ANTIGENIC SPECIFICITIES ON MURINE SARCOMA CELLS

Reciprocal Relationship between Normal Transplantation Antigens (H-2) and Tumor-Specific Immunogenicity*,‡

By GERALD R. HAYWOOD,§,‖ Ph.D., and CHARLES F. McKHANN, M.D.

(From the Departments of Microbiology and Surgery, University of Minnesota Medical School, Minneapolis, Minnesota 55455)

(Received for publication 16 November 1970)

The antigenic structure of tumor cells may be critical in determining their growth characteristics and metastatic properties. Moreover, the immunologic response to the tumor can act as a powerful selective force influencing the final nature of these properties. The antigenic alterations which take place on the surface of normal cells when they are transformed may be qualitative, as exemplified by the gain of tumor-specific transplantation antigen(s) (TSTA)[1] or the complete loss of a normal antigen, or they may be quantitative, as in the modulation of normal histocompatibility antigens. The quantity of H-2 isoantigen on the surface of different normal and tumor cells of the mouse has been shown to vary depending on the age of the animal (1–3), type of tissue or tumor (1, 4–6), and the immune state of the host (7–9). Recent serological studies (10, 11), the fluorescent antibody study of Cerottini and Brunner (12), and the immunoferritin study of Aoki et al. (13) all suggest that the antigenic specificities within the H-2 system are located in "patches" on the cell surface. Klein (14) proposed that the total amount of surface H-2 antigens may change but that a minimum concentration is necessary for cell viability. Within these limits, alterations in individual H-2 antigens in carcinogenesis could carry important genetic implications depending on whether there is a loss or decrease of one specificity on all tumors, a completely random change in the various specificities, or a coordinated alteration of all specificities.

The relationship between the TSTA and normal H-2 antigens is of interest because of the possible role that these surface components may play in cellular recognition. A reciprocal relationship between the thymus–leukemia (TL) and normal transplanta-

* Supported in part by American Cancer Society grant T-428, and U. S. Public Health Service grants CA-10910, Ca-08832, and AI-0090.

‡ Parts of this paper were presented at the 10th International Cancer Congress, Houston, Texas, May 22–29, 1970.

§ Deceased March 27, 1971. Please address reprint requests to C. F. McKhann.

‖ This work was done in partial fulfillment of the requirements for the Ph.D. degree at the University of Minnesota.

[1] *Abbreviations used in this paper:* 199 BSA, medium 199 containing 0.1% bovine serum albumin; LNC, Lymph node cells; MCA, methylcholanthrene; MEM, minimal essential medium; TL, thymus–leukemia; TPD-50, tumor-producing dose; TSTA, tumor-specific transplantation antigen.

tion antigens has been demonstrated by Boyse, Stockert, and Old (15) on leukemia cells where the new phenotypic expression of the TL antigen was associated with a concurrent loss of H-2 antigens. Fenyo et al. (9) utilized immunized mice to select Moloney lymphoma cells with a decrease in Moloney-specific surface antigen. They also reported preliminary studies demonstrating a reciprocal increase in H-2 antigens.

Most of the quantitative studies on H-2 antigens on the surface of cells have been carried out using multispecific isoantisera against several alloantigenic specificities. Therefore, little is known about the variation of individual antigens within the H-2 system or about their relationship to tumor-specific antigens. The present study was undertaken to compare the antigenic make-up of several methylcholanthrene (MCA)-induced tumors in mice. The amounts of normal H-2 antigens were determined by quantitative absorption of monospecific antisera and by the sensitivity of the tumors to the cytotoxic action of antibody and C′. The immunogenicity of the tumor-specific antigens were estimated on the same tumors by a standardized in vivo immunization and challenge. A characteristic reciprocal relationship was found to exist between the normal H-2 antigen concentration and tumor-specific immunogenicity in the series studied. Moreover, the pattern of cellular antigenicity correlated well with a behavioral property of the individual tumors, namely their capacity for early metastasis to the lung.

Materials and Methods

Animals.—C3H/HeJ inbred female mice, 6-12 wk old, were used in all of the studies. This strain carries the H-2^k allele with the following alloantigenic specificities: **1**, 3, 5, 8, 11, 25, 32 (16). Inbred DBA/2 and A.SW mice were used for immunization with C3H/HeJ cells. All mice were purchased from the Jackson Laboratories, Bar Harbor, Maine. They were housed in air conditioned animal rooms, fed Purina lab chow, and watered ad libitum.

Irradiation.—The mice were irradiated with 350 rads whole body irradiation to the midline in a rotating plastic holder with a General Electric Maximor 220 machine. The radiation characteristics were 220 kvp, 15 ma, 60 cm tube to surface distance, with a 1 mm Al and a 0.25 Cu filter delivered at a dose rate of 70.6 rads per min. with full wood backscatter for 4.25min.[2]

Tumors.—Five different MCA-induced sarcomas were used. These were induced with 0.5 mg MCA dissolved in 0.1 ml trioctanoin injected intramuscularly in the hind legs of C3H mice (17). The tumors were numbered MC-1, MC-2, MC-3, MC-4, and MC-5, banked in liquid N_2, and transplanted in isogeneic mice. The earliest tumor used was transplanted from the original host 10 wk, and the latest 18 wk, after injection of MCA. The tumors were used between the 12th and 22nd in vivo transfer.

Preparation of Tumor Cells for Study.—Using sterile technique, tumors were removed from the mouse, minced, and treated with 0.5% trypsin in Eagle's minimal essential medium (MEM) tissue culture media for 1 hr at 25°C.The tumor cells were then cultured in plastic Petri plates in F-10 media supplemented with 20% fetal calf serum, 100 units penicillin, and 100 mg

[2] The irradiation was carried out by Dr. Richard Kenyon in the Department of Radiotherapy, University of Minnesota Hospital.

