[CANCER RESEARCH 35, 2049-2055, August 1975]

Neoantigens on Chemically Transformed Cloned C3H Mouse Embryo Cells[1]

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SUMMARY

Using an *in vitro* cytotoxicity test for cell-mediated immunity and a membrane immunofluorescence test, the appearance of new antigens was detected on cloned C3H mouse embryo cells undergoing malignant transformation *in vitro* following treatment with 3-methylcholanthrene or 7,12-dimethylbenz[a]anthracene. These antigens were recognized by specifically immunized syngeneic mice and were individually unique for each of eight chemically transformed cell lines tested, all of which were derived from the same control parent clone. Very few cross-reactions were seen between lymphoid cells or antibody from mice immunized against a given cell line and target cells of other cell lines. New antigens could not be detected on two spontaneously transformed lines.

Lymphoid cells from multiparous pregnant or embryo-immunized mice were used to search for fetal antigens on control and transformed cells. Fetal antigens were detected on seven of the chemically transformed cell lines and one spontaneous transformant, but not on nontransformed control cells. It is concluded that individually specific new antigens are characteristic of chemically transformed cells, but the expression of fetal antigens may be a more common feature of transformed cells in general.

INTRODUCTION

Tumors induced by carcinogenic hydrocarbons in inbred animals are characterized by individually unique antigens capable of inducing a tumor rejection response in immunized hosts (15, 16). Antigens with the same individual specificity can be detected by *in vitro* cytotoxicity assays for cell-mediated immunity (12) or by indirect immunofluorescence assays for tumor-specific antibody (1). These antigens may be identical with the tumor rejection antigens. With *in vitro* methods it is also possible to demonstrate cell surface-expressed fetal antigens on many experimental tumors, including sarcomas induced by polycyclic hydrocarbons in the rat and mouse (3, 9, 20). These antigens are recognized by multiparous pregnant females, are common for many tumors, and appear to be distinct from those that induce tumor-specific immunity (4).

Individually distinct antigens have also been detected on mouse prostate cells undergoing malignant transformation *in vitro* (11, 14) and on 3T3 cells transformed in diffusion chambers following exposure to carcinogens (7). As in the case of tumors induced *in vivo*, these antigens could be demonstrated by their ability to induce immunity to an inoculum of cells, which grows in untreated animals (7, 14), or by 3 *in vitro* methods (11). From these studies it was concluded that the antigens detected had newly arisen as a result of carcinogen action and were not selected from already existing antigens, since different transformed clones derived from a single parent control clone had different antigens (7, 11). However, the relationship of these antigens to fetal antigens, which are not normally expressed on adult cells, was not investigated.

The studies in this report were designed to search for and to compare both individually distinct antigens and fetal antigens on cloned cells undergoing carcinogen-induced transformation *in vitro*. The system used was a quantitative system developed by Reznikoff *et al.* (18, 19) that is based on cloned C3H mouse embryo cell lines that are sensitive to postconfluence inhibition of cell division and are designated C3H/10T1/2 cells.

MATERIALS AND METHODS

Mouse Embryo Cells. C3H/10T1/2 clone 8 mouse embryo cells (19) and malignant cells derived from them following treatment in culture with MCA[4] or DMBA (18) were used. Four clones were transformed by MCA, 4 were transformed by DMBA, and 2 clones of spontaneously transformed cells were chosen as malignant lines. The criteria for transformation have been carefully defined (18). Controls were either untreated 10T1/2/CL8 cells, subclones of them, or acetone-treated controls. For these studies large numbers of cells were grown in roller bottles or in Falcon tissue culture flasks using BME with 10% heat-inactivated fetal calf serum (Grand Island Biological Co., Grand

[1] Supported in part by Grant CA-07175 and by Contract 72-2022 from the Virus Cancer Program, National Cancer Institute, NIH, and by Grant BS-2C from the American Cancer Society.

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Received February 11, 1975; accepted April 23, 1975.

[4] The abbreviations used are: MCA, 3-methylcholanthrene; DMBA, 7,12-dimethylbenz[a]anthracene; BME, Eagle's basal medium; PBS, phosphate-buffered saline [NaCl (8 g/l), KCl (0.2 g/l), Na_2HPO_4 (1.15 g/l), KH_2PO_4 (0.2 g/l)]; LNC, lymph node cells.

