CELL SURFACE ANTIGENS OF CHEMICALLY INDUCED SARCOMAS OF THE MOUSE
I. Murine Leukemia Virus-Related Antigens and Alloantigens on Cultured Fibroblasts and Sarcoma Cells: Description of a Unique Antigen on BALB/c Meth A Sarcoma*

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The first evidence for tumor-specific antigens came from transplantation studies of sarcomas induced by methylcholanthrene in inbred mice (1–5). Growth of a sarcoma transplant in syngeneic recipients, followed by its removal, increased resistance to subsequent challenge with the same sarcoma. The surprising feature that emerged from the study of these transplantation antigens is that they are distinct for each tumor, no two tumors having been convincingly shown to share the same antigen. Although repeated immunization with tumor cells can lead to substantial resistance to tumor challenge, antibody to the unique antigens of chemically induced sarcomas has been difficult to demonstrate in mice and, where positive results have been obtained, the analysis was not sufficiently detailed to indicate the class of surface antigen being detected. The fact that murine leukemia virus (MuLV)* and its antigens are often found in chemically induced sarcomas (6–8) and that normal mouse serum frequently contains naturally occurring antibody to MuLV antigens (9–11) adds a serious complication to the serological study of these tumors and makes it virtually impossible to interpret past studies where this was not taken into consideration. Apparently, MuLV-related antigens on chemically induced tumors are not effective in eliciting transplantation resistance or cross reactions between different sarcomas would have been a far more common finding.

To initiate a serological analysis of the unique surface antigens of chemically induced sarcomas, we have established a number of fibroblast and sarcoma cell lines in tissue culture and have defined the surface phenotype of these cells with regard to known MuLV and alloantigenic markers. With this background, it has been possible to characterize a unique cell surface antigen on Meth A, the widely studied BALB/c methylcholanthrene-induced sarcoma.

Materials and Methods

Animals. Female mice, 2–5 mo old, were obtained from the following sources: A/Thy-1.1 and 129 strain mice were from our breeding colony, BALB/c, C57BL/6, and other inbred strains from

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Abbreviations used in this paper: FBS, fetal bovine serum; GSCA, Gross cell surface antigen; MS, methylcholanthrene sarcoma; MuLV, murine leukemia virus; RDDP, RNA-dependent DNA polymerase; TNF, tumor necrosis factor.
The Jackson Laboratory, Bar Harbor, Maine, and random bred Swiss ICR from Camm Research Institute, Inc., Wayne, N. J.

Tumors. Sarcomas were induced in 2-mo-old female mice by a single subcutaneous injection of 0.1 mg 3-methylcholanthrene (Eastman Kodak Co., Rochester, N. Y.) dissolved in 0.1 ml sesame oil. They were maintained by serial transplantation in the strain of origin or stored in liquid nitrogen. With the exception of the Meth A and Meth 4 sarcomas, the newly derived sarcomas are distinguished by an initial letter or letters indicating their strain of origin (C = BALB/c, B6 = C57BL/6, CB6 = [BALB/c x C57BL/6]F1) and by the abbreviation MS (methylcholanthrene sarcoma).

Meth A was induced in a BALB/c female mouse in 1960 (5). An ascites variant of the solid tumor was obtained in 1961 and has been in continuous in vivo transplantation since that time. The original solid Meth A sarcoma has been stored at intervals in liquid nitrogen and was used in the present studies during in vivo passages 110-140.

Meth 4 was induced in a C57BL/6 female mouse in 1972 (12), and studied during in vivo passages 1-10. CMS1,2,4,5,7,14, and 21 were induced in BALB/c mice in 1975, and studied during in vivo passages 1-20. CB6MS1,2,4, and 5 were induced in C57BL/6 mice in 1975, and studied during in vivo passages 1-10. CB6MS1 and 2 were induced in [BALB/c x C57BL/6]F1 mice in 1975, and studied during in vivo passage 1.

The F9 and PYS culture lines were derived from a teratocarcinoma of 129/Sv origin and were provided by Dr. K. Artzt. The other tumor lines used in this study were serially passaged in mice and have been described in previous publications (13-15).

Culture Medium. Eagle's complete minimum essential medium was supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 100 U penicillin/ml, 100 μg streptomycin/ml, and 10% fetal bovine serum (FBS).

Cell Cultures. Cell cultures were derived from whole fetuses (14 days), lung fibroblasts of 12- to 16-wk-old mice or transplanted sarcomas as described by Shiku et al. (12). Meth A(a) and Meth A(s) refer to culture lines established from Meth A ascites and Meth A solid sarcoma.