TABLE I

Production of Monospecific H-2 Antisera against Alloantigens on C3H Cells

Alloantigenic specificity	Recipient/donors
H-2.1, 19	(B10.D2 × C3H.NB)/A.SW
H-2.1, 3, 19	(A.BY × WB/Re)/A.SW
H-2.5, 33	(B10.D2 × A.CA)/B10.A(5R)
H-2.8, 9	(B10 × A.SW)/B10.M
H-2.11, 25	(B10.D2 × C3H.NB)/LP.R III
H-2.23, 32	(B10 × LP.RIII)/B10.BR
H-2.32	(B10 × A)/B10.BR

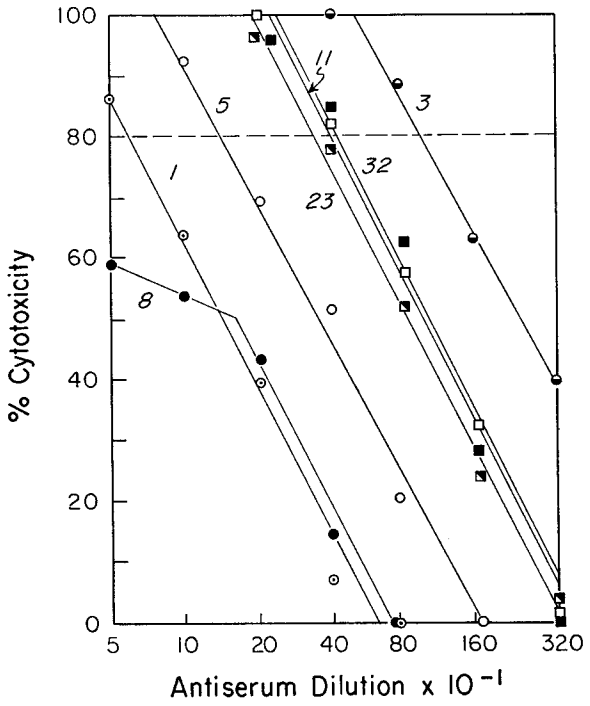

FIG. 1. Titration of monospecific H-2.1, 3, 5, 8, 11, 23, and 32 antisera against C3H lymph node cells.

streptomycin/milliliter in a humidified, gassed (5% CO_2, 95% air) 37°C incubator. After 24 hr of culture the plates were washed with media to remove dead cells and the tumor cells were removed by pipetting them off with 4 ml of Versene-trypsin, (0.2% ethylenediaminetetraacetic acid [EDTA], 0.02% trypsin, 0.04% KCl, 0.8% NaCl, and 0.005% phenol red, pH 7.4). Within 4 min the cells were placed in an equal volume of MEM containing 20% fetal calf serum. The cells were then washed three times in 199 tissue culture media containing 0.1%

bovine serum albumin (199 BSA), by centrifuging at 650 *g* for 6 min in a refrigerated centrifuge. The tumor cells were resuspended in 199 BSA.

Antisera.—The "monospecific" antisera used in this study were produced by Dr. George Snell at the Jackson Laboratories (National Institutes of Health contract PH 43-66-483). Table I gives the donor and recipient combinations used in the production of these antisera. It should be noted that recipients were F_1 hybrids in which one of the parents was from a strain congenic with the donor. This assures that, within the genetic limits of the congenic resistant animals, no non-H-2 antibodies are present in the serum. As can be seen in Table I the sera may contain antibodies against more than one specificity. However, they are monospecific for only one antigen represented on the C3H cells. Anti-H-2.3, H-2.11, and H-2.23 did show cross-reactivity with one of the other C3H specificities as detected by agglutination or cytotoxicity (M. Cherry. Jackson Laboratories. Personal communication.). H-2.25 was excluded from this study because the available antisera was not cytotoxic. Individual monospecific antisera was titrated with ^{51}Cr-labeled lymph node cells (LNC). Fig. 1 shows the titration curves of the antisera. The per cent of ^{51}Cr released was directly proportional to the killing of cells as measured by vital dye exclusion. The dilution of antisera which gave 50% release of ^{51}Cr was defined as the titer.

Multispecific antisera were produced by immunizing mice with spleen cells. Recipient mice were given donor spleen cells subcutaneously once a week for 6 wk. 10 days after the last injection the recipients were bled from the retroorbital plexus. All sera were stored in 0.25 ml volumes at −70°C.

Complement.—Sera from rabbits were screened for high complement (C′) activity and low natural cytotoxicity. Selected sera were absorbed once for 5 min at −2°C with minced liver and spleen tissue. After absorption the C′ was titrated with excess antibody and stored in 0.5 ml samples at −70°C.

Cytotoxic Test.—The cytotoxic test, measuring release of ^{51}Cr from labeled lymph node cells, was adapted directly from the technique of Wigzell (18). LNC, teased from the inguinal, axillary, popliteal, and mesenteric lymph nodes, in medium 199 were labeled with 200 μl of $Na_2{}^{51}CrO_4$ (1.1 mCi/ml from the Radiochemical Centre, Amersham, England) for 45 min at 37°C. After washing, the labeled cells were counted and tested for viability with trypan blue. Only preparations with greater than 90% viable cells were used. The cells were diluted to 500,000/50 μl in 199 BSA. Twofold serial dilutions of antiserum were prepared in 199 BSA with 50 μl of each dilution per tube. 50 μl of labeled cells were then added to each tube, mixed, and incubated for 10 min at 37°C. 50 μl of C′ was added to each tube and the tubes incubated for 1 hr at 37°C with frequent mixing. After incubation 1.5 ml of phosphate-buffered saline was added, the tubes mixed and centrifuged. 1 ml of the supernate was carefully drawn off and counted in a Tracerlab gamma-radiation counter (Tracerlab Div., LFE Electronics, Richmond, Calif.).

