Tumor Development after 3-Methylcholanthrene in Immunologically Deficient Athymic-Nude Mice

Abstract. *Athymic-nude* (nu/nu) *mice and normal* (nu/+) *mice showed no differences in either latent period or incidence of local sarcomas or lung adenomas within 120 days after administration of 3-methylcholanthrene at birth. However,* nu/nu *mice were incapable of rejecting allogeneic skin grafts for the duration of the experiment. These results argue against an active role of thymus-dependent immunity as a surveillance mechanism preventing tumor development.*

The availability of athymic-nude mice (*1*)—that is, mice in which the thymus fails to develop—offered a new possible model for testing the effects of the absence of thymus-dependent immune functions on tumor development. The demonstration of tumor-associated transplantation antigens in the majority of the malignancies studied (*2*) and the presence in the tumor-bearing host of concomitant immune reactions against those antigens (*3*) led to theories in which the immune system plays a major role as deterrent against malignancy (*4*). Thus, in all the presentations of the "immune surveillance" theory, the effects of immunodepression (thymectomy and other procedures and drugs) favoring tumor development are quoted as experimental evidence that some sort of control on tumor development is exerted by the intact immune system (*4*).

Such immune surveillance theories would predict a high risk for tumor development in the athymic-nude mice. However, our results do not support that prediction, since tumor incidence after exposure to 3-methylcholanthrene (MC) at birth was similar in the immunologically normal nude heterozygotes and the immunologically deficient athymic-nude homozygotes.

The effects of administration of MC at birth on subsequent tumor development in the athymic-nude (*nu/nu*) and in the normal heterozygotes (*nu/+*) were studied. MC was administered at birth since such procedure shortens the actual length of the experiment (*5*), the short life-span of the *nu/nu* being a major problem for long-term studies in these mice. By preserving the mice in a relative pathogen-free environment, life was prolonged in the *nu/nu* to more than 6 months (mean ± S.D., 184 ± 39 days). Thus, adequate numbers of animals remained alive for periods useful for the study of oncogenic agents with long latency, such as MC.

The *nu/+* mice were obtained [from Dr. H. H. Wortis (Tufts University School of Medicine, Boston)] in a CBA background. The animals used in our experiments were derived from the mating of fertile *nu/nu* males with normal pathogen-free CBA/H females from our colony, thus obtaining *nu/+* heterozygotes that were subsequently mated for production of the *nu/nu* and *nu/+* animals used in the experiments. These animals, derived from conventional *nu/+* carriers, were given foster nursing by pathogen-free CBA/ mice, kept in plastic isolators (Germfree Laboratories, Miami, Florida), and fed sterilized chow. Newborn CBA/H mice kept in similar conditions were also studied.

In all the experiments, the mice were treated at birth with 0.1 mg of 3-methylcholanthrene (Eastman Organic) in 0.02 ml of corn oil, injected subcutaneously in the left inguinal region. All the animals were observed three times a week for tumor development, weaned at 30 days of age, grafted with allogeneic DBA/2 skin (*6*), and finally killed at 120 days of age. This age for termination of the experiment was selected because (i) it is 2 standard deviations below the mean life-span of the *nu/nu* in our colony and permitted the study of reasonable numbers of animals without the influence of obvious disease and before mortality reduced those numbers; and (ii) the available data on MC administration at birth shows that at such age 10 to 50 percent of the animals, depending on the strain, show local tumor development (*5*).

No significant differences in tumor incidence nor latent periods for tumor appearance were observed (Table 1, compare lines 1 and 3). All the tumors were fibrosarcomas originating in the MC-injection site. No tumors were observed in the oil-injected controls. No difference in MC toxicity (expressed by the difference between the total number of mice injected at birth and the number of mice weaned) was observed between *nu/+* and *nu/nu* mice. All the results are expressed for males and females combined, since no significant differences in incidence were observed between sexes (that is, fibrosarcoma incidence in *nu/+* was 3 of 18 for females and 4 of 21 for males, and in *nu/nu* it was 3 of 13 for females and 2 of 14 for males). Local tumor incidence and latent periods were also comparable to the above data in the inbred CBA/H mice (Table 1).

