[CANCER RESEARCH 32, 1129–1132, June 1972]

RNA Tumor Virus *gs* Antigen and Tumor Induction by Various Doses of 3-Methylcholanthrene in Various Strains of Mice Treated as Weanlings[1]

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SUMMARY

The effect of various doses of 3-methylcholanthrene on s.c. tumor induction and the occurrence of the murine C-type RNA group specific (*gs*) viral antigen in tumor tissue were studied in weanling mice of 8 genotypically different strains. Tumor incidence was found to be related to the dose of 3-methylcholanthrene. On the other hand, the incidence of *gs* antigen in the induced tumors was independent of the dose of 3-methylcholanthrene and reflected the natural *gs* antigen expression of the mouse strain. Histopathological examination showed no relation of tumor type to carcinogen dosage or mouse strain. The majority of the tumors examined were sarcomas. These studies confirm earlier findings, which suggested that the *gs* antigen expression induced in tumors is dependent on host-regulatory controls and that such controls of virogene (*gs* antigen) and oncogene (tumor induction) expressions of C-type RNA viral genome are independently affected by a carcinogen.

INTRODUCTION

A working hypothesis has been set forth that chemical carcinogens induce tumors by direct or indirect derepression of endogenous oncogenic information (2, 5, 15, 16). Our associates and we have proposed that inherited, largely covert, C-type RNA tumor viral genomes, postulated to be present in all vertebrate cells, provide this oncogenic information (4, 5, 15, 16). Previous communications have reported the results of 3MC[2] carcinogenesis studies in 12 inbred and 4 random-bred genotypically different mouse strains with differing natural expressions of the C-type RNA tumor virus genome (5, 16). Further studies demonstrated significant differences in tumor induction but not in tumor *gs* antigen incidence with 3MC, 7,12-dimethylbenz(a)-anthracene, and benzo(a)pyrene, depending on the mouse strain genotype, age, route, or dose of chemical treatment (15).

In the present studies, weanling mice of 8 genotypically different substrains were treated with varying doses of 3MC. Tumor incidence, tumor latency, and the incidence of *gs* antigen in the resulting tumors were compared to demonstrate the relationship of these responses to varying doses of 3MC and the natural expression of the C-type RNA tumor virus genome of the different mouse strains.

MATERIALS AND METHODS

Mice. All mice used in these studies were 4-week-old females, with the exception of C57L/J from The Jackson Laboratory, where only males were available. The 3 sources of C57BL/6 mice were Cumberland View Farms, Clinton, Tenn. (designated Cum); The Jackson Laboratory, Bar Harbor, Maine; and Microbiological Associates, Inc., Bethesda, Md. The C3H/fMai (Bittner-Andervont origin), BALB/cCr[3] (8), and BALB/cSPF[4] mice were from Microbiological Associates, Inc. The C3H/He (Heston origin) mice were obtained from the NIH Animal Colony, Bethesda, Md.

Preparation of the 3MC in trioctanoin and the s.c. inoculation procedures were the same as described previously (15, 16); the procedures for housing, feeding, and observation of experimental animals were also described previously.

Tissue Preparation and CF Tests. The preparation and storage of specimens for CF tests and histopathological examination were described previously, as were Antisera Pools IX322 and 26 used for *gs* antigen determinations (1, 5, 15, 16). MSV Pool IX322 was furnished by Dr. Rodger Welsnack, Huntington Research Center, Inc., Baltimore, Md. Highly specific *gs-1* guinea pig antiserum (furnished by Dr. R. Gilden, Flow Laboratories, Rockville, Md.), made with electrofocused, purified *gs* antigen (10), was also used for a limited number of parallel tests in order to check the specificity and sensitivity of the MSV rat antisera (IX322 and 26) used.

Extracts of selected specimens were treated with ether for

[1] This study was conducted under Contracts NIH-70-2068 and NIH-43-67-697 within the Special Virus-Cancer Program of the National Cancer Institute, NIH, USPHS at Microbiological Associates, Inc., and the Council for Tobacco Research.

[2] The abbreviations used are: 3MC, 3-methylcholanthrene; CF, complement fixation; MSV, murine sarcoma virus; TD_{50}, carcinogen dose producing tumors in 50% of the animals in 8 months; CI, carcinogenic index.

Received January 11, 1972; accepted February 24, 1972.

[3] These mice were provided under Contract PH-43-67-697 within the Special Virus-Cancer Program of The National Cancer Institute, NIH, USPHS.

[4] These mice were provided under Contract PH-43-66-914 within the Special Virus-Cancer Program of The National Cancer Institute, NIH, USPHS.

gs-1 identifications. One part 10% sonically extracted tumor extract was mixed with 10 parts ether in a 50-ml beaker and agitated at room temperature until the ether evaporated (approximately 3 hr). The specimens were treated again in the same manner. When all ether had evaporated, the specimens were held at −70° until tested by CF procedures for *gs* antigen content.

