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Rank Order of Sarcoma Susceptibility Among Mouse Strains Reverses with Low Concentrations of Carcinogen

Abstract. *Ten mouse strains in which aryl hydrocarbon hydroxylase can be induced, or F_1 hybrids of these strains, were ranked according to their sarcoma susceptibility when exposed to a high concentration (5 percent) of the chemical carcinogen 3-methylcholanthrene. This rank order was reversed when the concentration of 3-methylcholanthrene was reduced to 0.05 percent.*

Tumor induction with chemical carcinogens is known to differ among various inbred mouse strains (*1*). An important determinant in tumor susceptibility is the inducibility of the enzyme aryl hydrocarbon carboxylase (AHH; locus gene symbol *Ah*). Mice that carry the dominant Ah^b allele respond to the injection of aromatic hydrocarbons (including 3-methylcholanthrene) by producing increased concentrations of AHH and a high incidence of solid tumors. In contrast, mouse strains homozygous for the recessive allele Ah^d are not AHH inducible and do not easily produce solid tumors (*2*).

In addition to the large differences in tumor inducibility due to the *Ah* locus, there are smaller differences in tumor inducibility among the AHH-inducible mouse strains. It has been thought that these small differences may have an immunological basis because the AHH system itself does not account for all findings (*3*).

While producing tumors with two different concentrations of 3-methylcholanthrene (MCA) in six inbred mouse strains and four F_1 hybrids (all AHH inducible), we discovered a paradoxical result that we discuss in this report: the mouse strains most susceptible to tumor induction with a high concentration of MCA were the least susceptible at a low concentration. Conversely, the mouse strains least susceptible to tumors with a high concentration of MCA were the most susceptible at low concentrations.

Four- to five-week-old mice (Animal Resources, Jackson Laboratory) were used throughout the study. The inbred mice were females of the strains C3H/HeJ, CBA/J, BALB/cJ, BALB/cByJ, A/J, and males and females of the C57BL/6J strain. The following F_1 hybrid mice also were used: (C57BL/6J × C3H/HeJ)F_1, (C3H/HeJ × C57BL/6J)F_1, and (C57BL/6J × BALB/cByJ)F_1 females and (BALB/cByJ × DBA/2J)F_1 males.

The tumors were induced according to the method of Bartlett (*4*). Briefly, Millipore filter strips were saturated with either 5 or 0.05 percent MCA in paraffin. Disks (6 mm in diameter) were punched from these strips with a ticket punch and kept refrigerated in the dark until used (within 1 month). The disks used in experiment 1 were made at the Institute for Cancer Research, Philadelphia, and shipped to the Jackson Laboratory. The disks in other experiments were made and used at the Jackson Laboratory. The mice were anesthetized with Nembutal alcohol and the MCA disks were inserted dorsally into the subcutaneous space through a small incision in the midline. The incision was closed with a wound clip. The mice were examined weekly by palpation for the presence of tumors. The date was recorded when a tumor had reached a diameter of 5 mm. If the tumor subsequently killed the mouse, that date was used in the calculations as the end of the latency period.

All tumors, and all tumor-free mice, at the end of each experiment were examined during autopsy for the presence of the MCA disk. If the disk was missing, the mouse was discarded from the experiment because it was impossible to know how long the mouse had been exposed to the MCA. Mice of strains C3H/HeJ, C57BL/6J, and A/J rejected from 32 to 50 percent of the 5 percent MCA disks. However, since the two strains that were most different in tumor inducibility, C3H/HeJ and C57BL/6J, were equal in this respect, the sloughing of the MCA disks did not appear to have been a factor in the results. In one of the experiments, designated EL, the mice were not examined for the presence of the MCA disk at the end of the experiment. The tumor-free mice in experiments 1 and 2 were observed for 365 days; in experiments 3 and EL for 245 days. We used these dates for the calculations of the tumor-free days for the mice that did not develop tumors. We used tumor-free days rather than tumor latency in order to be able to use all of the data. The trend of the data was the same if we calculated tumor latency using only the tumorous mice. The Mann-Whitney U test was used for the statistical analysis of the data.