The surface phenotype of the cultured cells was determined after the cells achieved a stable growth pattern in vitro and most tests were conducted on lines between the 10th and 30th passage generation.

Antisera to MuLV-Related Antigens and Known Alloantigens. Details of the serological typing systems used for determining the cell surface antigens of fibroblast and sarcoma cells are given in Table I. The following antisera were used in microcytotoxicity tests: goat anti-MuLV (Rauscher) p30, goat anti-MuLV (Rauscher) gp70, and (W/Fu × BN)F1 anti-W/Fu leukemia (C58NT)D = anti-NTD. (The goat anti sera were obtained from Dr. R. Wilsnack, Huntington Research Laboratories, Brookville, Md.) The specificity of these MuLV antisera has been verified by extensive studies in our laboratory.

Antisera to Meth A Antigen. Two methods of antisera production resulted in antibody to the unique antigen of Meth A sarcoma. The first method involved intraperitoneal injections of 5 × 10⁶ irradiated Meth A ascites cells once weekly for 5 wk in BALB/c or [BALB/c x C57BL/6]F1 recipients followed by five weekly intradermal challenges of increasing numbers of nonirradiated Meth A ascites cells (2 × 10⁶-1 × 10⁷ cells). The second method took advantage of the sensitivity of Meth A sarcoma to the tumor necrosis factor (TNF) present in the serum of Bacillus Calmette-Guérin-infected mice injected with endotoxin (for details of TNF production see reference 25). (BALB/c x C57BL/6)F1 mice received 3 × 10⁴ Meth A ascites cells injected intradermally. When tumors reached approximately 7-8 mm in average diameter, the mice received 0.5 ml TNF serum injected i.v. Approximately 25% of TNF-treated mice showed complete regression of their tumors and these animals were subsequently injected intraperitoneally at 2-wk intervals with viable Meth A ascites cells starting with 5 × 10⁶ and increasing to 5-10 × 10⁶. Mice that remained tumor-free through the immunization period were bled individually and their serum titered on Meth A ascites cells and Meth A(a) cultured cells.

Cytotoxic Tests. (a) For cells in suspension, the conventional cytotoxic test was used (13, 17, 26). (b) For monolayer cultured cells, a modification of the microcytotoxicity test described by Bloom was used (27). Monolayer cells were detached with 0.05% Trypsin (Armour Pharmaceutical Co., Chicago, Ill.) in Fack's saline A solution, washed in culture medium, and the concentration of viable cells adjusted to 2 × 10⁶ cells/ml in culture medium. A 10-μl cell suspension containing 200 target cells was distributed with either a microliter syringe and dispenser (Hamilton Company
### Table I

**Description of Serological Typing Systems for Detecting Cell Surface Antigens of Fibroblast and Sarcoma Cell Lines by Absorption Tests**

| Antigenic system | Antiserum (dilution)* | Standard test cell | Complement source (serum dilution) | Reference |
|------------------|-----------------------|--------------------|------------------------------------|-----------|
| **MuLV-Related antigens** | | | | |
| Gx | Rat (W/Fu × BN)F₁, anti-W/Fu leukemia (C58NT)D (anti-NTD) (1:150) | 129 thymocytes | Rabbit (1:12) | 16, 17 |
| GCSA | C57BL/6 anti-transplanted spontaneous AKR leukemia K36 (1:10) | EJ G2 leukemia cells | Guinea pig (1:2) | 13 |
| **Alloantigens** | | | | |
| H-2.2 (H-2D*) | (BALB/c × HT1)F₁, anti-BALB/c lymph node cells | C57BL/6 lymph node cells | Guinea pig (1:4) | 18 |
| H-2.33 (H-2K*) | (B10.A × HTG)F₁, anti-BALB/c lymph node cells | C57BL/6 lymph node cells | Guinea pig (1:4) | 18 |
| H-2.4 (H-2D*) | BALB.G anti-BALB/c lymph node cells | BALB/c lymph node cells | Guinea pig (1:4) | 18 |
| H-2.31 (H-2K*) | (A × BALB.B)F₁, anti-BALB/c lymph node cells | BALB/c lymph node cells | Guinea pig (1:4) | 18 |
| In.7 | A.TH anti-A.TL (1:20)§ | BALB/c lymph node cells | Guinea pig (1:4) | 19, 20 |
| Lyt-1.2 | C3H anti-CE thymocytes (1:50) | C57BL/6 thymocytes | Rabbit (1:15) | 21, 22 |
| Lyt-2.2 | (C3H × B6-Ly-2.1)F₁, anti-ERLD (1:100) | C57BL/6 thymocytes | Rabbit (1:15) | 21, 22 |
| PC.1 | (DBA/2 × C57BL/6)F₁, anti-MOPC-70A (1:8) | MOPC-70A | Guinea pig (1:2.5) | 15 |
| Thy-1.2 | (A-Thy-1.1 × AKR-H-2)F₁, anti-ASL1 (1:160) | A thymocytes | Rabbit (1:15) | 22, 23 |
| TL.2 | (A-γ-TL × C57BL/6)F₁, anti-ASL1 (1:400) | BALB/c thymocytes | Rabbit (1:15) | 24 |