All *nu/+* and *nu/nu* mice were grafted at 40 days of age with allogeneic DBA/2 skin. Mean skin graft rejection times were 11.6 ± 1.2 for *nu/+* injected with MC and 11.3 ± 0.9 for *nu/+* injected with oil. As was described for other mouse strains (*6*), MC had no effect in prolonging allogeneic skin graft rejection times at the dose used in the present experiments. On the other hand, all *nu/nu* mice in the MC or oil-injected groups retained viable grafts until the end of the experiment at 120 days of age, that is, 80 days after skin grafting. Such immune deficit has been described in *nu/nu* mice (*7*).

Table 1. Incidence and age of appearance of local subcutaneous sarcomas within 120 days after administration of 3-methylcholanthrene (MC) in oil to *nu/+*, *nu/nu*, and CBA/H mice. Animals were injected subcutaneously within 24 hours of birth in the left inguinal region with 0.02 ml of either corn oil alone or corn oil containing 0.1 mg of MC. The tumor incidence and percentage is expressed as number of mice with tumors per number of mice weaned at 30 days of age and not as the percentage of total mice injected at birth with MC. The early deaths after injection were usually within the first week of life. The age in days of the first detectable tumors is presented individually and the mean in days is in parentheses.

Strain	Group	Mice injected	Mice weaned	Tumor incidence		Age at tumor appearance (days)
				No.	Percent	
nu/+	MC	43	39	7	17	85, 90, 90, 95, 95, 103, 110 (95)
nu/+	Oil	29	28	0		
nu/nu	MC	32	27*	5	18	80, 85, 93, 95, 100 (90)
nu/nu	Oil	11	9	0		
CBA/H	MC	38	34	4	13	87, 95, 100, 110 (98)
CBA/H	Oil	26	23	0		

* Only one animal died, without tumor, at 70 days of age and is not included in the group.

The relatively low incidence of local tumors after MC reflects mainly the short observation time of the experiment (*5*). Control groups of *nu/+* and CBA/H injected at birth with MC developed 50 to 100 percent local sarcomas at 212 ± 72 and 189 ± 81 days, respectively (each group of 25 mice), indicating that the strains are not genetically resistant to the oncogenic action of MC (*6*). Local sarcoma incidence in an additional group of *nu/nu* mice observed for 170 days (this experiment is still in progress) was 15 of 30 (50 percent), while a similar group of 30 *nu/+* siblings developed 17 local sarcomas (56 percent) after administration of 0.1 mg of MC at birth. This evidence suggests that even at the 50 percent tumor incidence level the *nu/nu* mice do not differ significantly from their immunologically normal *nu/+* siblings.

Table 2 shows the results of autopsies performed in almost all the experimental animals at the termination of the experiment (120 days after MC administration at birth). Only data on lung adenomas are described since they were the main tumor type observed in these mice. The only other malignancy observed was a thymic lymphocytic lymphoma detected at autopsy in a *nu/+* male. Table 2 shows that (i) there are no important differences in lung adenoma incidence nor are there significant differences in number of tumors per mouse between *nu/+* and *nu/nu* after MC administration; (ii) both *nu/+* and *nu/nu* animals had the same low spontaneous incidence of lung adenomas (lung tumors in the oil-injected controls); (iii) no sex differences were observed: lung adenoma incidence was 9 of 18 for *nu/+* females, 9 of 21 for *nu/+* males, 6 of 13 for *nu/nu* females, and 5 of 14 for *nu/nu* males; and (iv) CBA/H had higher spontaneous incidence of lung adenomas that increased after MC administration.

Metastases of the fibrosarcomas in the regional nodes were observed in 1 of 7 *nu/+* and in 3 to 5 *nu/nu* animals. No other metastases were detected. The majority of the fibrosarcomas infiltrated the muscle of the abdominal wall.