All of the mice, with the exception of some of the (BALB/cByJ × DBA/2J)F_1 hybrids and two C3H/HeJ mice in experiment EL, developed solid tumors when 5 percent MCA disks were used for tumor induction (Tables 1 and 2). Although the average tumor-free time varied considerably among the strains with 5 percent MCA, both the tumor incidences and the observed tumor-free time varied when the induction was done with the 0.05 percent MCA.

The results of experiment 1 are given in Table 1 and Fig. 1. The three inbred strains and the four F_1 hybrids are listed in ascending order of tumor-free days with 5 percent MCA, that is, from the most susceptible to MCA (C3H/HeJ) to the least susceptible (C57BL/6J). The most susceptible strain, C3H/HeJ, became the least susceptible with the low concentration of 0.05 percent MCA; with the low concentration their tumor incidence was only 31 percent with an average of over 300 tumor-free days. In contrast, the strain least susceptible to the high concentration of MCA, C57BL/6J had, with the lower concentration, an 80 percent incidence and less than 250 days tumor-free. The BALB/cBy strain was intermediate between these two ex-

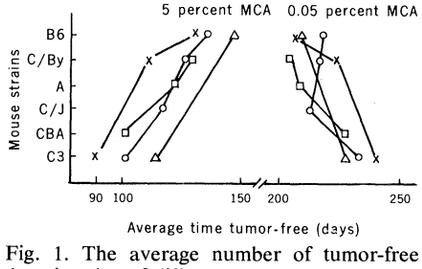

Fig. 1. The average number of tumor-free days in mice of different genotypes. For purposes of comparison, the data plotted are based on an observation period of 245 days for each experiment. Abbreviations of the mouse strains are the same as in Table 2. Symbols: X, experiment 1; ○, experiment 2; □, experiment 3; △, experiment EL.

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tremes. All tumor latencies or tumor-free days were statistically different among the three mouse strains, though not between male and female C57BL/6J mice. The first three listed F_1 hybrids showed the same inverse susceptibility pattern as the inbred strains, although the differences were smaller. The fourth F_1 hybrid, (BALB/cBy × DBA/2)F_1, did not seem to show the inverse relationship in susceptibility, and instead had a relatively long tumor latency with both concentrations of MCA. The mice of strain (BALB/cBy × DBA/2)F_1 were the only ones in this study heterozygous for the Ah^b allele, a fact that may explain why they were different.

Because the paradoxical tumor induction results were surprising, we repeated the experiment two more times using the original three mouse strains and the three additional Ah^b homozygous strains, BALB/cJ, CBA/J, and A/J. At the same time, 5 and 0.05 percent MCA disks were implanted into C3H/HeJ and C57BL/6J mice. Table 2 and Fig. 1 show the results of experiments 2, EL, and 3. Again the mouse strains are listed in ascending order of tumor-free days with 5 percent MCA (that is, descending order of tumor susceptibility). As in experiment 1, the order of tumor susceptibility among the mouse strains, with the possible exception of the BALB/cJ mice, was reversed when the 0.05 percent MCA disks were used. Although the differences among the mouse strains in experiment 2 were small, the trend was the same as in experiment 1. The reversal was clear in experiment EL and again in the results of experiment 3. Thus, our results show that among various Ah^b mouse strains or F_1 hybrids there is a general inverse susceptibility to carcinogenesis with concentration of the carcinogen.

Although not all the tumors in this study were examined histologically, past experience in this laboratory has shown that they are almost invariably soft-tissue sarcomas, sometimes identifiable as rhabdomyosarcomas. Past experience has also shown that paraffin disks of the size and type used in these experiments, in the absence of carcinogen, rarely, if ever, produce a tumor.