* Dilution used for absorption tests.
§ Obtained from Dr. F. Lilly, Albert Einstein College of Medicine, New York.
§ Obtained from Dr. D. C. Schreffler, Washington University Medical School, St. Louis, Mo.

Inc., Whittier, Calif.) or a 1-ml disposable glass syringe (no. 704S, Beckton-Dickinson & Co., Rutherford, N. J.) into the wells of Falcon Microtest Plates (no. 3034, Falcon Plastics Division of BioQuest, Oxnard, Calif.). The plates were then incubated at 37°C in 5% CO₂ in humidified air for 18 h. Medium was removed by blotting with a gauze sponge, and 10 µl of the appropriate dilution of antiserum and 10 µl selected rabbit serum (1/20-1/30) as complement source (each diluted in cultured medium with 10% heat inactivated FBS) were added. After further incubation for 18 h, the plates were again blotted with gauze sponge, fixed with absolute methanol, and stained with 2% Giemsa stain (Fisher Scientific Co., Pittsburgh, Pa.) in 0.08 M sodium phosphate buffer (pH 7.2).

For analysis of the Meth A antigen, sensitization with antiserum before addition of complement augmented cytotoxicity and resulted in higher antiserum titers. Thus in all tests recorded here, cultured Meth A(a) cells were preincubated with antiserum for 30 min at 37°C in 5% CO₂ in humidified air. Antiserum was then removed with a gauze sponge and complement was added followed by an 18-h incubation period, as above.
Fig. 1. Microcytotoxicity tests for MuLV-related antigens on fibroblast and sarcoma cell lines of BALB/c and C57BL/6 (B6) origin.

Tests were performed in duplicate along with appropriate media, antiserum, and complement controls. Plates were analyzed microscopically and the percentage cytotoxicity of a given antiserum dilution was calculated as follows:

\[
\text{Percent cytotoxicity} = \left( 1 - \frac{\text{number of cells in well treated with antiserum and complement}}{\text{number of cells in well treated with culture medium alone}} \right) \times 100.
\]

Antisera controls showed ≈ 5% cytotoxicity and complement controls were in the range of 5–15% cytotoxicity.

**Absorption Tests.** These were performed according to methods developed in our laboratory (13, 17, 26). The dilution of antiserum chosen for absorption tests was generally two doubling dilutions below its end point (50% cytotoxicity), as determined in preliminary tests. Equal volumes of diluted antiserum and packed washed cells were incubated together, with frequent shaking, for 30 min at 4°C. After removal of the absorbing cells by centrifugation, the residual cytotoxic activity of the serum was tested. Monolayer cells were harvested for absorption tests by treating cultures with 0.05% EDTA.

**XC Plaque Assay for Ecotropic MuLV.** Culture fluids were tested by the method of Rowe et al. (28).

**RNA-Dependent DNA Polymerase (RDDP) Assay.** Culture fluids were tested by the method of Stephenson et al. (29).

### Results

**MuLV Expression by Cultured Fibroblasts and Sarcomas.** The cultured cell lines were assayed for MuLV expression by (a) microcytotoxic tests with polyvalent anti-MuLV serum (anti-NTD) and with antiserum to MuLV structural components gp70 and p30 (Fig. 1), (b) absorption tests for two type-specific cell surface antigens specified by MuLV, GpX, and Gross cell surface antigen (GCSA) (Fig. 2). (c) XC assays for infectious MuLV ecotropic virus and (d) assays for
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GCSA

Positive Absorptions
- EGF2
- CMS 1
- CMS 3
- BALB/c adult lung fibroblasts (1)
- Meth A (s)

Negative Absorptions
- Meth A (a)
- AKR thymocytes
- CMS 1
- CMS 4
- CMS 5
- BALB/c adult lung fibroblasts (1)

GIX

Positive Absorptions
- Meth A (a)
- CMS 1
- CMS 3
- CMS 4
- CMS 5

Negative Absorptions
- Meth A (s)
- BALB/c adult lung fibroblasts (1)

Fig. 2. Typing of BALB/c fibroblast and sarcoma cell lines for GCSA and GIX cell surface antigens by absorption tests. EGF2, a GCSA+ MuLV (Gross)-induced leukemia of C57BL/6 origin, and AKR thymocytes, a GIX+ cell population, were included as positive control cells.