The amount of ^{51}CR in 1 ml of fully labeled cells was determined as a high control. Controls to determine the background, caused by antibody or C′ alone, were also included. C′ consistently gave the highest background and was used as the background control in the calculations. The per cent of ^{51}Cr released was calculated by the formula:

$$\frac{\text{cpm from sample–cpm from C′ control}}{\text{cpm from high control–cpm from C′ control}} \times 100$$

Quantitative Absorption.—The quantitative absorption technique was adapted from Boyse et al. (19). Tumor cells were counted and adjusted to the appropriate numbers needed to absorb the antisera as determined by preliminary studies. Only preparations of single cells in suspension were used. Tumor cells were serially diluted in 1 ml of 199 BSA, centrifuged, and the media carefully aspirated off. 170 μl of antiserum was added and the cells suspended by shak-

ing. The antiserum was absorbed for 30 min at 37°C followed by 30 min at 4°C, with frequent shaking. Control antisera were incubated at the same time without tumor cells. The tubes were centrifuged at 700 *g* and the absorbed antiserum carefully removed and stored at −70°C until all of the absorptions were complete. This provided enough absorbed antisera to allow three complete assays. All calculations were done with reference to 50 μl of antiserum, as used in the assay, i.e. when 170 μl of antiserum was used 3.4 times as many tumor cells were needed as would have been used to absorb 50 μl.

The size of the cells from these tumors varied slightly. Since the following experiments were based upon a comparison of the surface antigens, the number of cells used was corrected to give an equivalent surface area. Table II presents the data and calculation used in determining the correction factor. The surface area of the cell was calculated by the formula $A = \pi D^2$, assuming that the cells in suspension are spherical.

Calculation of Lethal Dose.—The method of Reed and Munch (20) as discussed by Davis et al. (21) was used to calculate a TPD-50, the number of cells needed to kill 50% of the mice receiving tumors. This method takes advantage of using small numbers of animals in a group. This was accomplished by assuming that if one animal dies at low dose it would have died at

TABLE II

Average Surface Areas of Cells of Different MCA Tumors

Tumor	Number of cells sized	Mean cell diameter	+ SE	Mean surface area	Correction factor
		(μ)		(μ²)	
MC-4	30	18.15	0.43	1034	1.00
MC-1	30	17.56	1.27	968	1.07
MC-2	30	16.92	0.58	899	1.15
MC-5	30	16.67	0.60	872	1.19
MC-3	30	14.92	0.46	698	1.48

the next higher dose and accumulating the values of the total number of animals that died or survived. The 50% point is then extrapolated between the two doses above and below the 50% point.

RESULTS

Quantitative Absorption of Monospecific Antisera with Different MCA Tumors.—The relative amount of each individual H-2 alloantigen was determined on five different MCA-induced tumors by quantitative absorption of monospecific antisera. Each antiserum was assayed for the amount of antibody remaining after quantitative absorption with the different tumors. The amount of antibody in the unabsorbed control, as measured in counts per minute released from the labeled LNC, was taken as 100%. The per cent of antibody remaining after absorption by various numbers of tumor cells was calculated from the counts per minute released in the cytotoxic assay of this sample divided by the control, times 100. The number of cells used for the absorptions and plotted on the graph was corrected to give an equivalent surface area. The quantitative absorption of anti-H-2.11 with the different tumors is shown in Fig. 2. From this graph the number of cells necessary to absorb 50% of the

antibody was determined. It can be seen that tumor MC-1 required about eight times more cells to absorb the same amount of antibody as did MC-5.

The number of cells necessary to absorb 50% of each monospecific antiserum was determined for each of the tumors, as shown in Table III. In order to make a relative comparison of the amount of each antigen on the surface of the tumors, tumor MC-3 was arbitrarily chosen as a standard. The average

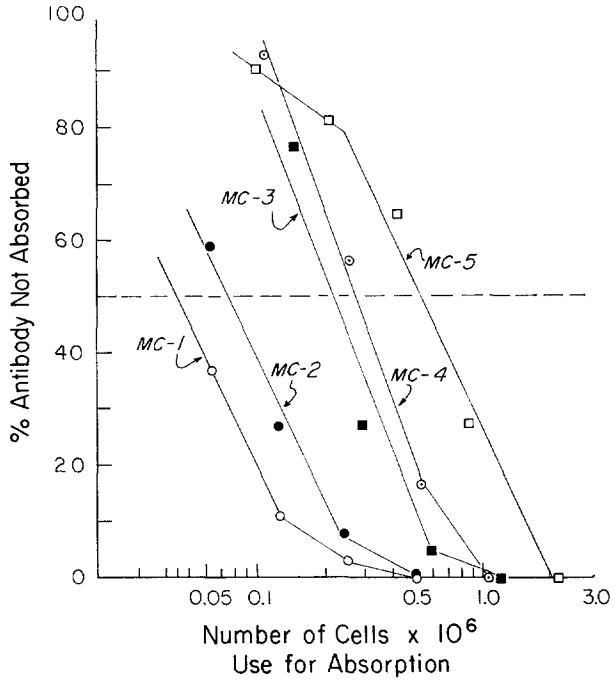

FIG. 2. Quantitative absorption of anti-H-2.11 sera with each of the five different MCA sarcomas, and tested for residual cytotoxicity. The number of cells necessary to absorb 50% of the antibody was determined from the graph.