Island, N. Y.). The transformed cells were previously shown to produce fibrosarcomas in irradiated (500 R) syngeneic mice, whereas the nontransformed cells did not (18).

Mice. Immunogenicity studies were carried out in inbred male C3H mice supplied by the Drug Development Center, National Cancer Institute, Bethesda, Md. Retired breeding females of this strain were used as multiparous mice and were mated with syngeneic males to produce pregnant donors. All mice were syngeneic with the animal from which the 10T1/2/CL8 cells were originally derived (19).

Immunization. Male mice were immunized i.p. with 2×10^6 live control or transformed cells at their 27th to 32nd passage, emulsified in 1 ml of PBS, pH 7.2, with 1 ml of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.). Ascites developed in treated mice after 7 to 10 days. Multiparous female mice that failed to become pregnant were similarly immunized with minced tissue of whole 15-day embryos in 1 ml of PBS emulsified with 1 ml of adjuvant.

LNC. Mice were sacrificed 11 to 18 days after immunization and the cervical, axillary, and mesenteric lymph nodes were taken. Cell suspensions were prepared by mincing the lymph node tissue with scissors and gently rubbing the minced tissue through a fine nylon mesh in a little BME. The cells were washed twice in BME before use.

LNC were prepared in a similar manner from multiparous pregnant mice at about 15 days of gestation. Untreated male mice served as controls for mice immunized with transformed or nontransformed 10T1/2/CL8 cells, and virgin females were used as controls for pregnant mice or those immunized with embryo tissue.

Serum and Ascites Fluid. Blood was taken from multiparous pregnant mice by cardiac puncture when they were sacrificed to obtain lymph nodes. Serum was prepared from the clotted blood and stored at −20°. Control serum was similarly obtained from untreated virgin female mice.

Ascites fluid was aspirated from mice immunized with cells, emulsified with Freund's complete adjuvant, and allowed to stand at 4° for a few hr so that lipid material could form a separate layer. The lipid-free portion was centrifuged at $1000 \times g$ to remove any suspended cells and was stored at −20°. Control ascites fluid was prepared from mice given injections of 1 ml of PBS emulsified with 1 ml of adjuvant.

Microcytotoxicity Tests. Cell-mediated immunity against cultured target cells was assayed as previously described (11). Briefly, 200 target cells/well were incubated in Linbro 15-FB-96 plates for 4 hr, or overnight, to allow attachment to the bottoms of the wells. The medium was then replaced with 0.2 ml of serum-free medium containing 2×10^5 LNC from control or immunized mice. After 45 min at 37°, 0.05 ml of 50% fetal calf serum in BME was added to each well, followed by a further 2 days of incubation at 37°. Dead or unattached cells were then gently washed off with 0.9% NaCl solution and the remaining cells were fixed with methanol, stained with Giemsa, and counted under ×30. The reduction of the number of target cells in wells treated with immune LNC compared with control LNC was expressed as percentage of cytotoxicity and was evaluated statistically by the Student *t* test.

Membrane Immunofluorescence Test. The technique used was a slightly modified version of the method previously described (11). Aliquots of 2 to 3×10^6 living cells were incubated for 15 min at room temperature with 0.05 ml of ascites fluid from control or immunized mice, diluted with 0.2 ml of BME. The cells were washed 3 times in BME and then incubated for 15 min at room temperature with 0.1 ml of a 1/20 dilution of fluorescein isothiocyanate-conjugated rabbit anti-mouse globulin (Hyland Laboratories, Los Angeles, Calif.). After 3 more washes in BME, the cells were resuspended in 0.1 ml of 50% glycerol in PBS and examined under a Leitz fluorescence microscope with incident UV illumination. Cells that showed fluorescence at the cell surface were scored as positive. The fluorescence index was expressed as the percentage of cells unstained in control minus the percentage unstained in the test ascites fluid, divided by the percentage unstained by control fluid.

In some tests, target cells were reacted with serum from pregnant multiparous mice, and in this case normal virgin female mouse serum was used in controls.