RDDP. Table II summarizes the results of these tests for MuLV and MuLV-related antigens.

Cell lines of BALB/c origin could be classified MuLV− or MuLV+ on the basis of these several assays, and the expression of MuLV antigens invariably correlated with the presence of infectious MuLV and RDDP activity. In contrast, cell lines of C57BL/6, A and (BALB/c × C57BL/6)F1 origin reacted with anti-gp70 even though they showed no other evidence of MuLV infection. Mice of these strains are known to express a gp70 molecule on the surface of their cells that is not dependent on productive MuLV infection (30). This selective expression of a portion of the viral genome is not seen with BALB/c cells, where either the full range of MuLV gene products were present or no viral markers could be detected.

In this study, 6 of the 11 lines of sarcoma cells typed MuLV+ by all assays. This does not appear to relate to length of time these tumors were passaged in vivo before tissue culture. For example, the line derived from the long-transplanted Meth A ascites (Meth A(a)) was MuLV−, whereas CMS1 and CMS3 of more recent derivation were MuLV+. No discernible difference in the growth or morphology of MuLV+ or MuLV− sarcoma cells was evident.

With regard to the stability of the MuLV phenotype of culture lines, 11 sarcoma cell lines were repeatedly tested over a 10-mo period with consistent
TABLE II
Expression of MuLV and MuLV-Related Antigens by Fibroblast and Sarcoma Cell Lines:
Summary of Results

| Strain       | Cell line         | Direct microcytotoxicity tests | Absorption tests | XC Assay for ecotropic MuLV | RDDP Assay |
|--------------|-------------------|-------------------------------|------------------|----------------------------|------------|
|              |                   | Anti-NTD | Anti- gp30 | Anti-gp70 | Gix | GCSA |                     |            |
| BALB/c       | Fetal fibroblasts | -        | -         | -         | -   | -   | -                   | -           |
|              | Adult lung fibroblasts (I) | -        | -         | -         | -   | -   | -                   | -           |
|              | Adult lung fibroblasts (II) | +       | +         | +         | +   | +   | +                   | +           |
|              | CMS1 sarcoma      | +        | +         | +         | +   | +   | +                   | +           |
|              | CMS3 sarcoma      | +        | +         | +         | +   | +   | +                   | +           |
|              | CMS4 sarcoma      | -        | -         | -         | -   | -   | -                   | -           |
|              | CMS5 sarcoma      | -        | -         | -         | -   | -   | -                   | -           |
|              | Meth A(a) sarcoma | -        | -         | -         | -   | -   | -                   | -           |
|              | Meth A(s) sarcoma | +        | +         | +         | +   | +   | +                   | +           |
| C57BL/6      | Fetal fibroblasts | -        | -         | +         | -   | -   | -                   | -           |
|              | Adult lung fibroblasts | -       | +         | -         | -   | -   | -                   | -           |
|              | Meth 4            | +        | +         | +         | +   | +   | +                   | +           |
|              | B6MS2 sarcoma     | +        | +         | +         | +   | +   | +                   | +           |
|              | B6MS5 sarcoma     | -        | +         | -         | -   | -   | -                   | -           |
| (BALB/c × C57BL/6)F₁ | CB6MS1 sarcoma | -        | +         | -         | -   | -   | -                   | -           |
|              | CB6MS2 sarcoma    | +        | +         | +         | +   | +   | +                   | +           |

A Adult lung fibroblasts - + - - - -

results. One BALB/c fibroblast line, however, showed a spontaneous MuLV⁻ to MuLV⁺ conversion after 6 mo in tissue culture. This was accompanied by changes associated with transformation (i.e., more rapid growth rate and greater saturation density of the cultured cells).

Cell Surface Alloantigens of Cultured Fibroblasts and Sarcomas. The fibroblast and sarcoma cell lines were typed for known cell surface alloantigens by absorption tests. Table III summarizes these results. (Because the typing antisera contain antibody to MuLV-related antigens, MuLV⁺ cell lines cannot be typed in direct cytotoxic tests. This does not interfere, however, with typing by absorption.)

H-2D and H-2K Alloantigens. All cell lines expressed the appropriate H-2D and H-2K private specificity.

