The American Journal of PATHOLOGY

May 1978 • Volume 91, Number 2

Effects of Whole-Body Irradiation on Neonatally Thymectomized Mice

Incidence of Benign and Malignant Tumors

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The individual and combined effects of neonatal thymectomy and whole-body irradiation on the prevalence of benign and malignant tumors in germ-free female mice of the Charles Rivers line were studied to determine if a portion of the tumorigenic effects of irradiation can be attributed to injury of the thymic-dependent component of the immune response. Neonatal thymectomy increased a) the incidence of benign and malignant tumors and b) the prevalence of multiple primary neoplasms in an individual mouse. Whole-body exposure to 700 rad at 6 weeks of age further increased the incidence of tumors, but the relative magnitude of this increase was less pronounced than in sham-operated controls. Thus, the cumulative effects of thymectomy plus irradiation are less pronounced than the sum of the individual effects. One of several possible explanations for this observation is that a portion of the carcinogenic effects of whole-body irradiation is mediated by suppression of the thymic-dependent component of the immune response. (Am J Pathol 91:217–228, 1978)

NEONATAL THYMECTOMY (nTx), which inhibits maturation of a major aspect of the immune response, renders some animals more susceptible to spontaneous and experimentally induced tumors.[1-5] In humans, several thymus-related immunologic deficiency diseases have been characterized by an increased frequency of malignant tumors, especially lymphomas.[3] These observations suggest that one of the functions of the thymic-dependent (T-cell) component of the immune response is to suppress abnormal clones of cells, including those with neoplastic potential.[6-9] Such suppression has been referred to as immunologic surveillance.[6-9]

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Supported by Grant CA14270 from the National Cancer Institute, US Department of Health, Education, and Welfare, and by the Albuquerque Veterans Administration Hospital.

Accepted for publication January 6, 1978.

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0002-9440/78/0510-0217$01.00

Whole-body exposure to ionizing radiation is immunosuppressive and tumorigenic. The immunosuppressive effects of such exposure relate in large measure to the exquisite radiosensitivity of lymphocytes, including those derived from the thymus.[10] Therefore, the carcinogenic effects of irradiation may be due to not only the direct effects of the physical agent on individual cells but also, and perhaps of equal importance, a general immunosuppressive effect involving particularly the T-cell component. If this hypothesis is correct, then the combined tumorigenic effects of nTx plus whole-body irradiation should be significantly less pronounced than the sum of the individual effects. The purpose of the present study is to evaluate the individual and cumulative effects of nTx and irradiation in germ-free mice of the Charles Rivers (CR) line. This outbred line was selected for two reasons: a) a reasonable but not excessive incidence of tumors (30 to 35% in nonirradiated germ-free animals), which encompass a variety of cell types, including several kinds of lymphomas,[11] and b) an increased prevalence of malignant tumors following nTx.[12] For comparative purposes, similar data were developed for conventional mice treated in similar fashion, but interpretation of these results is confounded by chronic runting among many of the experimental animals.

Materials and Methods

Animals

Germ-free mice of the CR line were subjected to thymectomy or sham thymectomy (STx) within 24 hours of birth, as described elsewhere.[13,14] Germ-free, in the context of this report, indicates that these mice were free of viable bacteria and fungi throughout the experiment and did not harbor antibodies to the following murine viruses: PVM, polyoma, K, Sendai, GDVII, rat, H-1, SV_5, mouse adeno, mouse hepatitis, and LCM. Antibody specific for Reo 3 virus was present throughout the experiment at a 1:20 dilution. At the time of weaning, male and female offspring were separated, and the latter were irradiated or sham irradiated.

Irradiation

At 6 weeks of age, 302 female germ-free nTx and STx mice were exposed to 700 rad whole-body irradiation, as described previously.[15] An equal number of nTx and STx were sham irradiated. This dose represents an $LD_{10(30)}$ for 6-week germ-free female nTx mice of this line. Mice which died of acute radiation injury (eg, within 30 days of exposure) were not included in this portion of the study. The residual 277 animals form the basis of this report.

