Production of Melanoma-associated Antigen(s) by a Defined Malignant Melanoma Cell Strain Grown in Chemically Defined Medium[1]

Darwin O. Chee, Arthur W. Boddie, Jr., Jack A. Roth, E. Carmack Holmes, and Donald L. Morton

Surgical Services, Oncology Research Section, Veterans Administration Hospital, Sepulveda, California 91343 [D. O. C., E. C. H., D. L. M.], and the Division of Oncology, Department of Surgery, University of California, Los Angeles, California 90024 [D. O. C., A. W. B., J. A. R., E. C. H., D. L. M.]

SUMMARY

A human malignant melanoma cell strain, UCLA-SO-M14 (M14), was adapted to grow in serum-free, chemically defined medium (CDM). The 3 M KCl extract prepared from the CDM-grown cells (M14-CDM) was assayed against leukocytes from melanoma patients, patients with other cancers, and normal donors by leukocyte migration inhibition (LMI). The leukocytes from 15 of 27 (56%) melanoma patients tested were LMI positive. In contrast, 4 of 18 (22%) other cancer patients and 5 of 30 (17%) normal donors' leukocytes were LMI positive. One of 14 melanoma patients' leukocytes were LMI positive for a control 3 M KCl extract from autologous muscle. Comparative studies were performed with the M14-CDM extract and a 3 M KCl extract from a freshly biopsied tumor specimen from the donor of the M14 cell strain. Seven of 12 (58%) melanoma patients' leukocytes were LMI positive to the M14-CDM extract, but only 2 of 12 (17%) were LMI positive to the autologous melanoma tissue extract. Furthermore, only 100 to 300 μg protein of M14-CDM extract were required to educe delayed cutaneous hypersensitivity response in 6 of 8 (75%) melanoma patients and 0 of 5 lung cancer patients, but 500 μg protein from biopsied autologous melanoma tissue extract were needed to produce delayed cutaneous hypersensitivity response in 24 of 42 (57%) melanoma patients and 7 of 28 (25%) nonmelanoma cancer patients. These data suggest: (a) the M14-CDM cells synthesized melanoma-associated antigen(s) (MAA) in CDM; (b) the 3 M KCl extraction procedure effectively removed the MAA from the M14-CDM cells; (c) the M14-CDM cells were a more potent source of MAA than the surgical autologous melanoma specimen; and (d) the M14-CDM cells provided a continuous source of standard MAA.

INTRODUCTION

Investigations with experimental animals have provided unequivocal in vivo evidence that tumors possess unique tumor-associated antigens that are absent on normal animal adult tissues and that these tumor antigens evoke an immune response that can influence tumor growth (9, 10, 22, 23, 28, 32).

A limited number of experiments with tumor autografts suggest that human tumors are immunogenic in the host (35), but for obvious ethical reasons, most investigations of human cancer immunology have been conducted with in vitro techniques. The data from these studies, generated by several laboratories, show a considerable degree of discrepancy (5, 6, 8, 13, 14, 18, 24-26, 29, 36, 39, 40). One of the most critical factors associated with these inconsistencies is the lack of reference tumor antigens with a high degree of activity (25). Surgical and necropsy tumor specimens vary in their activity and specificity (15) and are usually contaminated with extraneous tissues such as supporting mesenchymal and vascular tissues, blood cells, and plasma proteins that depreciate the quality of the tumor antigens prepared from these sources.

Tumor cells propagated in vitro offer a potential solution to the problem. However, recent evidence indicated that tumor cells cultured in medium supplemented with fetal calf serum acquire a heterologous membrane antigen that can be mistaken for a tumor-associated antigen (19, 20).

This report describes the successful adaptation to CDM[2] which synthesized MAA. Verification for MAA was demonstrated in an in vitro assay, LMI, and by in vivo DCHR in melanoma patients and in patients with other neoplasms.

MATERIALS AND METHODS

Cell Strain. A human malignant melanoma cell strain, UCLA-SO-M14, hereafter called M14, in its 18th passage, was obtained from Dr. H. L. Sulit, Division of Oncology, Department of Surgery, University of California, Los Angeles, Calif. The original culture was derived from an amelanotic lesion metastatic to the buttock of a 33-year-old patient. Tumorigenicity of the M14 cells was established by growing the cells in the cheek pouch of an immunodepressed Syrian hamster. Morphological characteristics in tissue culture (Fig. 1) and by electron microscopic examination (Fig. 2) identified the cell strain as melanoma.