of the duplicates in Table III was calculated and the number of cells of MC-3 necessary to absorb 50% of each monospecific alloantiserum was defined as unity. The number of cells needed to absorb a given amount of antibody was compared to the number of cells of MC-3 needed to absorb the same amount of antibody. This ratio expresses the quantity of antigen in terms of its capacity to absorb antibody relative to tumor MC-3. Table IV gives the relative amount of each antigen on the individual tumors compared with MC-3. These data are summarized in a bar graph in Fig. 3 from which it can be seen that tumor MC-1 has a relatively large amount of all antigens and MC-5 has the smallest

amount of all antigens. When the ratios of all of the antigens on an individual tumor are grouped together and compared, tumor MC-1 has 5.75 times more absorbing ability than MC-5. The other tumors fall in between in the order

TABLE III

*Number of Cells Necessary to Absorb 50% of the Antibody as Determined by Quantitative Absorption**

Tumor	Alloantigenic specificity						
	1	3	5	8	11	23	32
MC-1	1.1	1.6	0.64	1.9	0.74	0.94	2.5
	0.7	0.73	0.62	2.3	0.45	0.9	2.5
MC-2	1.5	2.1	1.4	2.3	1.26	2.1	6.4
	1.3	1.15	1.1	2.6	0.77	2.0	6.0
MC-3	3.6	4.9	3.2	5.8	2.8	4.4	8.8
	1.7	2.8	2.2	6.8	2.8	4.4	8.1
MC-4	4.3	5.7	1.6	7.6	3.1	5.4	9.5
	2.7	3.1	0.92	5.4	2.2	4.5	10.9
MC-5	7.0	8.1	6.4	6.0	6.4	4.4	7.8
	—	—	3.8	9.4	5.4	6.1	7.4

* Duplicate assays of absorption of all of the 7 mono-specific sera by each of the 5 tumors.

TABLE IV

*Absorbing Capacity of Different MCA Sarcomas Relative to MC-3**

Tumor	Alloantigenic specificity							Average of all antigen ± SE on each tumor	
	1	3	5	8	11	23	32		
MC-1	2.85	3.45	4.28	3.01	5.0	4.62	3.16	3.77	0.32
MC-2	1.85	2.38	2.15	2.57	2.75	2.08	1.37	2.16	0.18
MC-3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	—
MC-4	0.75	0.88	2.20	1.01	1.09	0.86	0.84	1.09	0.19
MC-5	0.51	0.61	0.54	0.83	0.48	0.84	1.11	0.76	0.11

* The average of the number of cells necessary to absorb 50% of each antibody was determined from Table III. Tumor MC-3 was chosen as a standard and the ratio determined for the number of cells of each tumor required to absorb 50% of the antibody as compared with MC-3.

MC-1 > MC-2 > MC-4 > MC-3 > MC-5. There was no gain or loss of any single specificity nor was there a random gain or loss of many different antigens. Instead, there was a quite uniform alteration in all of the antigens on an individual tumor.

Sensitivity of MCA Tumors to Cytotoxicity of Antibody and Complement.—A direct cytotoxic test was carried out on each of the five MCA-induced tumors using a multispecific anti-C3H antiserum. Tumor cell suspensions were prepared as for absorption and labeled with ^{51}Cr in the same way as LNC. 500,000 ^{51}Cr-labeled tumor cells were reacted with dilutions of antibody and excess C′.

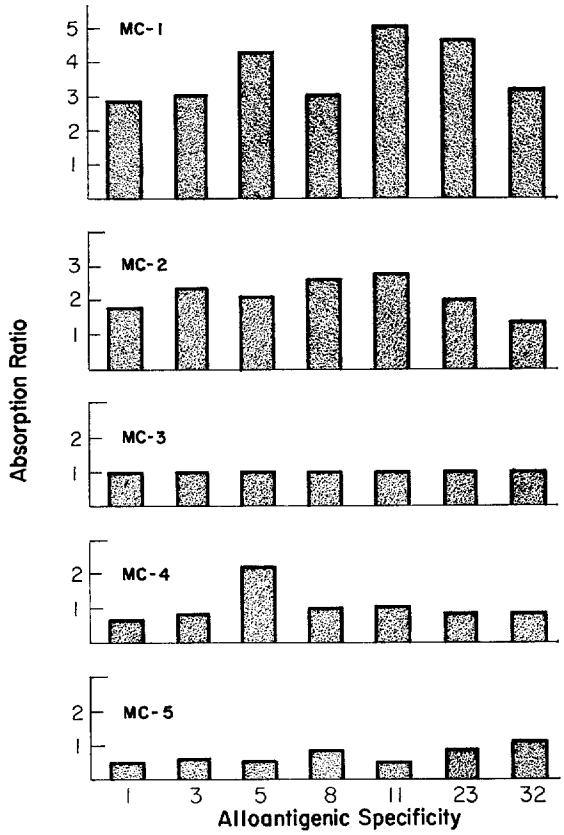

FIG. 3. Absorbing capacity of individual H-2 alloantigens on different MCA sarcomas relative to MC-3. No specificities are missing from any tumor and each tumor had its own coordinated level of antigenicity with no aberrant high or low levels of any one specificity.

From Fig. 4 it can be seen that there was a significant difference in the killing of different tumors with the same antibody and C′. The concentration of antigen on the surface of the tumor cells, as reflected by their sensitivity to antibody and C′, is again in the same order: MC-1 > MC-2 > MC-3 > MC-4 > MC-5.