Thy 1.2. Fig. 3 illustrates absorption tests for Thy-1.2. AKR thymocytes (phenotype Thy-1.1) and B6 thymocytes (phenotype Thy-1.2) were included as negative and positive control cells. On the basis of these tests, fibroblasts and sarcoma cells can be clearly typed Thy-1.2⁻ or Thy-1.2⁺.
### Table III

**Cell Surface Alloantigens of Cultured Fibroblast and Sarcoma Cell Lines as Determined by Absorption Analysis: Summary of Results**

| Strain     | Cell line          | H-2.4 (H-2D<sup>α</sup>) | H-2.31 (H-2K<sup>α</sup>) | H-2.2 (H-2D<sup>β</sup>) | H-2.33 (H-2K<sup>β</sup>) | Thy-1.2 | PC.1 | Lyt-1.2 | Lyt-2.2 | In.7 | TL.2 |
|------------|--------------------|--------------------------|----------------------------|--------------------------|--------------------------|---------|------|---------|---------|------|------|
| BALB/c     | Adult lung fibroblasts (I) | +                        | +                          | -                        | -                        | +       | +    | -       | -       | -    | -    |
|            | Adult lung fibroblasts (II) | +                        | +                          | -                        | -                        | +       | +    | -       | -       | -    | -    |
| CMS1 sarcoma |                     | +                        | +                          | -                        | -                        | -       | -    | -       | -       | -    | -    |
| CMS3 sarcoma |                     | +                        | -                          | -                        | +                        | -       | -    | -       | -       | -    | -    |
| CMS4 sarcoma |                     | +                        | +                          | -                        | -                        | -       | -    | -       | -       | -    | -    |
| CMS6 sarcoma |                     | +                        | +                          | -                        | -                        | -       | -    | -       | -       | -    | -    |
| Meth A(a)   |                     | +                        | +                          | -                        | +                        | -       | -    | -       | -       | -    | -    |
| Meth A(a)   |                     | +                        | +                          | -                        | -                        | +       | -    | -       | -       | -    | -    |
| C57BL/6     | Adult lung fibroblasts | -                        | +                          | +                        | +                        | -       | -    | -       | -       | -    | -    |
| B6MS2       |                     | -                        | -                          | +                        | +                        | -       | -    | -       | -       | -    | -    |
| Meth 4      |                     | -                        | -                          | +                        | -                        | -       | -    | -       | -       | -    | -    |

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**Thy1.2**

- Positive Absorptions
  - B6 thymocytes
  - BALB/c adult lung fibroblasts (I)
  - CMS 3
  - CMS 5
  - BALB/c adult lung fibroblasts (II)

- Negative Absorptions
  - AKR thymocytes
  - Meth A(a)
  - CMS 1

**PC.1**

- Positive Absorptions
  - B6 liver
  - Meth A(a)
  - CMS 1
  - CMS 3
  - CMS 4
  - CMS 5

- Negative Absorptions
  - BALB/c liver
  - Meth A(a)
  - CMS 1
  - CMS 3
  - CMS 4

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**Fig. 3.** Typing of BALB/c fibroblast and sarcoma cell lines for Thy-1.2 and PC.1 alloantigens by absorption tests. C57BL/6 (B6) thymocytes (Thy-1.2+) and AKR thymocytes (Thy-1.2-) were included as control cells for Thy-1.2 tests. BALB/c liver (PC.1+) and B6 liver (PC.1-) were included as control cells for PC.1 tests.

**PC.1.** Fig. 3 illustrates absorption tests for PC.1. B6 liver (PC.1-) and BALB/c liver (PC.1+) were included as negative and positive absorbing tissues. Two BALB/c lung fibroblast lines and Meth A(s), the cell line derived from the solid Meth A sarcoma, typed PC.1+. In contrast, Meth A(a), the line derived from the Meth A ascites tumor, was PC.1-.
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Figure 4. Microcytotoxicity tests of (BALB/c × C57BL/6) anti-Meth A serum on MuLV− fibroblast and sarcoma cell lines of BALB/c origin.

TL-2, LYT-1, LYT-2, Ia-7. These alloantigens were not demonstrable on any fibroblast or sarcoma cell line.

The Meth A Antigenic System. A number of syngeneic antisera have been prepared against individual BALB/c or C57BL/6 sarcomas. Regardless of the MuLV phenotype of the immunizing sarcoma, the resulting antisera invariably contained cytotoxic antibody to MuLV-related antigens and were reactive with MuLV+ fibroblast or sarcoma cell lines. Because of this, our search for sarcoma-specific antigens focuses on MuLV− sarcoma lines of BALB/c origin so that reactions due to MuLV antibody will not confound the serological analysis. The first antigen to be defined in this way is the unique antigen of Meth A sarcoma.