Histology

Mice were housed in plastic isolators and given autoclaved food and water.[16] The isolators were observed at least three times daily. Autopsies were performed immediately after death, and tissues were obtained for subsequent histologic evaluation, which routinely included examination of heart, lungs, stomach, small and large intestine, liver, spleen, mesenteric lymph node, bone marrow, kidneys, ovaries, and skin. A section was

also taken routinely from the region of the thymus; the 2 mice with evidence of residual thymus were not included in the data analysis. Sections were also obtained from all lesions noted grossly.

Tumors were classified on the basis of histologic appearance. Benign, in the context of this report, indicates a well-circumscribed tumor with little, if any, pleomorphism; few, if any, mitotic figures; and no evidence of local invasion or distant metastases. Included in this category were cavernous hemangiomas, thecal cell tumors, and tubular adenomas of the ovary.

Results

The effect of irradiation on the total numbers of benign and malignant tumors developed by nTx and STx germ-free mice is shown in Table 1. Neonatal thymectomy results in a 63.5% increase in "spontaneous" neoplasms. Subsequent whole-body irradiation at 6 weeks of age further increases the prevalence of such tumors but only by 5.0% (59.5% vs 64.5%). Thus, whereas irradiation increases the prevalence of tumors by 101.4% in the STx group, the comparable figure for the nTx group is 8.4%.

The prevalence of the tumors of Table 1 is shown in Table 2 by broad histologic classification as a function of experimental group. In contradistinction to the STx groups, the influence of radiation in nTx mice is due almost entirely to increased numbers of histologically benign tumors.

Not reflected in Tables 1 and 2 is the observation that a significant number of mice developed more than one primary malignant tumor. The rate of occurrence of multiple primary malignant tumors by experimental group is shown in Table 3. Such tumors are largely confined to the nTx groups, in which the added effect of irradiation has no apparent influence. In addition to multiple primaries, the nTx group is also characterized by significant numbers of tumors which are unusual in this line of mice (discussed later).

A correction for mice with multiple primaries is made in Table 4,[17] and the influence of irradiation on the incidence of benign and malignant tumors in nTx and STx germ-free mice is illustrated. The increased incidence of tumors occasioned by nTx persists, although the increment is

Table 1—Effect of Irradiation on Total Numbers of Benign and Malignant Tumors in Neonatally Thymectomized Germ-Free Mice

Experimental group	Radiation dose		Percent increase
	0 rad	700 rad	
Sham thymectomy	36.4	73.3	101.4
Neonatal thymectomy	59.5	64.5	8.4

Results are expressed as percent of mice in group with indicated tumor; animals with multiple tumors are included more than once.

Table 2—Prevalence of Benign and Malignant Tumors by Histologic Type

Type of tumor	Sham thymectomy			Neonatal thymectomy		
	Nonirradiated	Irradiated	Percent increase	Nonirradiated	Irradiated	Percent increase
Leukemia/lymphoma	22.7	43.7	92.8	39.2	39.5	0.8
Other malignant tumors	7.6	14.1	85.5	16.2	15.8	—
Benign tumors	6.1	15.5	154.1	4.1	9.2	124.4
Total	36.4	73.3	101.4	59.5	64.5	8.4

Results are expressed as percent of mice in group with indicated tumor; animals with multiple tumors are included more than once.

Table 3—Effect of Irradiation on Prevalence of Multiple Primary Malignant Tumors in Neonatally Thymectomized Germ-Free Mice

Experimental group	Radiation dose (rad)	No. of primary tumors		
		1	2	3
Sham thymectomy	0	30.3	0	0
	700	56.3	1.4	0
Neonatal thymectomy	0	45.9	9.5	0
	700	48.7	3.9	1.3

Results are expressed as percent of mice in group with indicated number of malignant tumors.

less pronounced than that noted in Table 1 (26.3% vs 63.5%). Irradiation increases the incidence of benign and malignant tumors in both groups, but the increment is much more pronounced with the STx than the nTx group. The latter observation is reflected in the statistical observations of Table 4, in which the association between radiation and neoplasia is much stronger in the STx than in the nTx group.