[2] The abbreviations used are: CDM, chemically defined medium; MAA, melanoma-associated antigen(s); LMI, leukocyte migration inhibition; DCHR, delayed cutaneous hypersensitivity response; CEM, Chee's modification of Eagle's minimum essential medium; SCEM, CEM supplemented with 10% fetal calf serum; PVP, polyvinyl pyrrolidone; M14-CDM, M14 cells adapted to grow in CDM.

Cell Culture. The cells were subsequently grown in a medium (CEM) (Associated Biomedic Systems, Buffalo, N. Y.) modified from Eagle's minimum essential medium and supplemented with 10% fetal calf serum, 100 units penicillin, 50 µg streptomycin, and 100 µg kanamycin (SCEM). Confluent monolayers of the M14 cells grown in plastic T-flasks (no. 3013; Falcon Plastics, Oxnard, Calif.) were subcultured by discarding the spent medium and adding 0.5 ml EDTA-trypsin solution, composed of 0.15% tetrasodium EDTA (Calbiochem, San Diego, Calif) and 0.25% trypsin in Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} (Microbiological Associates, Bethesda, Md.). The flask was incubated at 37° for 2 to 4 min before the addition of 9.5 ml SCEM. The cells were dispersed gently by pipetting up and down, and a 5-ml cell suspension was then placed into each of 2 Falcon T-flasks. These flasks were incubated in a 37° incubator with 5% CO_2. This regimen was followed for serially subculturing the M14 cell strain.

Mass Culture. M14 was grown as mass culture in a CR-111 roller vessel (Forma Scientific, Inc., Marietta, Ohio). Six confluent monolayers of cells in Falcon T-flasks were treated with EDTA-trypsin solution and pooled into the roller bottle with 50 ml SCEM. The contents were incubated at 37° and rolled by a Model CR-5 Cel-Roll (Forma Scientific). The medium was changed weekly until confluency was reached.

Spinner Culture. Stationary cultures of M14 cells were adapted to grow as spinner cultures by subculturing the confluent M14 cells from the roller bottle into a 3-liter Bellco hanging bar spinner flask (Bellco Glass, Inc., Vineland, N. J.) containing 250 ml SCEM. The culture was gently agitated with a Bel-Stir magnetic stirrer (Bellco Glass). A volume of 250 ml fresh SCEM was added every 3 to 7 days until a total volume of 3 liters was obtained.

CDM Spinner Culture. A volume of 1.5-liters of M14 cell suspension was taken from the spinner culture and centrifuged for 30 min. The medium was decanted and the pellet of cells was suspended in 500 ml CEM containing 1.4% PVP-360 (Sigma Chemical Co., St. Louis, Mo.) (PVP-CEM) as a source of macromolecules (21) in a 3-liter spinner flask. PVP-CEM in volumes of 250 to 500 ml was added weekly. After adaption to this medium (approximately 8 weeks), the cells were centrifuged for 60 min, the medium was discarded, and the cells were resuspended in 500 ml CEM without PVP in a 6-liter spinner flask. CEM (500 ml) then was added weekly for 11 weeks. At this time, the M14 cells appeared to be fully adapted in CDM by morphological criteria. Microscopic examination (Nikon model MS phase-inverted microscope; Kogaku K.K., Tokyo 100, Japan) showed the cells as compact and refractile with a central area of granulation occupying 90% or more of the visible cell surface. A thin transparent zone surrounded the central area and a thinner dark margin demarcated the cell periphery. Doublet cells verified cytokinesis. These M14 cells then were designated as M14-CDM. An aliquot of M14-CDM suspension was removed from the 6-liter volume of cells, and viable cells were counted by the trypan blue exclusion procedure. The cell density at this time was 6 × 10⁴ cells/ml.

Antigen Preparation. A modification of the 3 M KCl procedure for the preparation of soluble HL-A antigens was used

(33) for the extraction of tumor antigen(s) from M14-CDM cells. A population of 2 × 10⁸ viable cells in 3.3 liters of M14-CDM cell suspension was harvested by centrifugation for 60 min. The pellet of cells containing viable and dead cells was resuspended in 3 M KCl-0.15 M phosphate-buffered solution, pH 7.4. Ten ml of 3 M KCl were added to each 10⁷ viable cells and the mixture was agitated for 16 to 24 hr at 4°. The mixture then was spun at 40,000 × g for 60 min at 4° in a Beckman Model L5-50 ultracentrifuge, and the supernatant was dialyzed extensively against Dulbecco's phosphate-buffered saline [mg/liter: $CaCl_2$ (anhydrous), 100; KCl, 200; KH_2PO_4, 200; $MgCl_2 \cdot 6H_2O$, 100; NaCl, 8000; $Na_2HPO_4 \cdot 2H_2O$, 1150; Microbiological Associates]. Protein concentration was determined by the biuret method. The yield of protein from 2 × 10⁸ viable M14-CDM cells was 2.0 mg. The extract was concentrated with an Amicon ultrafiltration unit and UM 10 membrane, and the concentrated extract was sterilized by filtering through a 0.22-µm filter (Millipore Filter Corporation, Bedford, Mass.). Final protein concentration of the extract was adjusted by the addition of Dulbecco's phosphate-buffered saline. Extracts from both a biopsied melanoma specimen and a skeletal muscle specimen from the same patient from whom M14 was derived, i.e., autologous to M14, were prepared by the 3 M KCl extraction procedure. The specimens were trimmed, minced, and forced through a 60 mesh stainless steel sieve. Viable cells were counted by the trypan blue exclusion method and the cells were processed as described above for the M14-CDM cells.