Analysis of Data. The latency period, tumor, and *gs* antigen incidence were computed by the methods of Shimkin and Andervont (12) as previously described (15). The TD_{50} dose was determined by the method of Reed and Muench (11) and expressed as the antilog of the log TD_{50}. The CI, a value suggested by Iball (6), was determined by the formula $P/T \times 100$, in which P is the percentage of mice developing tumors in 8 months and T is the average latent period in days for tumors to develop to 1.5 to 2.0 cm. CI equates latency to

Table 1

Comparison of tumor incidence, CI, and gs antigen incidence in various mouse strains treated s.c. with 3MC at 4 weeks of age

Mouse strain	3MC (μg)	Tumor incidence		TD_{50} (μg)	CI	*gs* antigen				
						Tumor		Mean	Normal muscle	
		Tu/T[a]	%			*P/T*[b]	%	%	*P/T*	%
C57BL/6Cum	0	0/28	0						0/10	0
	9.38	10/28	36		21	1/5	20			
	18.75	13/30	43		29	3/11	27			
	37.50	14/27	52	26.37	32	2/13	15	18	0/72	0
	75.00	20/28	71		55	4/17	24			
	150.00	26/27	96		83	0/11	0			
C57BL/6Mai	0	0/30	0						0/10	0
	37.50	12/30	40	60.60	28	6/8	75	43	0/21	0
	150.00	20/27	74		50	3/13	38			
C57BL/6J	0	0/28	0						0/10	0
	37.50	14/29	48	63.97	34	7/8	88	65	0/20	0
	150.00	16/27	59		44	6/12	50			
C57L/J	0	0/28	0						0/10	0
	37.50	5/29	17	127.9	8	0/3	0	7	0/15	0
	150.00	13/28	46		31	1/12	8			
C3H/fMai	0	0/28	0						0/10	0
	9.38	12/27	44		25	5/9	56			
	18.75	18/29	62		42	6/10	60			
	37.50	25/30	83	15.56	74	7/10	70	57	0/65	0
	75.00	25/27	93		70	7/18	39			
	150.00	27/30	90		90	10/14	71			
C3H/He (NIH)	0	0/26	0						0/10	0
	9.38	15/26	58		33	6/10	60			
	18.75	12/26	46		27	7/9	78			
	37.50	16/24	67	23.01	54	5/11	45	52	0/50	0
	75.00	20/28	71		57	7/11	64			
	150.00	20/26	77		76	5/15	33			
BALB/cCr[c]	0	0/48	0						0/10	0
	9.38	3/30	10		7	2/3	67			
	37.50	17/28	61	34.36	45	7/17	41	34	0/19	0
	75.00	23/28	82		71	2/12	17			
BALB/cSPF[d]	0	0/28	0						0/10	0
	37.50	14/24	58	39.36	57	3/12	25	23	0/22	0
	150.00	26/31	84		98	2/10	20			

[a] *Tu/T*, tumor-bearing mice/total mice on test for 8 months.

[b] *P/T*, positive/total extracts for *gs* antigen. Positive samples gave ≥3+ CF at a 1:20 final dilution of tissues tested against MSV Antiserum Pool IX322. Samples giving ≤2+ CF at a 1:20 final dilution were considered negative.

[c] BALB/cCr strain was established at Microbiological Associates, Inc., as an inbred colony in 1958 from mice received from Dr. Andervont at NIH, Bethesda, Md.

[d] BALB/cSPF strain was established at Microbiological Associates, Inc., as a random-bred colony in 1968 from mice obtained from Life Science Laboratories, Fort Lauderdale, Fla.

tumor incidence, thereby making it possible to obtain 1 value for the carcinogenicity of a compound providing an expedient means of comparing varying dosages of the same or different carcinogens and to compare these effects in different hosts. Calculation of the correlation coefficient is that of Snedecor and Cochrane (13).

RESULTS AND DISCUSSION

The tumor incidence, TD_{50} dose, CI, and *gs* antigen incidence in tumor and normal muscle tissues are summarized in Table 1. A higher incidence of tumors in each strain was generally associated with higher doses of 3MC. The tumor dose responses, expressed as TD_{50} and CI, indicated that the C3H, BALB/c, and C57BL/6Cum mouse substrains were most sensitive to 3MC carcinogenesis.

With the exception of several squamous cell carcinomas involving the cutaneous area and attributable to inadvertent injection in that tissue, all tumors were sarcomas. No histological differences were observed in the tumor types induced with different doses of 3MC or in the various genotypic strains and substrains of mice studied.