Direct Cytotoxic Tests with Meth A Antisera. Antisera prepared in BALB/c or (BALB/c × C57BL/6)F1 mice by repeated inoculations of Meth A ascites cells were cytotoxic for Meth A ascites cells as well as for the MuLV− tissue culture line derived from Meth A ascites sarcoma. The antisera had little or no cytotoxic activity for other MuLV− cultured cells of BALB/c origin (Fig. 4).

Absorption Analysis of Meth A Antisera. Fig. 5 illustrates absorption tests with Meth A antisera and Table IV summarizes the results of the extensive absorption analysis that defines the Meth A system. The Meth A antigen is restricted to the ascites and solid forms of Meth A sarcoma and the culture lines derived from them. It has not been found on any other normal or neoplastic cell type.

Tests for Meth A Antigen on Normal Lymphoid Cells. It has recently been suggested that the transplantation antigens of chemically induced tumors represent derepressed or altered products of the H-2 complex (31–33). The absorption tests summarized in Table IV give no support to the idea that the Meth A antigen is related to histocompatibility antigens normally expressed by other mouse strains, since the spleen cells of 14 different inbred strains lacked Meth A antigen. These strains represent six independent H-2 haplotypes and the majority of known private and public specificities defined by the H-2 complex.
exclude the possibility that the Meth A absorption system detects more than one
determinant and that normal cells express only one of them and therefore fail to
absorb Meth A reactivity, the Meth A antiserum was tested for direct cytotoxic-
ity on normal lymphoid cells from spleen and thymus. No cytotoxic reactions
were observed with cells from the following strains of mice: A, BALB/c, B10.A,
C3H/An, C57BL/6, RF, PL, SJL, Swiss, SWR, and 129.

Discussion

In contrast to the detailed knowledge we have of the surface antigens of
normal and neoplastic lymphoid cells of the mouse (34), far less is known about
the antigens that characterize the surface of fibroblast and sarcoma cells. The
greater sensitivity of lymphoid cells to cytotoxic antibody and the ease of
working with cells that remain in free suspension (rather than with cells like
fibroblasts that adhere to glass) clearly account for the past emphasis on
lymphoid populations. Serological analysis of lymphocytes, particularly those of
thymic derivation, has uncovered a series of surface antigens that are restricted
to cells that have undergone this pathway of differentiation (hence the designa-
tion differentiation antigens to distinguish antigens that characterize different
cell populations). Alloantigens belonging to the Lyt family, of which four
separate systems have now been defined and several others identified but not as
yet analyzed, are prime examples of differentiation antigens on T lymphocytes
(35). The presumption is that these surface molecules play a critical role in the
interactions involved in differentiated cellular function and that all specialized
cell types will be marked by a uniquely characteristic display of surface anti-
### Table IV

**Absorption Analysis of (BALB/c × C57BL/6)F₁ Anti-Meth A Serum: Summary of Results**

| Source of cells | In vivo | In vitro | Source of cells | In vivo | In vitro |
|-----------------|---------|----------|-----------------|---------|----------|
|                 | Normal  | Tumor    | Chemically-induced sarcomas | Normal  | Tumor    |
| Spleen          | A       | ASL1     | CMS1 BALB/c      | A       | CMS1 BALB/c |
|                 | AKR     | K36      | CMS3             | AKR     | CMS3 BALB/c |
|                 | BALB/c  | SL-2     | CMS4             | BALB/c  | CMS4 BALB/c |
| Meth A (solid)  | (BALB/c × C57BL/6)F₁ | C57BL/6 | CMS7             | (BALB/c × C57BL/6)F₁ | C57BL/6 |
|                 | C3H/He  | Radiation-induced leukemias | CMS14 | C3H/He |
| In vitro        | DBA/2   | RADA1 | CMS21 | MA/My |
|                 | LF      | RL-11   | CMS21 | Meth 4 Teratocarcinoma |
| Meth A(a)       | MA/My   | ERLD    | CMS21 | Fetal fibroblasts |
| Meth A(a)       | NZB/B1  | EARAD1 | CMS21 | Fetal fibroblasts |
|                 | PL      | (C57BL/6 × AFI) | CMS21 | Fetal fibroblasts |
|                 | RF      | Chemically-induced leukemia | CMS21 | Fetal fibroblasts |
| Spleen          | SJL     | BP8     | BALB/c         | BALB/c |
| Kidney          | SWR     | C3H     | CMS1 BALB/c | CMS1 BALB/c |
| Thymus          | AKR     | MuLV (Gram-positive-induced leukemia) | CMS1 BALB/c | CMS1 BALB/c |
|                 | BALB/c  | E.62    | C57BL/6 | C57BL/6 |
| Fetus           | Spleen (11 day) | Spontaneous mammary tumor | CMS1 BALB/c | CMS1 BALB/c |
|                 | BALB/c (15 day) | Myeloma | CMS1 BALB/c | CMS1 BALB/c |
|                 | Xenogeneic | Sheep erythrocytes | CMS1 BALB/c | CMS1 BALB/c |
|                 | Human erythrocytes (types AB, O) | CMS1 BALB/c | CMS1 BALB/c | CMS1 BALB/c |
|                 | Human fetal brain | CMS1 BALB/c | CMS1 BALB/c | CMS1 BALB/c |
|                 | Guinea pig kidney | CMS1 BALB/c | CMS1 BALB/c | CMS1 BALB/c |