LMI Assay. The LMI in agarose gel assay (4) was modified to test the M14-CDM extracts and M14 autologous tumor and muscle extracts for their antigenic activity. The procedure was described in a recent paper (2). A migration index of <0.71 was considered positive. A protein concentration of 3.5 mg/ml of the M14-CDM extract was established as optimum and was used routinely in these studies. Extracts from the autologous tumor and muscle were tested at 8.0 and 10.0 mg protein per ml, respectively. Preliminary data indicated that at higher concentrations these 2 extracts gave nonspecific reactions, whereas at lower protein concentrations both were consistently negative.

Skin Test. DCHR was used to assay the in vivo activity and specificity of the M14-CDM and autologous tumor extracts. M14-CDM extract (100 to 300 µg), or 500 µg of the tumor extract were injected intradermally into the volar aspect of the left arm of melanoma patients and of patients with other neoplasms. After 24 hr, the diameter of the reaction site was measured. A 10-mm or greater diameter induration was considered positive (17).

Statistical Method. All data were statistically analyzed by Student's t test.

RESULTS

Relative Activity of 3 M KCl Extract from M14-CDM Cells Compared to 3 M KCl Extract from an Autologous Melanoma Specimen. Seven of 12 (58%) melanoma patients' leukocytes were inhibited in the presence of the extract from M14-CDM cells. By contrast, only 2 of 12 (17%) of these

same patients' leukocytes were LMI positive with the preparation made from an autologous biopsy tumor specimen, although the protein content was at least 2 times greater than that in the extract from M14-CDM cells.

The difference in reaction between these 2 preparations was statistically significant, $p < 0.0005$ (Table 1). These results suggest that the 3 M KCl extraction technique solubilized antigen(s) from M14-CDM cells and that the antigen(s) obtained from M14-CDM cells was at least twice as active as the antigen(s) present in the autologous melanoma tissue extract.

Tumor-associated Activity of M14-CDM Extract. The leukocytes of 15 of 27 (56%) melanoma patients were inhibited by the M14-CDM extract compared to 1 of 14 (7%) assayed against autologous muscle extract. These differences in activity were statistically significant at $p < 0.005$ (Table 2). These data suggest that M14-CDM cells synthesized tumor-associated antigens that were removed by 3 M KCl.

Melanoma-associated Activity of M14-CDM Extract. Table 3 shows that the M14-CDM extract was melanoma associated. The leukocytes of 56% of melanoma patients were inhibited whereas only 4 of 18 (22%) leukocytes from the

patients with other neoplasms were inhibited. Furthermore, the leukocytes of only 5 of 30 (17%) normal donors were LMI positive. The difference in inhibition between melanoma patients and nonmelanoma cancer patients was statistically significant at $p < 0.0025$. Likewise, the reaction of the leukocytes from melanoma patients was significantly different than that of normal donors ($p < 0.005$).

Melanoma-associated Activity of M14-CDM Extract by DCHR. Six of 8 (75%) melanoma patients displayed DCHR to the KCl extracts from M14-CDM cells, whereas 0 of 5 lung cancer patients reacted positively to the extract (Table 4). The difference between these 2 populations was statistically significant ($p < 0.005$).

The LMI data in Tables 1 to 3 suggest that M14-CDM was a more potent source of MAA than the fresh surgical melanoma specimen autologous to M14-CDM cells. However, it could be argued that the 3 M KCl extraction procedure failed to release MAA from the tumor specimen, resulting in borderline activity of the preparation in the LMI assay. This possibility was excluded by skin testing melanoma patients and patients with other cancers with the preparation from the fresh tumor specimen. Twenty-four of 42 (57%) mela-

Table 1

Activities of 3 M KCl extracts from M14-CDM cells and autologous tumor by the LMI test with leukocytes from melanoma patients

Extract from	mg protein/ml	No. of patients tested[a]	No. positive	% positive	Migration index[b]
M14-CDM	3.5	12	7	58	0.63 ± 0.004[c]
Autologous Tumor	8.0	12	2	17	0.88 ± 0.05

[a] Extract of M14-CDM cells compared with autologous tumor extract, $p < 0.0005$.
[b] A migration index of <0.71 is considered positive for all leukocyte samples tested in these studies.
[c] Mean ± S.E.

