Characterization of a Murine Ovarian Reticulum Cell Sarcoma of Histiocytic Origin

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ABSTRACT

We have studied the M5076 tumor, a transplantable murine reticulum cell sarcoma that arose spontaneously in the ovary of a C57BL/6 mouse. This tumor displays functional and ultrastructural characteristics indicating that it is of macrophage origin. Cells from the M5076 tumor are phagocytic, form rosettes with sheep red blood cells, mediate antibody-dependent cellular cytotoxicity against 51Cr-labeled red blood cells, and display macrophage-like cytotoxicity against syngeneic tumor target cells but do not exhibit any natural killer cell activity. The tumor cells possess lysozyme, nonspecific esterase, and phosphatase activities comparable to that seen in rodent macrophages. Ultrastructural examination revealed phagocytic vacuoles and a lack of tight junctions typical of macrophage morphology. Karyotype analysis showed that M5076 tumor cells are hypodiploid with a high percentage (>80%) of metacentric chromosomes that serve as an excellent marker for identification of these tumor cells.

INTRODUCTION

In the past, the terms reticulum cell sarcoma and histiocytic lymphoma have been applied to a variety of lymphomas believed to have originated from macrophages (10, 33). Classification of these neoplasms formerly was based on morphological criteria alone (33), although histological identification of reticulum cell sarcomas is extremely difficult (19, 21). Recent studies, in which immunological and functional markers were used, have revealed that many of the histiocytic lymphomas originated from lymphocytes rather than monocytes (1, 7, 14, 19, 21, 35). Relatively few murine or human tumors have been shown definitively to have originated from monocyte macrophages (1, 7, 14, 19, 21, 35).

In this paper, we report on the in vitro characterization of a spontaneous ovarian tumor from a C57BL/6 mouse which, by functional, immunological, and ultrastructural criteria, appears to have originated from histiocyte macrophages. The in vivo behavior and organ-specific metastatic properties of this tumor line are described in the accompanying paper (12).

MATERIALS AND METHODS

Mice. Specific-pathogen-free adult mice (6 to 8 weeks old) of the inbred strain C57BL/6 were obtained from the Frederick Cancer Research Center's Animal Production Area.

Media. Eagle's minimal essential medium (Auto-Pow) was obtained from Flow Laboratories, Inc. (Rockville, Md.). This medium was supplemented with 5% heat-inactivated, endotoxin-free fetal bovine serum (Reheis Chemical Co., Kankakee, Ill.), sodium pyruvate, sodium bicarbonate, nonessential amino acids, L-glutamine, 2-fold vitamin solution, and 50 µg gentamicin per ml and designated CMEM.3 The M5076 cells were grown in RPMI Medium 1640 (Grand Island Biological Co., Grand island, N. Y.), supplemented with 17% heat-inactivated equine serum (Flow Laboratories), L-glutamine, sodium pyruvate, and 50 µg gentamicin per ml. HBSS, pH 7.2, was obtained from Grand Island Biological Co.

Cell Cultures. The murine tumor M5076 arose spontaneously in the ovary of a C57BL/6 mouse in the laboratory of Dr. W. F. Dunning of the Papanicolaou Research Institute in Miami, Fla. We obtained the tumor from Dr. D. P. Griswold of the Southern Research Institute, Birmingham, Ala. The tumor was frozen and supplied to us in the 136th serial passage in syngeneic mice.

The B16 malignant melanoma variant cell line (F1) was derived from a spontaneous tumor of a C57BL/6 mouse in this laboratory by Dr. I. J. Fidler (8). The cell line YAC-1 is a T-cell lymphoma induced by Moloney leukemia virus in A/Sn mice (obtained from Dr. R. Burton, Massachusetts General Hospital, Boston, Mass.). These cell lines were propagated in CMEM and subcultured at a ratio of 1:10 weekly. The M5076 tumor cells were propagated in RPMI Medium 1640, supplemented as described above, and subcultured at a ratio of 1:2 each week. The cell lines were incubated at 37° in a humidified atmosphere of air and 5% CO2.

To establish primary cultures of M5076 tumor cells, we removed ascites-passaged cells aseptically by lavaging the peritoneal cavity of tumor-bearing mice with HBSS. The cells were washed twice in HBSS and cultured in 100- x 20-mm tissue culture plates (Falcon, Oxnard, Calif.). Tumors used in these experiments were examined for and found free of Mycoplasma and the following murine viruses: reovirus type 3, pneumonia virus of mice, K virus, Theiler's virus, Sendai virus, minute virus, mouse adenovirus, mouse hepatitis virus, lym-phocytic choriomeningitis virus, ectromelia virus, and lactate dehydrogenase virus (Microbiological Associates, Inc., Walkersville, Md.).