Amyloidosis of liver or spleen (or both) was found in 5 of 27 *nu/nu* injected with MC and in 1 of 39 *nu/+*. No amyloidosis was observed in either the oil controls or the CBA/H mice. Amyloidosis was detected as described and its presence in mice thymectomized at birth has been discussed (*8*).

Table 2. Incidence of lung adenomas at autopsy, 120 days after administration of 0.1 mg of 3-methylcholanthrene (MC) at birth to *nu/+*, *nu/nu*, and CBA/H mouse sublines. Animals were injected at birth subcutaneously with 0.02 ml of corn oil alone or with the same dose of oil containing 0.1 mg of MC.

Strain	Group	Mice with tumors*		Lung nodules per mouse (No.)	Nodules per mouse (mean No.)
		No.	Percent		
nu/+	MC	18/39	46	2, 4, 4, 4, 5, 5, 5, 5, 5, 6, 8, 8, 10, 12, 12, 12, 14, 15	7.5
nu/+	Oil	2/28	7	2, 4	3.0
nu/nu	MC	11/27	40	5, 6, 6, 7, 8, 8, 8, 9, 10, 10, 16	8.4
nu/nu	Oil	1/9	11	3	3.0
CBA/H	MC	12/30	40	3, 4, 4, 5, 5, 5, 7, 8, 10, 10, 11, 14	7.1
CBA/H	Oil	4/20	20	2, 3, 3, 4	3.0

* Per total number of mice examined.

Although the groups are not very large, it is apparent that no significant differences in tumor incidence or in latent periods for tumor development could be detected between athymic *nu/nu* mice and the normal heterozygote *nu/+* counterparts. However, *nu/nu* differed from *nu/+* animals regarding immune competence, since they were absolutely unable to reject allogeneic skin grafts for the duration of the experiment. The results suggest that the absence of an intact thymus-dependent immune system may not affect significantly the risk for solid tumor development after exposure to MC at birth.

The interpretation that immune surveillance in this case is exerted via antibodies and the thymus-independent B cell system would not be valid in view of much of the experimental data on immunity to solid tumors [(*9*); also (*3*)]. Two possible exceptions have been described in which cells that are not thymus dependent seem to be the main effectors of tumor immunity in vitro: sarcomas induced by the Moloney sarcoma virus (MSV) in mice (*10*) and human urinary bladder carcinomas (*11*). Human tumor immunity is still being studied, and evaluations of or generalizations concerning the bladder tumor data are difficult to make. However, the mouse experiments with MSV may represent the exception since the system, contrary to other experimental solid tumors, is extremely sensitive to the effect of antibodies (*12*).

The data on the effects of immunosuppression on tumor induction by MC or similar compounds in mice show both facilitation of tumor development (*13*) or no significant effects (*6, 14*), indicating that the assumption of a direct correlation between depressed immune functions and high tumor risk was not absolute. On the other hand, the data on induction of lung adenomas in mice, mainly by urethan, is concordant with a possible role of immune functions on the development of such tumors (*15*). However, in our experimental system, the athymic *nu/nu* mice show that the incidence of both subcutaneous sarcomas and lung adenomas did not differ significantly from that observed in the immunologically normal *nu/+* animals.

Our data show that mice with a genetic absence of the thymus and thymus-dependent immune functions do not show an inordinate incidence of spontaneous or chemically induced malignancies. This is true even when the athymic animals are kept under conditions that permit their observation during useful periods of time, by elimination of early mortality due to infections and also by avoiding the abnormalities that result from more absolute gnotobiotic conditions (*16*). Although the evidence for an immune response to developing tumors is quite definitive (*3*), the experimental evidence for immune mechanisms acting as a surveillance device for the prevention of tumor development is not as clear cut. Immune surveillance apparently exists in nature and may be efficient in preventing the action of certain oncogenic viruses, mainly of DNA and herpes type (*17*). Also immune surveillance is not universal and probably does not apply to every instance of malignant transformation. This seems especially so with chemical carcinogens of the polycyclic hydrocarbon type, in which both our data and that on the effects of immunosuppressive treatments on tumor development (*6, 14*) indicate practically no immunological control over tumor incidence or latency for development.