Tumor-Specific Immunogenicity.—Tumor-specific immunity is usually dem-

onstrated by optimal immunization of animals and then challenge with different doses of viable tumor cells. In these studies the immunogenicity of the tumors was evaluated under more standard conditions by setting up a constant single immunization and challenge schedule for all of the tumors. Large groups of mice were immunized by giving 10^6 viable tumor cells intramuscularly in the leg. 7 days after the injection the legs with tumors were amputated. 5 days later the mice were divided into groups of five and challenged with serial doses of

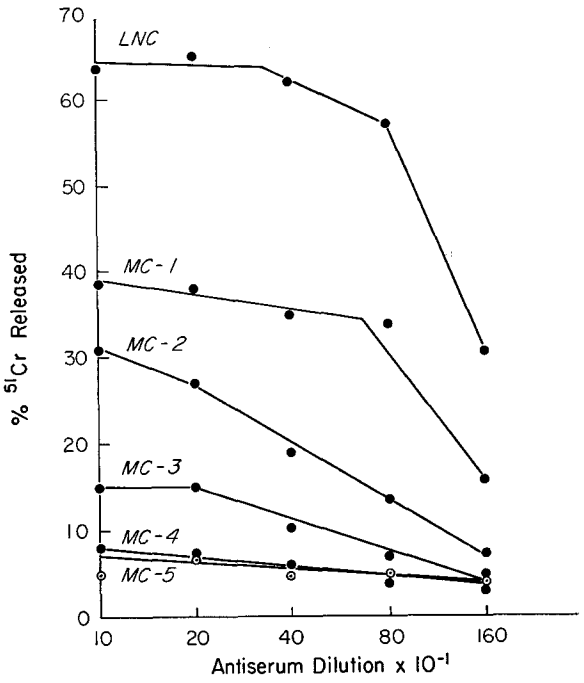

FIG. 4. Direct cytotoxic effect of multispecific H-2 antisera (DBA/2 anti-C3H) against different MCA-induced tumors and normal lymph node cells.

tumor cells by subcutaneous injection on the back. All immunized mice were challenged with the same tumor with which they were immunized. Cells from the same tumor suspension were used to challenge groups of unimmunized control mice. The number of mice developing tumors over the number of mice challenged is presented in Table V. The 50% tumor-producing dose (TPD-50) was computed to give a quantitative comparison of the immunogenicity of the different tumors. Table V also shows the TPD-50 for each of the tumors in control and immunized mice. This experiment demonstrated no immunogenicity for tumors MC-1, MC-2, or MC-3. However, there was a 10-fold increase in the number of tumor cells needed to produce tumors in 50% of the immune mice

with tumors MC-4 and MC-5. The TPD-50 in the controls also gives a measure of immunogenicity. The TPD-50, shown in the control column of Table V for tumors MC-3, MC-4, and MC-5, was 10 times greater than for tumors MC-1 and MC-2. From these data it was determined that the immunogenicity of the five tumors is in the order: MC-5 = MC-4 > MC-3 > MC-2 = MC-1.

Metastasis.—The role of the antigenic structure of tumor cells on the biological properties of the tumor is not well defined. If the autochthonous animal has an immune response to the tumor, the strength of the tumor-specific antigen could play an important role in the ability of a tumor to metastasize.

An experiment was designed to test the ability of the various tumors to metastasize to the lung. Groups of four animals were given 10^6 viable tumor cells

TABLE V

*Incidence of Tumor Growth in Immunized Mice**

Tumor	Number of cells used for challenge								TPD-50	
	10^5		10^4		10^3		10^2			
	Control	Immune	Control	Immune	Control	Immune	Control	Immune	Control	Immune
MC-1	5/5	4/5	4/5	5/5	3/5	4/5	2/5	0/5	120	120
MC-2	5/5	2/4	5/5	4/4	4/5	4/5	1/5	0/5	120	110
MC-3	3/5	4/5	4/5	4/5	4/5	3/5	0/5	1/5	1000	1000
MC-4	5/5	2/5	3/5	1/5	2/5	1/5	2/5	0/5	1280	11450
MC-5	2/3	2/4	3/4	2/5	1/5	0/5	1/5	0/5	1290	12700

* Mice were immunized with 10^6 tumor cells 12 days before challenge with various doses of cells from the same tumor. Tumors growing from the immunization were removed by amputation 5 days before challenge. The table gives the number of mice developing tumors/the number of mice challenged. Last column indicates number of cells of each tumor required to kill 50% of normal or immunized mice, used to establish sequence of tumor-specific immunogenicity.

subcutaneously. 14 days later the mice were sacrificed and the lungs removed. The lungs from each animal were minced and injected subcutaneously into C3H mice previously given 350 R of whole body X-irradiation. For 3 months the irradiated mice were watched for the development of tumors. The results in Table VI show that tumors MC-1 and MC-2 had metastasized in all of the animals by 2 wk. These were the same tumors with the greatest amount of H-2 antigen and the lowest tumor-specific immunogenicity. Tumors MC-3, MC-4, and MC-5 showed only a slight degree of metastasis at this time. If the tumors were allowed to grow for 3 or 4 wk they all metastasized to the lung but also killed the animals by progressive growth of the original tumor. The results of these experiments are summarized in Table VII.

DISCUSSION

The variation among normal and tumor-specific transplantation antigens on the surface of cells transformed by carcinogens suggests that they play a

critical role in the tumor–host relationship. Quantitative absorption and sensitivity to cytotoxicity of antibody can both be used to demonstrate H-2 antigenic differences between tumors. In the present experiment quantitative absorption of monospecific H-2 antisera was used to study the variation of individual H-2 antigenic determinants on five MCA-induced tumors. No pattern of individual antigenic gain or loss could be detected. Such a picture would have been predicted if the TSTA had replaced one of the H-2 determinants or if

TABLE VI

*Incidence of Pulmonary Metastasis in Mice with Different MCA Tumors**

Tumor	Day of lung transfer		
	14	21	31
	Number lung metastases/mouse		
MC-1	4/4	4/4	—
MC-2	4/4	3/3	4/4
MC-3	2/4	4/4	3/3
MC-4	1/4	1/2	1/1
MC-5	0/4	2/2	2/2

* Mice were given 10^6 viable tumor cells 14, 21, and 31 days before the lungs were transferred to immunologically suppressed animals. All groups had lungs injected into four mice and the table gives the number of mice developing tumors per number of mice receiving lungs.