*Absorbed antisera assayed for residual cytotoxicity on Meth A(a) cultured cells.
† Strain of origin.
gens. Whether this conclusion can be extended to other cell populations is now the subject of considerable interest. Because the fibroblast establishes itself in vitro with relative ease and can be derived from different organs and from mice of different ages, it is a particularly attractive model for such studies. In addition, as much of our current thinking about malignant transformation has come from in vitro studies with fibroblasts, the development of a comprehensive serology for the cell surface antigens of fibroblasts would provide valuable immunogenetic probes with possibly greater resolving power than lectins to analyze the surface modifications that accompany the malignant change.

With the widespread occurrence of MuLV in the mouse, the possible contribution of MuLV antigens must always be considered in interpreting results of immunological analysis of mouse cells, whether normal or malignant, and this is true whether one is working with serological techniques or those measuring cell-mediated immunity. Although it has been known for some time that sarcomas induced by chemical carcinogens may express cell surface antigens related to MuLV, this has not been the subject of any detailed study. Before attempting to define new surface alloantigens or tumor-specific antigens on sarcoma cells, it is essential to characterize the MuLV phenotype of the target cell. All mouse serum, whether from immunized donors or not, must be suspected of having antibodies to surface MuLV antigens, and we have not as yet found any reliable or routine method to eliminate them. Cells expressing MuLV are, therefore, generally unsuitable as target cells for detecting non-MuLV antigens in direct cytotoxic tests; positive reactions with mouse sera may be due to the presence of MuLV antibody and appropriate absorption tests are necessary to resolve the issue of specificity. For this reason, the availability of MuLV- fibroblast and MuLV- sarcoma lines greatly facilitates search for surface antigens unrelated to MuLV.

What significance can be attributed to the appearance of MuLV in sarcomas arising in strains (such as BALB/c) that normally do not express these endogenous viral genes? Does it reflect the general tendency of malignant cells to show instability in genetic control of the differentiated state, resulting in the activation of ordinarily silent genes, or is it of etiological significance, with MuLV playing a critical role in chemical carcinogenesis, a suggestion that continues to attract attention? The fact that some sarcomas express MuLV and others do not, even though they all presumably carry MuLV genes, indicates that MuLV activation is not an invariable consequence of malignant transformation. Nor is MuLV activation restricted to sarcomas, since some morphologically normal fibroblast lines become MuLV+ during passage in tissue culture. In BALB/c mice, full expression of the viral genes and the antigens they determine appears to be the case under conditions of MuLV activation. Thus, the surface phenotype of MuLV+ sarcoma or fibroblast lines is G\(_{\alpha}^+\) GCSA+ gp70+ p30+. We did not find any instance of partial expression of MuLV (e.g., G\(_{\alpha}^+\) GCSA-) as if only part of the viral genome was activated. In contrast, C57BL/6 fibroblasts and sarcomas carry a gp70-like molecule on their surface, whether they express other MuLV traits or not. This gp70 of C57BL/6 cells is most likely related to the 0-gp70 molecule that has been detected on normal C57BL/6 lymphoid and leukemia cells (30) and appears to be the product of a separate MuLV locus in this strain. With the recognition that there is far more MuLV polymorphism than was
originally suspected, it will be important to characterize the MuLV from fibroblast and sarcoma cultures to determine whether they can be distinguished from MuLV derived from normal or leukemic lymphoid tissues.

