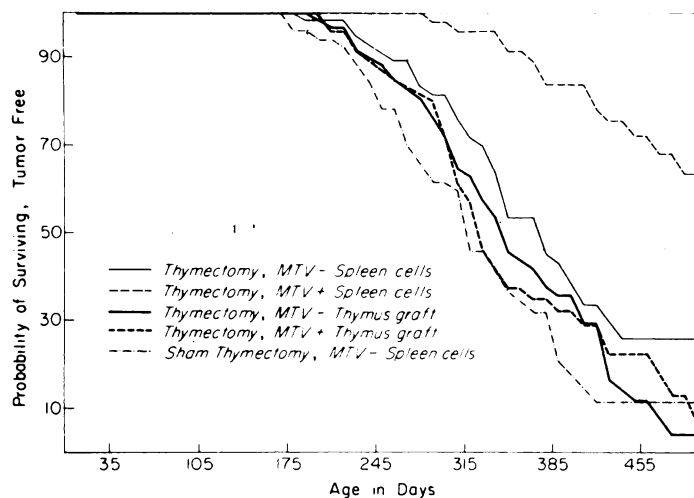


Chart 1. Probability of surviving without tumor development, with all other causes of death removed.

for mouse leukemia (18). In the latter situation, the thymus is the site of development of the leukemic process, and the amount of virus is correlated with the frequency of cell division which occurs maximally in the thymus (18). Other factors interfering with the virus cycle may be involved since it has been shown that infection with an enzyme-elevating virus induces a decrease of spontaneous mammary tumors in mice (26). A more detailed study of this possibility is presently in progress.

It has been reported that pregnancy influences neonatally thymectomized mice (25). Presumably the thymus of the fetuses can partially restore the immunologic capacity of neonatally thymectomized mice by releasing into the fetal-maternal circulation of a "humoral" factor (25). If this be true, our experiments indicate that the thymus of the host itself, and

not merely a "humoral" substitution, must be present for development of spontaneous mammary adenocarcinoma in mice. However, the effect of thymectomy on MTV could occur and be determined prior to the first pregnancy. Animals of each group had several litters which, according to the investigations of Osoba (25), should have restored, at least in part, immunologic functions.

We observed a marked difference in development of mammary adenocarcinoma in neonatally thymectomized mice reconstituted immunologically with spleen cells derived from MTV⁺ and MTV⁻ donors. These data, at face value, would appear to indicate that immunologic deficiencies produced by neonatal thymectomy are not primarily responsible for delayed development of spontaneous mammary adenocarcinoma, since spleen cells from either source would be expected to restore immunologic function in neonatally thymectomized

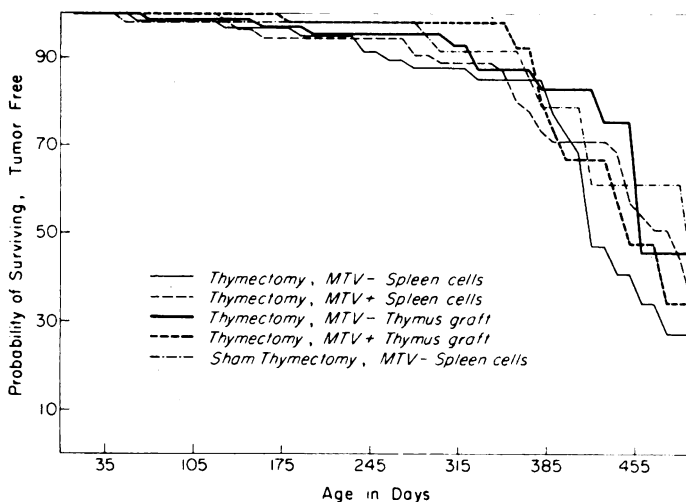


Chart 2. Probability of normal survival, tumor risk removed.

mice. (Comparisons of the capacity of spleen cells from MTV⁺ and MTV⁻ mice donors in graft-versus-host assay indicate that the spleen cells from the two sources have similar immunologic capacity.)

Since a tolerant state to the virus and/or virus-induced antigen(s) exists in C3H MTV⁺ mice (21, 22), our results can be interpreted to indicate that the thymus may be important in the process of developing negative adaptation (tolerance) to the virus. In this respect, the state of tolerance to the MTV may be comparable to the asymptomatic state of mice with neonatal lymphocytic choriomeningitis infection introduced in the neonatal period (12).

It could be that tolerance induction to MTV or to MTV-induced antigens occurs following virus entrance immediately after birth. After a period of time, breakdown in tolerance may occur, following which virus-host cell interaction, possibly combined with damage associated with immune response, may lead to cancer cell formation (18). Thymectomy may decrease the rate of tolerance breakdown as has been suggested from studies of mouse leukemia (18). Thus, thymectomy interfering with breakdown of tolerance may lead to a prolongation of the latent period for cancer development and a reduced incidence of cancer. Thymectomy is known to maintain experimentally induced immunologic tolerance to some antigens (8). However, in contrast to the effect of thymectomy on the development of spontaneous mammary cancer, other investigators have found that thymectomy increases the incidence of tumors induced by some chemicals (3, 10, 19, 24), polyoma virus (15, 16, 20), and SV40 virus (1). Attempts to produce tolerance in newborn animals with SV40 virus have failed (1). These viral-induced tumors contain antigens foreign to the host, which may stimulate intact animals to become immune. This may result in the elimination or suppression of tumor cells containing these antigens. Neonatal thymectomy in such cases may increase the incidence of induced tumors by lowering the immunologic reactions of the host.

The increase of tumor incidence in thymectomized mice injected with MTV⁻ syngeneic lymphoid cells as compared to the thymectomized group injected with MTV⁺ syngeneic cells is a most provocative finding. Although current data do not permit complete understanding of these relationships, it seems to us that appropriate analysis may derive from considering possible immunologic relationships in terms of negative rather than positive adaptations.

For example, a population of immunologically competent nontolerant C3H lymphoid cells, when exposed to virus, might undergo an immune response productive of antibody capable of enhancing establishment of malignant cells similar to that reported in other experiments (2, 11). Alternatively, it may be that the tolerant state required for development of mammary adenocarcinoma is a specific negative adaptation which results from virus-lymphoid cell interactions within the thymus (27). Development of the malignancy may actually be the result of the tolerant state. A neonatally thymectomized mouse may not be able to develop this negative adaptation, and treatment with tolerant lymphoid cells, as from MTV⁺ mice, might then be expected to facilitate development of the malignancy; whereas treatment with MTV⁻ cells would be expected to permit or facilitate resistance to development of this malignancy.

Exactly the opposite was observed. MTV⁻ cells facilitated the development of malignancy and MTV⁺ cells were much less effective. Therefore, our results cannot be explained so directly. They seem to us to favor the view that development of this form of malignancy depends upon the existence in the host, first of a state of tolerance to the agent followed by a breakdown of tolerance and perhaps production of an immunologic reaction.

Whatever be the final explanation, each of the hypotheses based on immunologic considerations is susceptible to testing which may shed light on the host-parasite relationships and host-tumor cell relationships responsible for this type of malignancy.

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