RESULTS

Cell-mediated Immunity Tests. LNC from mice immunized with control or transformed cells were tested for their ability to inhibit survival of the immunizing cell line or other cell lines using the microcytotoxicity assay (Table 1). Mice that were immunized with 5 control lines (including the parent 10T1/2/CL8 line), 2 subclones (10T1/2/CL8/SC3 and 10T1/2/CL8/SC5), and 2 acetone-treated 10T1/2/CL8 controls (55AC/CL1 and 58AC/CL1) failed to show any significant cytotoxicity against plated control target cells. Animals immunized with chemically transformed cells, however, reacted against the immunizing cell line in all cases such that LNC from the immune mice reduced the number of target cells surviving in microtest plates compared with LNC from normal control mice. This target cell reduction ranged from 20 to 70% cytotoxicity in 23 separate tests on chemically transformed cells. In addition to being tested against the cell line used for immunization, immune LNC preparations were tested against other target cells. Cross-reactions were unusual and were seen in only 6 of 87 cross-tests between transformed cells, these cases being observed mainly with DMBA-transformed cells, which were less fibroblastic in appearance than the MCA-transformed cells. LNC from 1 mouse treated with 10T1/2/CL8/SC5 control cells were reactive against spontaneously transformed 58AC/CL2 cells and DMBA-transformed 55DMBA/CL12 cells, but this was the only animal immunized with control cells that showed any reactivity against transformed target cells. Mice immunized against 2 spontaneous transformants (58AC/CL2 and 55AC/CL3) did not react against the immunizing cell line, indicating a lack of immunogenicity of the spontaneously transformed cells in comparison with the chemical transformants, as we had previously observed in mouse prostate fibroblasts (11).

Included among the MCA-transformed cells was a line designated 58MCA/CL15/TU, which was derived from a

Table 1

Microcytotoxicity against transformed and nontransformed C3H/10T1/2/CL8 cells by LNC from immunized mice

Target cells	Immunization of LNC donors: Cell line	Immunization of LNC donors: Days after immunization	Cytotoxicity (%)[a]
Nontransformed			
10T1/2/CL8	10T1/2/CL8	15	7.8, 5.2
	58MCA/CL15	19	0
	55DMBA/CL2	19	0
	55DMBA/CL10	19	0
10T1/2/CL8/SC3[b]	10T1/2/CL8/SC3	15	10.8, 15.0
10T1/2/CL8/SC5[b]	10T1/2/CL8/SC5	15	10.5, 15.8, 16.3
55AC/CL1[c]	55AC/CL1	12	0
	58AC/CL1	15	0
	55AC/CL3	12	0, 0
	55DMBA/CL2	19	0
	55DMBA/CL10	19	0
58AC/CL1[c]	58AC/CL1	12, 15	0, 0, 6.5
	55AC/CL3	12	0, 0
	58MCA/CL15	19	0
	55DMBA/CL2	19	0
	55DMBA/CL10	19	0
Chemically transformed			
58MCA/CL13	58MCA/CL13	13, 14	22.8,[d] 25.4,[d] 45.9[e]
	58MCA/CL14	13, 14	3.1, 9.8, 17.0[d]
	58MCA/CL15	16, 17	1.3, 7.3
	58MCA/CL16	16	2.9, 3.3
	55DMBA/CL2	17	6.8
	55DMBA/CL10	17	8.9
58MCA/CL14	58MCA/CL13	13, 14	0, 0, 0
	58MCA/CL14	13, 14	34.8,[d] 36.8,[f] 36.8[f]
	58MCA/CL15	16, 17	0, 9.3
	58MCA/CL16	16	0, 0
	55DMBA/CL2	17	0
	55DMBA/CL10	17	0
58MCA/CL15	10T1/2/CL8/SC3	14	0
	10T1/2/CL8/SC5	14	0, 0
	58MCA/CL13	13, 14	0, 0, 0
	58MCA/CL14	13, 14	0, 0, 0
	58MCA/CL15	16, 17	45.4,[f] 47.7,[e] 51.2[e]
	58MCA/CL16	16	0, 0
	55DMBA/CL2	17	5.5
	55DMBA/CL10	17	0
58MCA/CL15/TU[g]	58MCA/CL13	13	0, 0
	58MCA/CL14	13	0, 2.2
	58MCA/CL15	16	38.6,[f] 38.6[f]
	58MCA/CL16	16	5.1, 5.7
58MCA/CL16	10T1/2/CL8/SC3	14	3.1, 10.5
	10T1/2/CL8/SC5	14	0
	58MCA/CL13	13, 14	0, 0, 0
	58MCA/CL14	13, 14	0, 0, 0
	58MCA/CL15	16, 17	0, 0
	58MCA/CL16	16	34.0,[f] 43.3[e]
	55DMBA/CL2	17	0
	55DMBA/CL10	17	0

[a] Probability that values are due to chance is indicated by: [d] $p < 0.05$; [e] $p < 0.001$; [f] $p < 0.01$. Unmarked values are not significant. Values <0 are given as 0 for clarity; these were not statistically significant.