Of the differentiation alloantigens studied, Thy-1 and PC.1 have been detected on some fibroblast and sarcoma cell lines. These antigens may not be stable markers for cultured fibroblasts since we have observed the loss of Thy-1 and PC.1 from fibroblast cultures that in earlier passages typed Thy-1+ and PC.1+ by absorption. Another explanation for this finding could be that fibroblasts are dimorphic for these alloantigens and that the emergence of Thy-1− or PC.1− cultures represents the outgrowth of one cell type from an originally mixed population. Either explanation (instability and loss of antigen expression in vitro or dimorphic populations of normal fibroblasts) could account for the finding of Thy-1+ or− and PC.1+ or− sarcoma lines. As expected, alloantigens belonging to the TL and Lyt systems that characterize cells of thymic origin were not detected on fibroblast or sarcoma cells. Given this information on known alloantigens, it should be possible to develop a series of planned immunizations in mice of various genotypes to uncover new surface alloantigens of fibroblasts and sarcoma cells.

A remarkable and baffling feature of chemically induced sarcomas is the extensive range of antigens that have been detected by transplantation immunity in syngeneic mice. Since the discovery of this class of tumor antigens, sarcomas induced by a variety of chemical carcinogens have been the object of intense scrutiny by tumor immunologists and yet not a single instance of a bona fide sharing of antigen by two tumors, even when induced in the same mouse (36), has been established. There has been much discussion concerning the basis of this extensive antigenic diversity. Whether these unique surface antigens have their origin in carcinogen- or malignancy-induced changes in structural or regulatory genes, or arise from epigenetic errors in membrane synthesis is not known. Clearly, their analysis would be greatly facilitated if serological methods for detecting them were devised. The present study of BALB/c Meth A sarcoma represents an attempt to define serologically the strong transplantation antigen carried by this tumor. The availability of an ascites variant of this tumor was particularly useful, providing an abundant source of cells for immunization and a target cell population for cytotoxic tests with >95% viability that does not require removal of dead cells by proteolytic enzymes. Another advantage is that Meth A ascites (in contrast to the original solid tumor) does not express MuLV antigens, and cultured lines derived from it remain MuLV−. The disadvantage of this system, of course, is that Meth A is a long-transplanted tumor that has had ample time to diverge from the strain of origin during the 17-yr since its induction. However, as the original solid tumor and the ascites variant which have had a separate passage history for the past 16 yr continue to share the same antigen, this would indicate a high degree of coding stability for Meth A antigen. Nevertheless, the conclusions derived from the study of Meth A will have to be confirmed with a series of newly derived sarcomas.

Absorption analysis has not as yet given any clues regarding the origin of the Meth A antigen. There has been speculation that the specific antigens of chemically induced tumors and other tumors as well might represent derepressed fetal antigens, modified antigens of the H-2 complex, or derepressed...
alloantigens (e.g., anomalous appearance of TL antigens in leukemias of TL- strains [34]). Another possibility in view of the extensive polymorphism of MuLV is that MuLV genetic information (e.g., from recombinational or mutational events) might be responsible for the unique antigens of chemically induced tumors. The fact that no other normal or malignant tissue, adult or fetal, MuLV+ or MuLV-, expressed Meth A antigen provides no help in distinguishing these possibilities. Biochemical characterization of Meth A antigen now that serological reagents are available may be more revealing.

Summary
As background for a serological definition of the unique antigens of chemically induced sarcomas, we have typed a series of fibroblast and sarcoma cell lines of BALB/c and C57BL/6 origin by cytotoxicity and absorption tests for murine leukemia virus (MuLV)-related cell surface antigens and known alloantigens. 7 of the 17 cultured lines expressed the range of cell surface antigens associated with MuLV (G~x, GCSA, gp70, p30), and this was invariably associated with MuLV production. In nonproducer lines of C57BL/6 (but not BALB/c) origin, a MuLV-gp70-like molecule was found on the surface of fibroblasts and sarcoma cells. The alloantigenic phenotype of these MuLV+ and MuLV- cell lines was H-2D+, H-2K+, Thy-1.2+ or-, PC.1+ or-, Lyt-1.2-, Lyt-2.2-, Ia.7-, and TL.2-. A unique antigen was defined on the BALB/c ascites sarcoma Meth A with antisera prepared in BALB/c or (BALB/c × C57BL/6)F1 mice. Tissue culture lines derived from this tumor were MuLV-, which facilitated serological study of the antigen. Absorption analysis indicated that the antigen was restricted to Meth A; it could not be detected in normal or fetal BALB/c tissue, MuLV+ or MuLV- fibroblast lines, 12 syngeneic or allogeneic sarcomas, or normal lymphoid cells from 13 different inbred mouse strains.

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