Table 2

Tumor-associated activity of the M14-CDM extract by the LMI test with leukocytes from melanoma patients

Extract from	mg protein/ml	No. of patients tested[a]	No. positive	% positive	Migration index[b]
M14-CDM	3.5	27	15	56	0.71 ± 0.04[c]
Autologous Muscle	10.0	14	1	7	0.93 ± 0.04

[a] M14-CDM extract compared with autologous muscle extract, $p < 0.0005$.
[b] A migration index of <0.71 is considered positive for all leukocyte samples tested in these studies.
[c] Mean ± S.E.

Table 3

Melanoma-associated activity of the M14-CDM extract by the LMI test

Leukocytes from	No. of patients tested[a]	No. positive	% positive	Migration index[b]
Melanoma patients	27	15	56	0.71 ± 0.04[c,d]
Nonmelanoma cancer patients	18	4	22	0.86 ± 0.06[c]
Normal donors	30	5	17	0.84 ± 0.03[c]

[a] Melanoma patients compared with nonmelanoma cancer patients, $p < 0.0025$; melanoma patients compared with normal donors, $p < 0.005$.
[b] A migration index of <0.71 is considered positive for all leukocytes tested in these studies.
[c] Mean ± S.E.
[d] Same data as in Table 2.

Table 4
DCHR of melanoma and lung cancer patients to the M14-CDM extract[a]

Melanoma patients		Lung cancer patients	
Patient	Induration (mm)[b]	Patient	Induration (mm)[b]
B. B.	25.0 (+)	J. B.	0.0 (−)
D. B.	30.0 (+)	W. D.	5.0 (−)
G. C.	10.5 (+)	F. F.	0.0 (−)
J. F.	10.0 (+)	F. M.	4.0 (−)
J. G.	10.0 (+)	M. S.	0.0 (−)
R. H.	10.0 (+)		
R. L.	6.5 (−)		
J. I.	4.5 (−)		
Positive/total 6/8 (75%)		0/5 (0%)	

[a] Melanoma patients compared with lung cancer patients, $p = 0.01$.
[b] Mean of 2 perpendicular diameters. An induration of >10.0 mm is considered positive in these studies.

noma patients reacted positively to the extract, whereas only 7 of 28 (25%) patients with other neoplasms demonstrated DCHR to the extract. The difference between these 2 groups of patients was statistically significant ($p < 0.0005$).

DISCUSSION

The present series of experiments was undertaken to test the hypothesis that M14 cells adapted to grow in serum-deficient medium synthesize MAA.

The approach used in these studies was to propagate a human malignant melanoma cell strain M14 in CDM, to prepare an extract from the CDM-grown M14-CDM cells, and to test the extract by an *in vitro* (LMI) and an *in vivo* (skin test) assay for MAA. The 3 M KCl extraction procedure was used to elute MAA from M14-CDM cells because of its simplicity and reproducibility in removing tumor antigens from animal and human tumors (3, 25–27).

The 3 M KCl extract from the CDM-grown melanoma cells was more active in the LMI test than the 3 M KCl extract obtained from a fresh autologous tumor specimen (Table 1). Although the extract from the M14-CDM cells contained less than one-half the protein concentration of the preparation made from the tumor specimen, 58% of the melanoma patients' leukocytes tested were migratory inhibited compared to 7% of the same patients' leukocytes exposed to the tumor extract.

There are several explanations for these findings: (a) immunoselection of weakly antigenic tumor cells (34) may have occurred *in vivo*, resulting in a preparation of low activity; (b) the tumor underwent antigenic modulation (31) before the surgical sample was taken; (c) blocking factors (12) reacted with the tumor antigen(s) and remained complexed to the tumor antigen(s) during the 3 M KCl extraction procedure and subsequent dialysis of the extract, rendering the preparation inactive at the concentrations used in this investigation; (d) inhibitors of LMI factors were present in the tumor preparations; (e) the tumor antigen(s) was degraded by enzymes present in the tumor mass, e.g., autoly-

sis; (f) the tumor preparation was contaminated with extraneous proteins that diluted the tumor antigen(s); (g) the CDM *in vitro* environment induced or favored the synthesis of tumor antigen(s); and (h) the CDM environment selected highly antigenic M14 variants. None of these possibilities were or could be excluded in these studies.