[b] Subclones of 10T1/2CL8.

[c] Acetone-treated control cells.

[d,e,f] See Footnote *a*.

[g] Cells grown from tumor arising from *in vivo* cell inoculation.

Table 1—*continued*

Target cells	Immunization of LNC donors: Cell line	Days after immunization	Cytotoxicity (%)[a]
55DMBA/CL2	58MCA/CL15	18	12.3
	55DMBA/CL2	14, 18	23.0,[d] 24.4,[d] 41.2[f]
	55DMBA/CL4	11	0, 9.7
	55DMBA/CL10	14, 18	0, 33.1,[f] 36.1[f]
	55DMBA/CL12	14	0, 1.2
55DMBA/CL4	58MCA/CL15	18	7.6
	55DMBA/CL2	14, 18	0, 0, 1.2
	55DMBA/CL4	11	27.4,[d] 28.5[d]
	55DMBA/CL10	14, 18	0, 9.4, 54.1[e]
	55DMBA/CL12	14	1.2, 2.8
55DMBA/CL10	10T1/2/CL8/SC3	14	0
	10T1/2/CL8/SC5	14	0, 0
	58MCA/CL15	18	0
	55DMBA/CL2	14, 18	4.8, 10.8, 13.7
	55DMBA/CL4	11	0, 2.9
	55DMBA/CL10	14, 18	48.2,[e] 58.3,[e] 67.2[e]
	55DMBA/CL12	14	10.3, 16.5
55DMBA/CL12	10T1/2/CL8/SC3	14	13.9
	10T1/2/CL8/SC5	14	14.9, 19.9[d]
	55DMBA/CL2	14, 18	0, 21.8[d]
	55DMBA/CL4	11	0.6, 6.5
	55DMBA/CL10	14, 18	0, 11.9
	55DMBA/CL12	14	66.6,[e] 69.2[e]
Spontaneously transformed			
55AC/CL3	55AC/CL1	12	0
	58AC/CL1	15	0
	55AC/CL3	12	0, 0
	58MCA/CL15	19	0
	55DMBA/CL2	19	0
	55DMBA/CL10	19	0
58AC/CL2	10T1/2/CL8	15	4.1, 6.5
	10T1/2/CL8/SC3	15	0, 15.2
	10T1/2/CL8/SC5	15	12.4, 20.5[d]
	58AC/CL2	12	0, 0, 0.4, 1.4, 2.1

tumor that grew *in vivo* after inoculation of 58MCA/CL15 cells into an irradiated (500 R) syngeneic C3H mouse. This line retained the specific antigen demonstrable on 58MCA/CL15 cells, as indicated by the fact that it provided a susceptible target for LNC from mice immunized against 58MCA/CL15, but not other cell lines.

Multiparous pregnant mice and multiparous mice immunized with 15- to 16-day embryo tissue were also tested for LNC-mediated cytotoxicity against transformed and control target cells to detect fetal antigens, as shown in Table 2. None of the LNC from these mice reacted significantly against nontransformed cells, but a high proportion were reactive against transformed cells compared with LNC obtained from virgin female controls. Thus, 8 of 14 samples of LNC from embryo-immunized mice and 10 of 14 from pregnant mice were cytotoxic to chemically transformed cells; all target cell lines except 55DMBA/CL4 were susceptible to their action. LNC from multiparous mice were also reactive against spontaneously transformed 58AC/CL2 cells, although there was no evidence of reactivity by LNC from 58AC/CL2-immunized mice (Table 1). The multiparous mouse LNC were found to be cross-reactive in a random fashion; individual mice reacted against some but not all target cells against which they were tested, possibly indicating a multiplicity of fetal antigens shared in a nonuniform fashion.