TABLE VII

Summary of Studies of H-2 Antigenicity, Tumor-Specific Immunogenicity, and Early Pulmonary Metastasis of Five MCA Sarcomas

	H-2		Tumor antigen	
	Absorption	Cytotoxicity	LD_{50}	Metastasis
MC-1	+++++	+++++	+	++++
MC-2	++++	++++	+	++++
MC-3	++	++	++	++
MC-4	++	+	+++	+
MC-5	+	+	+++	+

it had altered all but one. The results showed that all of the antigens on a given tumor were altered to the same relative degree. Whether or not there was an actual increase or decrease in the concentration of antigen on the surface of the cells would require comparison with the normal tissue from which the tumors derived. Assuming that the MCA fibrosarcomas were derived from fibroblasts, quantitative absorption studies were carried out on 18-day old mouse embryo fibroblasts cultured in vitro for 24 hr. There was so little H-2 antigen on these fibroblasts that the comparison was not meaningful. Quantitative studies with

adult fibroblasts or embryo fibroblasts maintained longer in culture were not done.

Sensitivity of the five tumors to the direct cytotoxic action of a multispecific anti–H-2 serum and C′ provided an alternate method for comparing the relative total antigenicity of the tumors. The cytotoxic sensitivity of the various tumors correlated well with the average of the amount of all antigens as determined by quantitative absorption. From these results it was possible to put the tumors in an arbitrary sequence with regard to their H-2 antigenicity (MC-1 through MC-5).

A coordinated change in all determinants from a given allele is consistent with the observations of Klein and Klein (22) and Hellstrom (23) on selected tumor variants in which a tumor from a F_1 hybrid may lose all of the H-2 antigenic expression of one or the other parent but not from both. The regions and specificities, with respect to the $H\text{-}2^k$ allele, are shown in Fig. 5. Region K is separated from E by a locus producing a serum protein and cross-overs have been recognized. In the studies of Old et al. (24) on the modulation of H-2

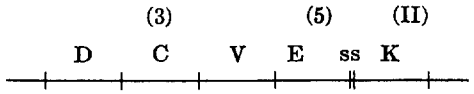

FIG. 5. A schematic map of the H-2 region of the IXth linkage group of the mouse showing the determinants used in this study (16).

antigens by TL antibody there was a quantitative alteration of the antigens representing the C region but not the K region. In the current study regions C, E, and K are expressed by antigens 3, 5, and 11. The finding of a coordinated alteration of the antigens for C and K in the present study and a noncoordinated change in the studies of Old et al. suggest that there are at least two levels of control of the expression of surface H-2 antigens.

To compare the tumor-specific immunogenicity of the same tumors, a standardized immunization challenge was carried out in isogeneic mice. Comparison of the TPD-50 in nonimmunized and immunized mice suggested that tumors MC-1, MC-2, and MC-3 had no immunogenicity. Tumors MC-4 and MC-5 demonstrated a 10-fold increase of the TPD-50 in the immune mice, suggesting a greater antigenicity. Tumors MC-3, MC-4, and MC-5 had a 10-fold greater TPD-50 in nonimmune mice compared with MC-1 and MC-2. Therefore the order of the tumors with respect to their tumor-specific immunogenicity was MC-5, MC-4, MC-3, MC-2, MC-1. The sequence is exactly the inverse of that related to H-2 antigenicity.

The concept that the surface of the cell is "organized as a mosaic" (25) was further advanced in a discussion by Boyse and Old (26) in which they viewed the cell surface as a precise grid, individuality being conferred by unique ar-