The data (Table 2) suggest that the antigen(s) extracted from the CDM-grown cells was tumor associated. The leukocytes of 56% melanoma patients were inhibited by the M14-CDM extract, whereas only 7% of leukocytes from melanoma patients were inhibited by autologous muscle extract prepared by the 3 M KCl procedure. Autologous muscle extract used as control excluded the possibility of histocompatability differences as an explanation for these results.

Autologous muscle might not have been the proper control. Tissue culture of autologous melanocytes presents an elusive technical problem and autologous fibroblasts failed to grow in CDM after repeated attempts (D. O. Chee, unpublished observations). Human neoplastic cells of different histological types would have provided other appropriate controls. However, human tumor cells are difficult to propagate in CDM and none were available when these studies were conducted. Organ- or tissue-specific antigens were ruled out since they are rapidly lost in tissue culture (7, 38), and the M14 cells were cultured *in vitro* for at least 6 months before these studies were conducted.

Cells grown *in vitro* for an extended period of time might have acquired neoantigens by derepression of silent genes coding for them. These putative antigens were not responsible for the reactivity observed with the 3 M KCl extract obtained from the M14-CDM cells (Table 2), since none of the patients used in this investigation were vaccinated with tissue culture cells.

Antigens shared by the M14-CDM cells with fungi, bacteria, and *Mycoplasma* are not a plausible explanation of these results (Table 2). The M14-CDM culture was inspected frequently for fungi and bacterial contamination and tested periodically for these microorganisms and for mycoplasma in appropriate microbiologic media. In every instance, the cultures were negative. Moreover, the patterns of LMI reactivity between melanoma patients and control groups were inconsistent for contamination with microbiologic agents. On a random basis, the leukocytes from both experimental and control groups should have reacted similarly in the LMI test. Therefore, the data in Table 2 suggest that the M14 cells grown in CDM synthesized a relatively high amount of tumor-associated antigen(s) which was extracted by the 3 M KCl procedure.

Further testing revealed that the M14-CDM preparation contained melanoma-associated antigen(s). Compared to 56% of melanoma patients' leukocytes that elaborated leukocyte migratory inhibition factors, only 22% of the leukocytes from patients with other neoplasms released LMI factors when incubated with the M14-CDM extract. Additionally, only 17% of normal donors were LMI positive with the extract from M14-CDM cells (Table 3). These results indicate that the antigen(s) released from the M14-CDM cells by treatment with 3 M KCl was melanoma associated.

Evidence to support this concept was obtained by DCHR

Table 5
DCHR of melanoma and nonmelanoma cancer patients to the extract from a surgical melanoma specimen autologous to M14-CDM cells

Population tested	No. of patients tested[a]	No. positive	% positive	Induration (mm)[b]
Melanoma patients	42	24	57	10.9 ± 1.3[c]
Nonmelanoma cancer patients	28[d]	7	25	5.6 ± 1.1

[a] Melanoma patients compared with nonmelanoma cancer patients, $p < 0.005$.
[b] Mean of 2 perpendicular diameters in all patients tested. An induration of >10.0 mm is considered positive in these studies.
[c] Mean ± S.E.
[d] Lung carcinoma, 8; head and neck tumor, 5; sarcoma, 5; breast carcinoma, 4; colon carcinoma, 4; renal cell carcinoma, 2.

to the M14-CDM extract in melanoma patients. In a small number of melanoma patients' skin tested with the preparation, 75% demonstrated DCHR, but none of 5 lung cancer patients was DCHR positive (Table 4).

The greater skin test activity to the extract from M14-CDM cells than to the autologous tumor extract was an unexpected finding. A concentration of 100 to 300 μg protein from the M14-CDM extract evoked DCHR in 75% of the melanoma patients tested (Table 4). In contrast, a protein concentration of 500 μg from the tumor specimen was required to elicit DCHR in 57% of the melanoma patients (Table 5). These data support the results obtained with the LMI assay (Table 1) and refute the suggestion that 3 M KCl was ineffective for removing tumor antigen(s) from the melanoma specimen. The data also imply that the skin test response to MAA was more sensitive than the LMI test. This is understandable, since the LMI test measures only one or a few mediators (lymphokines), whereas DCHR involves a complex series of interrelated reactions resulting in the production of several mediators and culminating in erythema, edema, and induration.