Immunofluorescence Tests. Ascites fluids from immunized mice were tested for antibody reacting with surface antigens of control and transformed cells by indirect membrane immunofluorescence (Table 3). In most tests with chemically transformed lines, ascites fluids from mice immunized with the tested cell line gave staining such that fluorescence indices of 0.3 or greater were obtained, indicating a significant reaction. However, 2 samples of fluid from mice immunized with 58MCA/CL15 cells were negative, as was the only sample from mice immunized with 55DMBA/CL12. No reactions were detected with control or spontaneously transformed cells. In some tests, positive ascites fluids were preabsorbed with 10T1/2/CL8 control cells (1.3×10^8 cells/ml for 1 hr at 37°), but this did not remove antibody

Table 2
Demonstration of fetal antigens on transformed 10T1/2/CL8 cells by LNC-mediated microcytotoxicity

Target cells	Cytotoxicity (%) by LNC from multiparous donors	
	Pregnant mice	Embryo-immunized mice
Nontransformed		
10T1/2/CL8	0, 0, 1.2, 11.6, 12.8	0
10T1/2/CL8/SC3	0, 0, 4.0, 8.5, 6.6	
10T1/2/CL8/SC5	0, 6.2, 8.1, 10.8	
55AC/CL1	0, 0	0
58AC/CL1	0, 0	0
Chemically transformed		
58MCA/CL13	6.4	30.7,[a] 34.2[a]
58MCA/CL14	42.5[b]	30.7,[c] 48.8[a]
58MCA/CL15	43.8,[a] 64.4[b]	0, 0
58MCA/CL15/TU	35.8[b]	
58MCA/CL16	26.5,[c] 26.9[a]	13.1, 15.8
55DMBA/CL2	18.5[c]	20.9,[c] 26.6[c]
55DMBA/CL4	0	6.0, 14.1
55DMBA/CL10	27.5,[a] 50.1[a]	28.9,[a] 43.1[b]
55DMBA/CL12	0, 0, 17.2, 36.5[a]	
Spontaneously transformed		
55AC/CL3	0, 0, 0	7.4
58AC/CL2	23.6,[c] 49.4,[a] 26.7,[c] 28.2[c]	

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

activity, confirming that the control cells did not possess antigens present on the chemically transformed cells.

Cross-tests were performed, and in most cases immune ascites fluids reacted only against the immunizing cell line. The only cross-reaction seen was between anti-58MCA/CL16 ascites fluid and 58MCA/CL14 target cells.

Fetal antigen could be detected on transformed cells in 6 of 29 tests with multiparous pregnant mouse serum and in 18 of 21 tests with ascites fluid from embryo-immunized multiparous mice (Table 3). The immunofluorescence test was somewhat less sensitive than LNC-mediated microcytotoxicity for detecting fetal antigens, but the reactions that were obtained showed cross-reactivity among several target cell lines.

Differentiation between Individual and Fetal Antigens. In an attempt to differentiate between individual antigens recognized by immunized mice and common fetal antigens, 3 target cell lines were incubated with serum from multiparous pregnant mice before being exposed to LNC from immunized and multiparous mice. As shown in Table 4, with 2 of 3 target cell lines (58MCA/CL13 and 55DMBA/CL12), reactivity by multiparous mouse LNC was blocked by multiparous mouse serum, whereas reactivity by immune LNC was unimpaired. With the 3rd line (55DMBA/CL10), the reduction of cytotoxicity was significantly greater for multiparous mouse LNC than for immune LNC, although the reactivity of the former was not completely abolished. These tests suggest that the fetal antigens and individual neoantigens are unrelated.

DISCUSSION

The results of this study indicate that separate transformed cell lines derived by chemical treatment of cells of a single parent clone of C3H mouse embryo cells acquire individually distinct neoantigens. This is in agreement with conclusions drawn from our previous studies in which multiple chemically transformed lines derived from clones of C3H mouse prostate fibroblasts were shown to have distinct antigens (11). Basombrío and Prehn (7) have shown similarly that the progeny of a single cell acquire different antigens after transformation by polycyclic hydrocarbons in diffusion chambers. These studies suggest that the appearance of a new cell-surface antigen is a unique event arising as a consequence of the carcinogen-induced neoplastic change and is not due to a selection of preexisting antigenically aberrant clones.