Thus, our results support some of the criticisms of the surveillance theory (*18*). On the other hand, according to the recently proposed "immune stimulation" theory (*19*), the *nu/nu* mice may have fewer tumors than their normal counterparts. This was not the case for the fibrosarcomas, although a slight decrease in the incidence of lung adenomas was observed in the *nu/nu* mice (see Table 2).

The relatively high incidence of lymphomas as well as epithelial solid tumors in humans with primary immune deficiencies (*20*) or in patients undergoing prolonged immunodepression for transplantation (*21*) suggests that an intact immune system may play an active role controlling malignant development (*4*). On the other hand, such association has not been observed in other immune deficiencies such as leprosy or some autoimmune disorders (*22*), while others contend that the high incidence of lymphomas in those patients may be a result of either the primary disease or the administered drugs (*18*).

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Piperidine Increase in the Brain of Dormant Mice

Abstract. *During a 2-hour period of dormancy (sleep), piperidine is accumulated in the mouse brain.*

Piperidine has been shown to have transmitter-like pharmacological properties in the molluscan central nervous system (*1*); it is physiologically present in the brains of many species including man (*2*). Some tissues of the mammalian brain were also reported to contain relatively greater quantities of piperidine than other tissue (*3*). The piperidine concentration in the brain of snails during experimentally induced hibernation increases severalfold (*4*), and piperidine might be involved in the process of hibernation in snails (*1*, *4*). We now report on piperidine measurements in the mouse brain, which were performed to determine whether any appreciable change in its concentration in mammalian brain can be detected during diurnal variations of behavioral activity.

Forty inbred female mice (3 months old) strain C57B1/6, were caged for 1 week, under constant illumination, before the experiment. Ten animals were randomly selected as "active" at a time when all animals were active; of the remaining mice, ten were randomly chosen as "dormant" at a time when all mice had been dormant for at least 2 hours. Since there is no absolute behavioral criterion of sleep (*5*), we refer here to a dormant mouse as

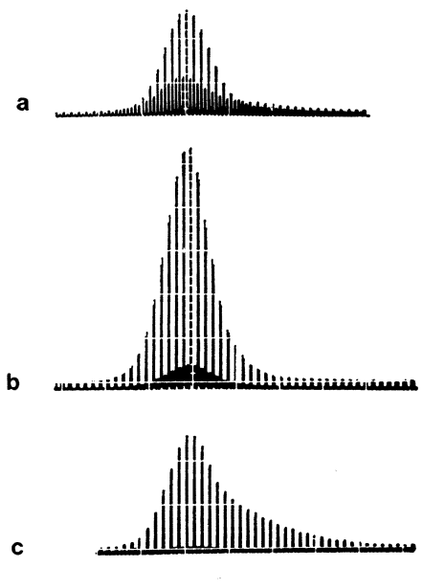

Fig. 1. Quantitative mass spectrometric measurement of piperidine. Evaporation profiles of dansylated pyrrolidine as an internal standard (1.65×10^{-10} mole in each sample), and dansylated piperidine from the mouse brain. The ion currents for *m/e* 304 and 318 were consecutively displayed on an oscilloscope (the two envelope curves in each record). In records (a) and (c) the pyrrolidine curves are higher than those of piperidine; in record (b) the piperidine curve is higher than the pyrrolidine standard. The records are from (a) brain of an active mouse, (b) brain of a dormant mouse, and (c) piperidine blank sample without tissue (the piperidine curve reaches only slightly above the lowest white line of the graticule). Relative amplifications are: (a) 4×; (b) 1×; and (c) 5×; time base, 15 seconds per division.