Extracts of normal muscle and s.c. tissues from control and tumored mice were all completely negative for *gs* antigen. Tumor extracts from all mouse strains were tested against 2 MSV antisera (Pools 26 and IX322). Each pool gave similar results, reacting almost exclusively with the intraspecies-specific *gs* antigen, as confirmed by testing random samples with *gs-1*-specific guinea pig serum and ether treatment of a representative number of tumor samples (2, 3, 15, 16). There was little difference noted in the incidence of *gs* antigen in the tumors induced with high and low doses of 3MC, except for those induced in the C3H/He and BALB/cCr strains (Table 1). In these 2 strains, a tendency toward higher incidences of *gs* antigen was noted in tumors that were induced with the smaller doses of 3MC and for which there were longer latency periods. The average tumor latency periods for 150 μg 3MC were 14.4 weeks for C3H/He mice and 16.4 weeks for the BALB/cCr, while those for 9.38 μg 3MC were 25.2 and 21.8 weeks, respectively. Since it is known that C-type RNA virus *gs* antigen expression in normal spleens and spontaneous tumors also increase in incidence with increasing age, it is possible that the appearance of 3MC tumors induced in older age reflects a similar tendency to greater RNA genome expression (Refs. 5 and 15; R. L. Peters, L. S. Rabstein, G. J. Spahn, C. E. Whitmire, J. W. Hartley, H. C. Turner, and R. J. Huebner, in preparation).

The actual CF results obtained with the C3H/He mice are as follows. 9.38 μg 3MC: 4/10, ≥4+ at 1:40; 2/10, 3+ at 1:20; 1/10, 2+ at 1:20; 3/10, 1+ at 1:20. 18.75 μg 3MC: 7/9, ≥4+ at 1:40; 1/9, 2+ at 1:20; 1/9, 1+ at 1:20. 37.5 μg 3MC: 3/11, ≥4+ at 1:40; 2/11, 4+ at 1:20; 3/11, 2+ at 1:20; 2/11, 1+ at 1:20; 1/11, 0 at 1:20. 75.0 μg 3MC: 3/11, ≥4+ at 1:40; 4/11, 4+ at 1:20; 4/11, 1+ at 1:20. 150.0 μg 3MC: 5/15, ≥4+ at 1:40; 3/15, 2+ at 1:20; 4/15, 1+ at 1:20; 4/15, 1+ at 1:20; 3/15, 0 at 1:20.

The actual CF results obtained with the BALB/c mice are as follows. 9.38 μg 3MC: 2/3, ≥4+ at 1:40; 1/3, 1+ at 1:20. 37.5 μg 3MC: 5/17, ≥4+ at 1:40; 2/17, 4+ at 1:20; 2/17, 2+ at 1:20; 5/17, 1+ at 1:20; 3/17, 0 at 1:20. 75.0 μg 3MC: 2/12, ≥3+ at 1:20; 2/12, 2+ at 1:20; 6/12, 1+ at 1:20; 2/12, 0 at 1:20.

Table 2

Relationship between average tumor gs antigen incidences and TD_{50} dose of 3MC for the 3 substrains of C57BL/6 mice

C57BL/6 substrain	TD_{50} μg 3MC	Mean % tumors with *gs* antigen
Cum	26.37	18
Mai	60.60	43
J	63.97	65

The C57BL/6 mice were obtained from 3 sources known to have differing incidences of natural *gs* antigen expressed in normal spleen tissues. The Cum strain showed very little spleen antigen expression, while the Mai and the J strains showed varying degrees of positive reactions (Refs. 7 and 9; C. E. Whitmire, unpublished data). Thus, the known low natural incidence of spleen antigen (8 to 20%) is perhaps reflected in lower tumor antigen expression (0 to 27%) in the Cum substrain. The Mai and J substrains showed 38 to 75% and 50 to 88% *gs* antigen-positive tumor reactions, respectively. These differences in tumor antigen expression no doubt reflect different segregations of genetic control of natural expression of *gs* antigens in normal spleen tissues due to variations in breeding programs (3–5, 14).

There was a relationship (r, 0.92) between the average tumor *gs* antigen incidences and the TD_{50} dose of 3MC for the 3 substrains of C57BL/6 mice. This relationship was based on the data in Table 2. Although r is relatively high, it is not high enough to be significant ($p > 0.10$) with this sample size of indexes.

The C57L/J mice, as expected from earlier studies, gave the lowest incidences of *gs* antigen in the 3MC tumors (2, 16). These mice responded with the lowest incidence of tumors, with a TD_{50} of 127.9 μg 3MC. The C57L/J mice were the only male mice used in these studies; therefore, the TD_{50} dose may be an expression of sex difference. Additional studies are in progress.

Extracts of normal muscle and s.c. tissues from control and tumored mice were all completely negative for *gs* antigen.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Mr. T. Black and his staff for preparation of the histopathological specimens and Dr. Louise S. Rabstein for histopathological diagnoses. The serological tests were performed by Mr. H. C. Turner and Mr. P. Hill and their staff in Dr. R. J. Huebner's laboratories at the National Cancer Institute, Bethesda, Md. We also wish to thank Dr. Huebner for his guidance in these studies and assistance in the preparation of this manuscript. The statistical analysis was done by Mr. F. McClure. We wish to thank the members of our staff, Mr. S. Zelnio, Mr. H. Ratrie, Mr. N. Baker, Mr. W. Boykins, Miss H. Sipe, and Miss N. Dunlop for their excellent technical assistance in the animal studies.

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