In addition to newly acquired individual antigens analogous to the tumor-specific antigens found on chemically induced tumors (1, 15, 16), most chemically transformed cell lines appeared to reexpress fetal antigens. These were detected by reaction with lymphoid cells or antibody from multiparous mice that were sensitized to fetal antigens. The presence of fetal antigens seems to be a common characteristic of many animal tumors (3, 6, 8, 9, 10, 21), and now we have demonstrated that they are also expressed on cells undergoing malignant transformation *in vitro*. Although in some tumor systems the fetal antigen and tumor-specific antigen may be identical (10), in chemically induced tumors

Table 3
Demonstration of antigens on transformed 10T1/2 cells by membrane immunofluorescence tests

Target cells	Fluorescence indices[a] with individual sera from: Specifically immunized mice	Multiparous mice
Nontransformed		
10T1/2/CL8	0.01	0
10T1/2/CL8/SC3	0.15	0, 0
10T1/2/CL8/SC5	0.01	0, 0.01
55AC/CL1		0.01, 0.08
58AC/CL2		0, 0.13
Chemically transformed		
58MCA/CL13	0.30, 0.34, 0.34	0, 0.24, 0.32, 0.53
58MCA/CL14	0.38, 0.46, 0.59	0.36, 0.44
58MCA/CL15	0.17, 0.17, 0.41, 0.56	0, 0.02, 0.13, 0.19
58MCA/CL16	0.36	0, 0.05, 0.16, 0.29
55DMBA/CL2	0.37, 0.49, 0.49, 0.49	0, 0.14, 0.27, 0.44
55DMBA/CL4	0.38	0.13, 0.20, 0.35
55DMBA/CL10	0.30, 0.34, 0.34, 0.48	0, 0.05, 0.09, 0.26
55DMBA/CL12	0.04	0.04, 0.08, 0.09, 0.13
Spontaneously transformed		
55AC/CL3	0	0, 0, 0, 0
58AC/CL2	0.10	0.22, 0.23, 0.49

[a] A fluorescence index of 0.30 is considered positive.

Table 4
Blocking of cell-mediated cytotoxicity against fetal antigens by exposure of target cells to serum from multiparous pregnant mice

Target cells	LNC donors	Cytotoxicity (%) following pretreatment with serum from: Normal female mice	Multiparous pregnant mice
58MCA/CL13	Embryo-immunized	34.2[a]	−6.6
	58MCA/CL13-immunized	45.9[a]	41.2[a]
55DMBA/CL2	Embryo-immunized	20.9[b]	−18.1
	55DMBA/CL2-immunized	41.2[a]	35.7[c]
55DMBA/CL10	Embryo-immunized	28.9[c]	14.8[b]
	55DMBA/CL10-immunized	58.3[a]	52.1[a]

[a] $p < 0.001$.
[b] $p < 0.05$.
[c] $p < 0.01$.

they appear to be distinct from each other (4, 8, 20). In chemically transformed mouse embryo cells these antigens are also different in type, since they differ both in their specificity and in their capacity to be blocked as targets for sensitized lymphoid cells following treatment with serum from multiparous pregnant donors. Multiparous serum protected target cells from attack by lymphoid cells sensitized to fetal antigens but not lymphoid cells sensitized to chemically induced individual neoantigens.

Fetal antigens were not detected on control clones although these were of embryonic origin. Fetal antigens are known to be expressed on embryos at certain stages of gestation in some species (5) so it is possible that the 10T1/2/CL8 cells were derived from embryos of later gestation than the period at which they are expressed. Alternatively, fetal antigens may have become lost from control cells upon prolonged culture, although they were stable on transformed cells tested up to the 33rd generation of transfer.

Individual antigens could not be detected on 2 spontaneously transformed lines, although 1 of these was shown by cell-mediated microcytotoxicity to have fetal antigens. This is again paralleled by some tumors arising in experimental animals, where fetal antigens may be detected in tumors apparently deficient in tumor-specific rejection antigens (2, 6). When present, individually specific neoantigens appear to be strongly associated with the malignant state since they are not present on carcinogen-treated but nontransformed cells (11) and are lost on reversion of malignant cells to a less malignant state (13). However, since such antigens are not present on all transformed cell lines, they may be a special characteristic of cells transformed by certain chemical carcinogens such as the polycyclic hydrocarbons used in this study, rather than a necessary requirement for cancer. Since we have recently demonstrated that chemical transformation of C3H/10T1/2 cells does not involve activation of an oncornavirus (17), it seems very unlikely that the neoantigens that we are measuring could be viral antigens. Therefore, it is likely that the expression of fetal antigens is a separate and more common event in neoplastic transformation, being a property of tumors of widely different etiology.

ACKNOWLEDGMENTS

The authors would like to thank Donald Palmer for his skilled technical assistance.

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