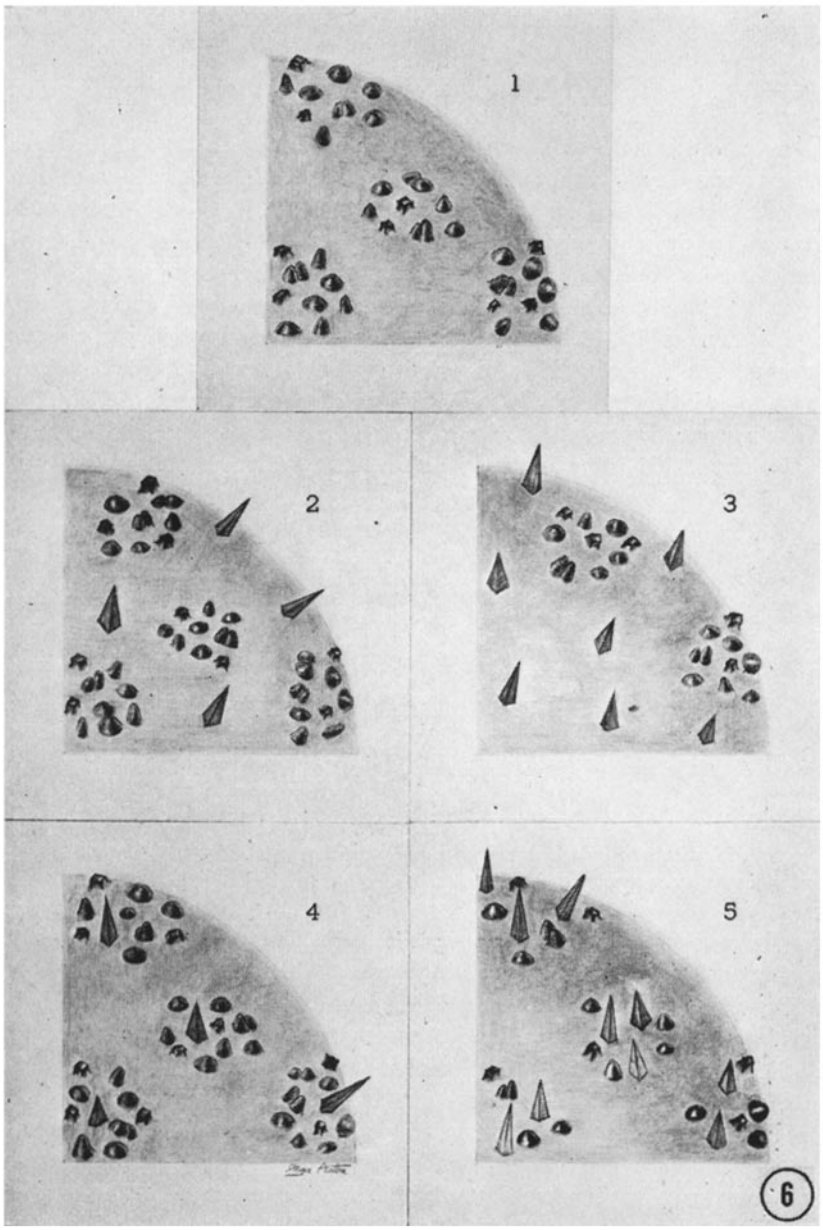

FIG. 6. Models of cell surface showing possible relationship between H-2 and tumor-specific antigeic sites. (*1*) Surface of normal cell, showing different H-2 specificities associated with each other in clusters. (*2 and 3*) Tumor-specific antigens remote from and replacing entire clusters of H-2 antigens. (*4 and 5*) Tumor-specific antigens intimately associated with and replacing H-2 antigens inside individual clusters. Each of these models would explain reciprocal relationships between H-2 and tumor-specific antigenic systems.

rangements of relatively few gene products. The present studies indicate an inverse relationship between the TSTA and the normal histocompatability antigens. This is consistent with the studies of Old et al. (24) and Fenyo et al. (9) demonstrating an alteration in the quantity of H-2 antigens by the modulation of either TL on leukemia cells or the TSTA on Moloney lymphoma cells. If the H-2 antigens are present on the cell surface in "patches," as indicated by recent studies, two different general configurations are suggested. (Fig. 6) (*a*) Tumor-specific sites may be independent of the normal antigens; with a large amount of TSTA the normal patches could be crowded out, producing fewer patches. (Fig. 6, sections 2 and 3) (*b*) The alternative would be for the TSTA to be intimately related to the H-2 patches (Fig. 6, sections 4 and 5) and in this way be expressed at the expense of normal antigens. If subsequent studies prove this latter model to be correct, two further alternatives will be

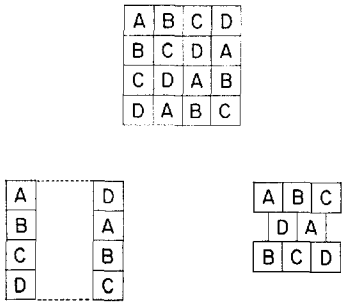

FIG. 7. Mechanisms by which primary deletions of normal antigens from clusters could result in new, tumor-specific specificities.

open for consideration. The tumor-specific antigen may be entirely new, having no relationship whatsoever to existing normal antigens on the cell, as depicted in Fig. 6. Alternatively, primary deletion of H-2 antigenic specificities from their normal position within the cluster may result in rearrangements and new relationships between existing specificities that result in entirely new, tumor-specific antigens, as shown in Fig. 7 and as suggested by Boyse and Old (26).

MCA-induced sarcomas metastasize to the lungs under appropriate experimental conditions. The primary tumor must remain in the host long enough to allow the circulating tumor cells to establish in the lungs, yet be removed soon enough that the primary tumor does not kill the animal (27). The studies presented here show that the ability of MCA-induced sarcomas to metastasize to the lung correlates with the antigenic make-up of the tumor. Tumors with relatively large amounts of normal H-2 antigen and low TSTA antigenic strength (MC-1 and MC-2) established viable tumor cells in the lung earlier than did tumors with the opposite relationship (MC-4 and MC-5). Other

studies on concomitant immunity, showing that an animal can reject an autograft from a progressively growing tumor, are consistent with these results (28–30). At what point in the course of exposure to a primary tumor metastasis develops depends upon a dynamic state between the immunologic capability of the host and the antigenic structure of the tumor.

SUMMARY

Five methylcholanthrene-induced sarcomas were compared for their capacity to (*a*) absorb monospecific H-2 antisera, (*b*) induce tumor-specific immunity in syngeneic mice, and (*c*) metastasize early to the lungs. Comparison of the uptakes of monospecific H-2 antisera by the five different tumors showed that each of the tumors had a high, intermediate, or low surface representation of all of the seven specificities tested. No antigenic specificity was completely absent from any tumor, and no tumor had an unusually large or small amount of any individual specificity. The tumors could be placed in the sequence from one to five with respect to their H-2 antigenicity. The same five tumors were also ranked with respect to their capacity to induce a tumor-specific immune response in syngeneic mice. The tumor-specific immunogenicity had an inverse relationship to the H-2 antigenicity in that highly immunogenic tumors were those that had quantitatively less H-2 antigen on their surface and vice versa. Early metastases to the lung was associated with low levels of tumor-specific immunogenicity and high levels of H-2 antigenicity.

The authors wish to thank Mrs. Sigrid Nelson and Miss Sharon Krueger for their excellent technical assistance.