However, our results do not necessarily imply that the MAA is tumor specific. Preparations from fetal lung and fetal colon have been shown to elicit DCHR (16, 39) and these embryonic antigens have been found to be components of neoplastic cells (1, 37). Thus it is possible that the MAA from the M14-CDM cells are embryonic antigen(s). Alternatively, the MAA from the M14-CDM cells might be specific and unique for malignant melanoma. Studies are underway to determine whether the M14-CDM cells produce one or both of these melanoma-associated antigen(s).

The M14-CDM cells may serve as a model for the production of MAA that could be used: (a) to develop a battery of standard cellular and humoral *in vitro* assays, (b) to develop a sensitive radioimmunoassay for the early detection of malignant melanoma and to monitor tumor burden, (c) as a standard skin test antigen; (d) as a vaccine for immunotherapy and immunoprophylaxis of malignant melanoma, and (e) to characterize the biochemical nature of MAA.

REFERENCES

1. Alexander, P. Foetal Antigens in Cancer. Nature, 235: 178-181, 1972.
2. Boddie, A. W., Jr., Holmes, E. C., Roth, J. A., and Morton, D. L. Inhibition of Human Leukocyte Migration in Agarose by KCl Extracts of Carcinoma of the Lung. Intern. J. Cancer, 15: 823-829, 1974.
3. Brannon, G. E., Adams, J. S., and Santos, G. W. Tumor-specific Immunity in 3-Methyl Cholanthrene-Induced Murine Fibrosarcomas. I. *In Vivo* Demonstration of Immunity with Three Preparations of Soluble Antigens. J. Natl. Cancer Inst., 53: 165-175, 1974.
4. Clausen, J. E. Tuberculin-Induced Migration Inhibition of Human Peripheral Blood Leukocytes in Agarose Medium. Acta Allergol. 26: 56-80, 1971.
5. Cochran, A. J., Mackie, R. M., Thomas, C. E., Grant, R. M., Cameron-Mowat, D. E., and Spilg, W. G. S. Immunity to Breast Cancer and Malignant Melanoma. Brit. J. Cancer, 28 (Suppl. 1): 77-81, 1973.
6. Cochran, A. J., Spilg, W. G., Mackie, R. M., and Thomas, C. E. Postoperative Depression of Tumor Directed Cell-Mediated Immunity in Patients with Malignant Disease. Brit. Med. J., 2: 67-70, 1972.
7. Dumonde, D. C. Tissue-Specific Antigens. Advan. Immunol., 5: 245-412, 1966.
8. Fass, L., Herberman, R. B., Ziegler, J. E., and Kiryabwire, J. W. Cutaneous Hypersensitivity Reactions to Autologous Extracts of Malignant Melanoma Cells. Lancet, 1: 116-118, 1970.
9. Foley, E. J. Antigenic Properties of Methylcholanthrene-induced Tumors in Mice of the Strain of Origin. Cancer Res., 13: 835-837, 1953.
10. Gross, L. Intradermal Immunization of C3H Mice against a Sarcoma that Originated in an Animal of the Same Line. Cancer Res., 3: 326-333, 1943.
11. Hayflick, L. The Limited *In Vitro* Lifetime of Human Diploid Cell Strains. Exptl. Cell Res., 37: 614-636, 1965.
12. Hellström, I., and Hellström, K. E. The Role of Immunological Enhancement for the Growth of Autochthonous Tumors. Transplant. Proc., 3: 721-724, 1971.
13. Hellström, I., Hellström, K. E., Sjögren, H. O., and Warner, G. E. Demonstration of Cell-mediated Immunity to Human Neoplasms of Various Histological Types. Intern. J. Cancer, 7: 1-16, 1971.
14. Herberman, R. B., and Oren, M. Delayed Cutaneous Reactions to Membrane Extracts of Human Tumor Cells. Clin. Res., 17: 403-412, 1969.
15. Hollinshead, A., Ariel, C., Stewart, T. H. M., and Herberman, R. B. Hypersensitivity Reactions to Soluble Membrane Antigens of Human Malignant Lung Cells. J. Natl. Cancer Inst., 52: 327-338, 1974.
16. Hollinshead, A., Glew, D., Bunnag, B., Gold, P., and Herberman, R. Soluble Antigen from Intestinal Cancer-Cell Membranes and Relationship to Carcinoembryonic Antigens. Lancet, 1: 1191-1195, 1970.
17. Holmes, E. C., Roth, J. A., and Morton, D. L. Delayed Cutaneous Hypersensitivity Reactions to Melanoma Antigen. Surgery, 78: 160-164, 1975.
18. Hughes, L. E., and Lytton, B. Antigenic Properties of Human Tumors: Delayed Cutaneous Hypersensitivity Reactions. Brit. Med. J., 1: 209-212, 1964.
19. Irie, R., Irie, K., and Morton, D. L. Natural Antibody in Human Serum to a Neoantigen in Human Cultured Cells Grown in Fetal Bovine Serum. J. Natl. Cancer Inst., 52: 1051-1058, 1974.
20. Irie, R., Irie, K., and Morton, D. L. Characteristics of Heterologous Membrane Antigen on Cultured Human Cells. J. Natl. Cancer Inst., 53: 1545-1551, 1974.
21. Katsuta, H., Takaoka, T., Hattori, K., Kawada, I., Kuwabara, H., and Kuwabara, S. The Growth Promoting Substances for Yoshida Sarcoma Cells in Tissue Culture. Japan. J. Exptl. Med., 29: 297-309, 1959.
22. Klein, G., Sjögren, H. O., and Klein, E. Demonstration of Host Resistance against Isotransplantation of Lymphoma Induced by the Gross Agent. Cancer Res., 22: 955-961, 1962.
23. Klein, G., Sjögren, H. O., Klein, E., and Hellström, K. E. Demonstration of Resistance against Methylcholanthrene-induced Sarcomas in the Primary Autochthonous Host. Cancer Res., 20: 1561-1572, 1960.
24. Lewis, M. G., McCloy, E., Blake, J. The Significance of Humoral Antibodies in the Localization of Human Malignant Melanoma. Brit. J. Surg., 60: 443-446, 1973.
25. McCoy, J. L., Jerome, L. F., Dean, J. H., Cannon, G. B., Alford, T. C., Doering, T., and Herberman, R. B. Leukocyte Migration by Tumor-Asso-