The effect of irradiation on the age-specific death rates of nTx and STx mice with malignant tumors is indicated in Text-figure 1. Although nTx increases the incidence of tumors and the prevalence of multiple primary malignant tumors, no effect is noted with respect to latent period. Irradiation accelerates the appearance of such tumors in both the nTx and the STx groups, but the effect is more pronounced in the latter group. This observation is especially pronounced in the midportion of the cumulative mortality curve, as shown in Table 5.

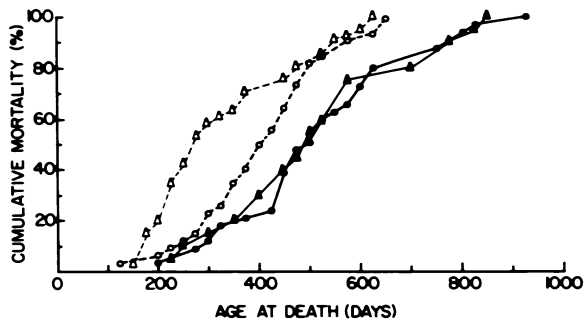
Results similar to those described above were observed with conventional mice of the same line treated in identical fashion (data not shown), although interpretation of data is confused by wasting, which is not unusual among nTx conventional mice of this line, and is further increased by whole-body irradiation. Therefore, mice thought to exhibit wasting were excluded from the data analysis. On this basis, whole-body exposure to 350 rad at 6 weeks of age ($LD_{10(30)}$ for conventional nTx CR mice) increased the incidence of benign and malignant tumors by 61.3% in STx mice in comparison with 11.1% in the nTx group. Multiple primary malignant tumors were not unusual in both the nonirradiated (15.9%) and irradiated (4.2%) nTx groups.

Irradiated and nonirradiated germ-free and conventional nTx mice also exhibited a moderate number of unusual (for this line) malignant tumors.

Table 4—Effects of Irradiation on Incidence of Benign and Malignant Tumors in Neonatally Thymectomized Germ-Free Mice

Experimental group	Radiation dose		Percent increase	Levels of significance		
	0 rad	700 rad		χ	G	*P*
Sham thymectomy	36.4	64.8	78.0	25.35	10.549	<0.001
Neonatal thymectomy	46.0	56.6	23.0	3.93	2.364	<0.05

Results are expressed as percent of mice in group with one or more tumors.
χ^2 = chi square test; G = goodness-of-fit test.[17]



TEXT-FIGURE 1—Effect of irradiation on cumulative mortality in neonatally thymectomized germ-free mice. STx, nonirradiated, ▲—▲ STx, irradiated, △—△, nTx, nonirradiated, ●—●: nTx, irradiated, ○—○. Results are expressed as percentage of mice in individual group dead with malignant tumor by indicated day.

Many of these were of soft tissue origin and included liposarcomas, fibrosarcomas, and neurofibrosarcomas. Unusual tumors of epithelial origin included a bilateral transitional cell carcinoma of the renal pelvis, an adenocarcinoma of the large intestine and a transitional cell carcinoma of the urinary bladder.

Discussion

The immunologic surveillance theory was apparently first suggested by Ehrlich[6] and later refined by Thomas[7] and Burnet.[8,9] The latter suggested "it is by no means inconceivable that small accumulations of tumors cells may develop and because of their possession of new antigenic potentialities provoke an effective immunologic reaction with regression of the tumor and no clinical hint of its existence." [18] Similarly, Thomas postulated that the "homograft rejection will turn out to represent a primary mechanism for natural defense against neoplasia." [7] The axioms on which the immune surveillance theory is based are a) tumor cells possess "new" antigens, determinants not present on normal adult cells; b) these neoantigens are antigenic to the autologous host; c) the immune response generated in response to these tumor-associated antigens protects the host by destroying tumor cells; and d) in the absence of such a response (eg, in a host with impaired immunity), the incidence of tumorigenesis is increased and the latency period may be reduced.[19]

Table 5—Mortality Data by Experimental Group

Experimental group	Age at death (days)		
	10th decile	50th decile	90th decile
STx, nonirradiated	250	490	775
STx, irradiated	170	265	545
nTx, nonirradiated	285	495	770
nTx, irradiated	180	400	570

Data from Text-figure 1

Several detailed analyses of the concept of the immune surveillance have been recently published;[1-5,20] the evidence *pro* and *con* will not be reviewed here. With respect to "spontaneous" tumors, the following points are of interest:

1. A moderate increase in the prevalence of malignant lymphomas was noted in thymectomized (C57BL × A)F_1 hybrids.[21]

2. A moderate decrease in the prevalence of adenocarcinoma of the breast as transmitted vertically by virus has been reported in thymectomized mice of several strains,[22-25] although the timing and completeness of the operative procedure appear to be of critical importance. Thus, incomplete nTx or complete thymectomy followed by implantation of syngeneic thymus in (C57BL × I)F_1 × C3H hybrid female mice results in a significant prolongation of the latent period before tumor development without altering the overall incidence.[26] Complete thymectomy at 9 to 12 days of life significantly reduced the incidence but had no effect on the latent period.[26]

3. No increase, and perhaps a decrease, in the prevalence of tumors has been noted among several colonies of congenitally athymic (nu/nu) mice,[27-29] although several of the studies were compromised by the short life span of the experimental groups.

4. No increase in incidence of spontaneous tumors was noted by Sanford et al[30] in nTx and STx mice (85% and 81%, respectively) of the BALB/c strain. However, the biologic behavior of the tumors as well as histologic type differed between the two groups. For example, 10% of the nTx mice develop tumors of a type not found in the STx group, including two myoepitheliomas, one squamous cell carcinoma, and a lipoma. In comparison with STx mice, the experimental group showed an increased incidence of hemangioendothelioma accompanied by a reduced frequency of mammary and lung tumors. Multiple lung tumors were noted in 10 of the 20 involved nTx mice, while only single tumors were found in the STx group. Similarly, Trainin and Linker-Israeli[31] reported an increased incidence of lung tumors in nTx SWR and Swiss mice. It is important to note that, although the data often appear contradictory, results from different laboratories utilizing the same strain of mice and identical immunosuppressive regimens are generally confirmatory.

Given the above, Stutman[4] questioned whether "some tumor types are more susceptible to immunologic control than others." Such a cautious approach appears particularly appropriate in view of the recent discovery of a nonlymphoid, naturally occurring killer (NK) cell which is apparently increased in relative and absolute numbers in nu/nu mice[32,33] and the definition of functionally distinct subpopulations of T cells.[33-44] For ex-

ample, administration of antilymphocytic serum (ALS) in mice apparently depletes the rapidly recirculating subpopulation of T cells more extensively than the more sessile subpopulation which homes preferentially to the spleen. The consequences of such an unbalanced situation with respect to tumorigenesis are unknown. Similarly, the tempo of the perinatal peripheralization of T cells in mice varies considerably among various strains, and the relative numbers of the various T-cell subpopulations within a single strain generally are in flux for the initial several weeks of life.[45] On this basis, the effects of neonatal thymectomy would be expected also to be dependent on the strain of mice employed and on the timing of the surgery. Finally, most inbred strains of mice appear to be susceptible to a limited number of types of tumors. Assuming that some tumors are more susceptible to immune control than others, strains susceptible to neoplasms which fall more closely under such surveillance would be expected to demonstrate an increased prevalence under nTx, while strains with tumors not under immune control would not be expected to be influenced.

The results of the present study should be interpreted with the above cautions in mind. Thus, although nTx is associated with an increased prevalence of benign and malignant tumors, much of the increment is due to animals with multiple primary malignant tumors (Table 3).

Similar caution appears appropriate with respect to the radiation data. Whole-body exposure of nTx germ-free female mice to 700 rad at 6 weeks of age increases the incidence and total numbers of benign and malignant neoplasms. The relative magnitude of this increase is significantly less pronounced than that noted with irradiated vs nonirradiated STx mice. Thus, the sum of the tumorigenic effects of nTx plus irradiation are less than the combined effect. This observation implies a relationship between these two factors. One interpretation is that a portion of the tumorigenic effects of whole-body irradiation is mediated via injury to one or more subpopulation of T cells. Other explanations are equally plausible, however. Therefore, the next step in testing this hypothesis is an evaluation of the effect on tumorigenesis of the adoptive transfer of syngeneic T cells after irradiation.

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Acknowledgments

We thank Laura Pogue, Chester Cook, and William E. Doughty for their contributions to this study.