BIBLIOGRAPHY

1. Moller, G. 1961. Studies on the development of the isoantigens of the H-2 system in newborn mice. *J. Immunol.* **85a:**56.
2. Moller, G. 1961. Phenotypic expression of isoantigens of the H-2 system in embryonic and newborn mice. *J. Immunol.* **90b:**271.
3. Schlesinger, M. 1965. Immune lysis of thymus and spleen cells of enbryonic and neonatal mice. *J. Immunol.* **94:**358.
4. Pizarro, O., G. Hoecher, P. Rubinstein, and A. Ramos. 1961. The distribution in the tissue and development of H-2 antigens of the mouse. *Proc. Nat. Acad. Sci. U.S.A.* **47:**1900.
5. Moller, E., and G. Moller. 1962. Quantitative studies of the sensitivity of normal and neoplastic cells to the cytotoxic action of isoantibodies. *J. Exp. Med.* **115:** 527.
6. Hammerling, U., I. V. Shigeno, L. J. Old, and E. A. Boyse. 1969. Labelling of mouse alloantibody with tritiated DL-alanine. *Immunology.* **17:**999.
7. Boyse, E. A., E. Stockert, and L. J. Old. 1967. Modification of the antigenic structure of the cell membrane by thymus-leukemia (TL) antibody. *Proc. Nat. Acad. Sci. U. S. A.* **58:**954.

8. Moller, E. 1962. Isoantigenic properties of tumors transgressing histocompatibility barriers of the H-2 system. *J. Nat. Cancer. Inst.* **33**:979.
9. Fenyo, E. M., E. Klein, G. Klein, and K. Sweich. 1968. Selection of an immunoresistant Moloney lymphoma subline with decreased concentration of tumor-specific antigens. *J. Nat. Cancer Inst.* **40**:69.
10. Boyse, E. A., L. J. Old, and E. Stockert. 1968. An approach to the mapping of antigens on the cell surface. *Proc. Nat. Acad. Sci. U. S. A.* **60**:886.
11. Cresswell, P., and A. R. Sanderson. 1968. Spatial arrangement of H-2 specificities: evidence from antibody adsorption and kinetic studies. *Transplantation.* **6**:996.
12. Cerottini, J. C., and K. T. Brunner. 1967. Localization of mouse isoantigens on the cell surface as revealed by immunofluorescence. *Immunology.* **13**:395.
13. Aoki, T., U. Hammerling, E. DeHarven, E. A. Boyse, and L. J. Old. 1969. Antigenic structure of cell surfaces. An immunoferritin study of the occurrence and topography of H-2, O, and TL alloantigens on mouse cells. *J. Exp. Med.* **130**:979.
14. Klein, G. 1967. Tumor Antigens. *In* The Specificity of Cell Surfaces. B. D. Davis and L. Warren, editors. Prentice-Hall, Inc., Englewood Cliffs, N. J. 165.
15. Boyse, E. A., E. Stockert, and L. J. Old. 1968. Isoantigens of the H-2 and TLA loci of the mouse: interactions affecting their representation on thymocytes. *J. Exp. Med.* **128**:85.
16. Snell, G. D., and J. H. Stimpfling. 1966. Genetics of tissue transplantation. *In* Biology of the Laboratory Mouse. E. L. Grenn, editor. McGraw-Hill Book Company, New York. 457.
17. Klein, G., H. O. Sogren, E. Klein, and K. E. Hellstrom. 1960. Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary anthochthonous host. *Cancer Res.* **20**:1561.
18. Wigzell, H. 1965. Quantitative titration of mouse H-2 antibodies using ^{51}Cr labelled target cells. *Transplantation.* **3**:523.
19. Boyse, E. A., M. Miyazawa, T. Aoki, and L. J. Old. 1968. Ly-A and Ly-B: two systems of lymphote isoantigens in the mouse. *Proc. Roy. Soc. Ser. B Biol. Sci.* **170**:175.
20. Reed, L. J., and H. Munch. 1938. A simple method of estimating fifty percent end points. *Amer. J. Hyg.* **27**:493.
21. Davis, B. D., R. Dulbecco, H. N. Eisen, H. S. Ginsberg, and W. B. Wood, editors. 1967. Microbiology. Harper and Row Publishers, Inc., New York.
22. Klein, G., and E. Klein. 1959. Cytogenetics of experimental tumor. *In* Genetics and Cancer. R. W. Cumley, M. Abbot, and J. McCay, editors. University of Texas Press. Austin, Texas.
23. Hellstrom, K. E. 1964. Possible use of H-2 antigens as possible selective markers in heterogeneous cell populations. *Nature (London).* **201**:893.
24. Old, L. J., E. Stockert, E. A. Boyse, and J. H. Kim. 1968. Antigenic Modulation: loss of TL antigen from cells exposed to TL antibody. Study of the phenomenon in vitro. *J. Exp. Med.* **127**:523.
25. Wallach, D. F. H. 1967. Isolation of plasma membranes of animal cells. *In* The Specificity of Cell Surfaces. B. D. Davis and L. Warren, editors. Prentice-Hall, Inc., Englewood Cliffs, N. J. 129.

26. Boyse E. A., and L. J. Old. 1970. *In* Immune Surveillance. R. T. Smith and M. Landy, editors, Academic Press, Inc., New York. In press.
27. Ketcham, A. S., J. J. Ryan, and H. Wexler, 1969. The shedding of viable circulating tumor cells by pulmonary metastasis in mice. *Ann. Surg.* **169**:297.
28. Milulska, Z. B., C. Smith, and P. Alexander. 1966. Evidence for immunological reactions of the host against its own actively growing primary tumor. *J. Nat. Cancer Inst.* **36**:29.
29. Gershon, R. K., R. L. Carter, and K. Kondo. 1967. On concomitant immunity in tumor-bearing hamsters. *Nature* (*London*). **213**:674.
30. Lausch, R. N., and F. Rapp. 1969. Concomitant immunity in hamsters bearing DMBA-induced tumor transplants. *Int. J. Cancer.* **4**:226.