D. O. Chee et al.

ciated Antigens in Soluble Extracts of Human Breast Carcinoma. J. Natl. Cancer Inst., 53: 11–17, 1974.

26. McCoy, J. L., Jerome, L. F., Dean, J. H., Perlin, E., Oldham, R. K., Char, D. H., Cohen, M. H., Felix, E. L., and Herberman, R. B. Leukocyte Migration by Tumor-Associated Antigens in Soluble Extracts of Human Malignant Melanoma. J. Natl. Cancer Inst., 55: 19–23, 1975.

27. Meltzer, M. D., Leonard, E. J., Rapp, H. J., and Borsos, T. Tumor-Specific Antigen Solubilized by Hypertonic Potassium Chloride. J. Natl. Cancer Inst., 47: 703–709, 1971.

28. Morton, D. L., Holmes, E. C., Eilber, F. R., and Wood, W. C. Immunological Aspects of Neoplasia. A Rational Basis for Immunotherapy. Ann. Internal Med., 74: 587–604, 1971.

29. Morton, D. L., Malmgren, R. A., Holmes, E. C., and Ketcham, A. S. Demonstration of Antibodies against Human Malignant Melanoma by Immunofluorescence. Surgery, 64: 233–240, 1968.

30. Old, L. J., Boyse, E., Clarke, D. A., and Carswell, E. A. Antigenic Properties of Chemically Induced Tumors. Ann. N. Y. Acad. Sci., 101: 80–106, 1962.

31. Old, L. J., Stockert, E., Boyse, E. A., and Kim, J. H. Antigenic Modulation: Loss of TL Antigen from Cells Exposed to TL Antibody. Study of the Phenomenon in Vitro. J. Exptl. Med., 127: 523–539, 1968.

32. Prehn, R. T., and Main, J. M. Immunity to Methylcholanthrene-Induced Sarcomas. J. Natl. Cancer Inst., 18: 759–778, 1957.

33. Reisfeld, R. A., Pellegrino, M. A., and Kahan, B. D. Salt Extraction of Soluble HL-A Antigens. Science, 172: 1134–1136, 1972.

34. Smith, R. T. Tumor-Specific Mechanisms. New Engl. J. Med., 278: 1207–1331, 1968.

35. Southam, C. M. Evidence for Cancer-Specific Antigens in Man. Progr. Exptl. Tumor Res., 9: 1–39, 1967.

36. Stewart, T. H. The Presence of Delayed Cutaneous Hypersensitivity Reactions in Patients toward Cellular Extracts of Their Malignant Tumors. Cancer, 23: 1368–1387, 1969.

37. Ting, C. C., Lavrin, D. H., Shiu, G., and Herberman, R. B. Expression of Fetal Antigens in Tumor Cells. Proc. Natl. Acad. Sci. U. S., 69: 1664–1668, 1972.

38. Weiler, E. A Cellular Change in Hamster Kidney Cultures: Loss of Tissue-Specific Antigens. Exptl. Cell Res. Suppl., 7: 244–262, 1959.