It is not clear what causes the paradoxical reversal of tumor susceptibility with concentration of MCA among various strains of mice. Differences in AHH-enzyme inducibility do not offer an easy answer, since all the genotypes are AHH-inducible. Furthermore, the level of enzyme inducibility is very similar in the two extreme examples, C3H/HeJ and C57BL/6J mice (*5*).

Another, perhaps more interesting way to rationalize the unexpected result is based on the biphasic nature of the immune response. It has been shown that the immune response can stimulate tumor growth if the immune interaction is weak (*6*) and that immunogenicity of chemically induced tumors tends to be directly correlated with the concentration of the carcinogen used for induction (*6*). Therefore, it might be predicted that in a mouse strain with great immunological capacity, the growth of tumors of low immunogenicity, produced by a very weak concentration of carcinogen, would be stimulated. Conversely, in the same mouse strain the growth of highly immunogenic tumors produced with a high concentration of carcinogen would be inhibited. A mouse strain with lower immunological vigor, on the other hand, might be completely unable to react to weakly immunogenic tumors but actually stimulate those induced by a high concentration of MCA. The result would be that the order of tumor susceptibility among mouse strains would reverse depending upon the concentration of the carcinogen.

Table 1. Tumors produced with 5 and 0.05 percent MCA disks in three different mouse strains and four F_1 hybrids.

Mouse strain*	5 percent MCA				0.05 percent MCA			
	Tumor incidence	Average time tumor-free (days)	P†		Tumor incidence	Percentage	Average time tumor-free (days)	P†	
C3	23/23	89.9	.0001	.0001	11/35	31.4	335.9	.0001	.0001
C/By	29/29	111.8			75/116	64.7	285.1		
B6‡	7/7	125.1	.04		18/29	62.1	279.3	.007	
B6	8/8	131.0			24/30	80.0	241.7		
B6C3F_1	14/14	112.3	.06	.03	15/34	44.1	307.9	.02	
C3B6F_1	14/14	112.4			20/34	58.8	291.6		
B6CF_1	17/17	124.8			21/32	65.6	266.6	.007	
CD2F_1‡	13/15	153.5			20/35	57.1	320.1		

*Abbreviations of the mouse strains are C3, C3H/HeJ; C/By, BALB/cByJ; B6, C57BL/6J; B6C3F_1, (C57BL/6J × C3H/HeJ)F_1; C3B6F_1, (C3H/HeJ × C57BL/6J)F_1; B6CF_1, (C57BL/6J × BALB/cByJ)F_1; and CD2F_1, (BALB/cByJ × DBA/2J)F_1. †Mann-Whitney U test. ‡Male.

Table 2. Tumors produced with 5 and 0.05 percent MCA disks in six different mouse strains.

Mouse strain*	5 percent MCA				0.05 percent MCA			
	Tumor incidence	Average time tumor-free (days)	P†		Tumor incidence	Percentage	Average time tumor-free (days)	P†	
Experiment 2‡									
C3	15/15	102	.01	.0001	13/34	38.2	313	.012	.09
C/J	23/23	118	.03		24/40	60.0	267		
C/By	22/22	127			19/36	52.8	292		
B6	15/15	136			19/37	51.4	286		
Experiment EL§									
C3	30/32	114	.0001		26/90	28.9	227		.0001
B6	20/20	147			66/119	55.5	209		
Experiment 3									
CBA	20/20	102	.003	.001	8/38	21.1	228	.014	.007
A	10/10	123			17/37	46.0	209		
C/By	19/19	129			19/39	48.7	206		

*Abbreviations of the mouse strains are C3, C3H/HeJ; C/J, BALB/cJ; C/By, BALB/cByJ; B6, C57BL/6J; CBA, CBA/J; and A, A/J. †Mann-Whitney U test. ‡Tumor-free mice were observed for 365 days in experiment 2 and for 245 days in experiments EL and 3. §The mice in experiment EL had been thymectomized as adults, x-irradiated with 800 R, and immunologically reconstituted by injecting 1×10^6 bone marrow cells and 1×10^7 spleen cells before MCA disk implantation. The purpose of this was unrelated to the present work.

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