39. Wells, S. A., Burdick, J. F., Christiansen, C., Ketcham, A. S., and Adkins, P. C. Demonstrtion of Tumor-Associated Delayed Cutaneous Hypersensitivity Reactions in Patients with Lung Cancer and in Patients with Carcinoma of the Cervix. Natl. Cancer Inst. Monograph, 37: 197–203, 1973.

40. Wolberg, W. H. Inhibition of Migration of Human Autogenous and Allogeneic Leukocytes by Extracts of Patients' Cancers. Cancer Res., 31: 798–802, 1971.
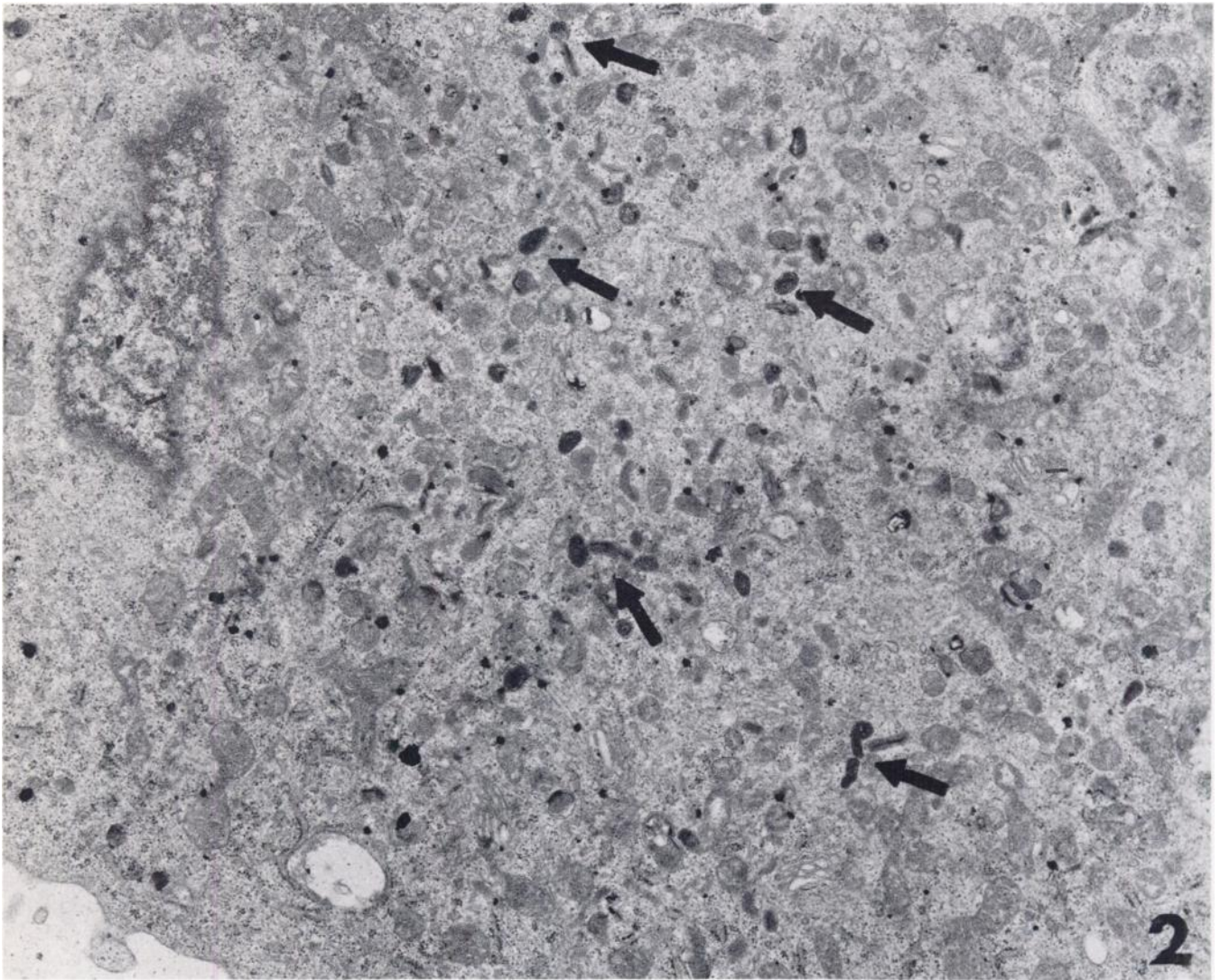
Fig. 1. Phase-contrast micrograph of UCLA-SOM-14 cells. × 100.

Fig. 2. Electron micrograph of UCLA-SOM-14 cells. *Arrows*, melanosomes.