Enzyme Assay. Lysozyme activity of cells and supernates was assayed by measuring the lysis of Micrococcus lysodeikticus (Sigma Chemical Co., St. Louis, Mo.) with a Zeiss spectrophotometer PM2 DL. Egg white lysozyme (Sigma) was the

1 Research sponsored by the National Cancer Institute Contract No1-Co-75380 with Litton Bionetics, Inc.
2 To whom requests for reprints should be addressed.
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3 The abbreviations used are: CMEM, complete Eagle's minimal essential medium; RPMI Medium 1640, Roswell Park Memorial Institute Tissue Culture Medium 1640; HBSS, Hanks' balanced salt solution; PEC, peritoneal exudate cells; LPS, lipopolysaccharide; SRBC, sheep red blood cells; mouse αSRBC, mouse anti-sheep red blood cells (sheep red blood cells coated with appropriate antiserum); rat αSRBC, rat anti-sheep red blood cells (sheep red blood cells coated with appropriate antiserum); ADCC, antibody-dependent cell-mediated cytotoxicity; NK, natural killer.
In Vivo Growth Rate. Mice given s.c. injections in the ear or in a caudal mammary fat pad were examined 3 times a week for tumor growth. Tumor diameters were measured in 2 dimensions at right angles to one another using a vernier caliper. Tumor volumes were calculated using the following formula: Volume = 0.4 x a x b², where a = the larger axis and b = the smaller axis (27).

In Vitro Growth Curves. Cells established in tissue culture flasks were harvested by tapping the flasks by hand, and the dislodged cells were washed in serum-free medium and plated at a density of 10⁵ cells/60-mm tissue culture dish (Falcon) in 5 ml complete RPMI Medium 1640. Cultures were incubated at 37° in a humidified atmosphere containing 5% CO₂. Triplicate cultures were counted every 48 hr following culture initiation using a hemocytometer and trypan blue exclusion.

Karyotyping and Banding. Cells for karyotyping were arrested in mitosis with 10 μg Colcemid (Sigma) per ml for 1 hr at 37°. The cells were pelleted and washed once in HBSS. Following hypotonic treatment with 0.5% aqueous KCl for 10 min at room temperature, these cells were pelleted gently and fixed in 3 changes of ice-cold methanol:acetic acid (3:1). The cells were stored at 20° for several days before spreads were prepared. Spreads were G-banded in 0.01% trypsin in Ca²⁺- Mg²⁺-free HBSS for 12 min at 37°, rinsed in ice-cold distilled water, and stained in a 5% solution of Giemsa blood stain type 620 (Harleco, Gibbstown, N. J.) in Gurr’s pH 6.8 buffer for 4 min. Nucleolar organizer regions (11) were stained by incubating slides in a 50% aqueous silver nitrate solution for 18 hr bating slides in a 50% aqueous silver nitrate solution for 18 hr at 56° in a humidified atmosphere. The silver stain was developed in 3% v/v pH 4.5 aqueous formalin solution for 15 min.

Collection and Culture of Macrophages. Resident peritoneal macrophages were collected by lavage from untreated C57BL/6 mice. Peritoneal macrophages were collected from mice injected with 2 ml of thioglycollate (Becton Dickinson, Cockeysville, Md.) 5 days before cell harvest.

PEC were washed and suspended in HBSS and plated into 96-well Micro Test II plates (Falcon). After cell attachment (60 min at 37°), the wells were rinsed thoroughly with HBSS to remove nonadherent cells and then refed with the appropriate medium.

Collection and Culture of Ascitic M5076 Cells. Ascitic M5076 cells were obtained by repeated peritoneal lavage. The cells so obtained contained less than 5% macrophages and less than 1% polymorphonuclear leukocytes as determined by using combined morphological and histocytotoxic criteria on cytocentrifuge preparations. These ascitic M5076 cells were washed twice in HBSS and processed in the same way as PEC for cytotoxicity assays.

In Vitro Cytotoxicity Assay. Thioglycollate-induced PEC, ascites-grown M5076 cells, or tissue-culture-propagated M5076 cells were assayed for their cytotoxic activity against B16-F1 melanoma target cells following 24-hr prior incubation in CMEM or CMEM containing 5 μg LPS per ml. Cytotoxicity was assayed using a radiorelease assay as described elsewhere (37). Briefly, B16-F1 melanoma target cells were labeled by incubation for 24 hr in CMEM supplemented with 0.3 μCi of 5-[³²]Iodo-2'-deoxyuridine (specific activity, 200 μCi/mol; New England Nuclear, Boston, Mass.). Following harvesting with a brief trypsinization (0.25% trypsin and 0.02% EDTA for 1 min at 37°), the melanoma cells were washed several times with CMEM to remove unbound label and then plated (10³ cells/well) into 96-well Micro Test II dishes containing effector cells (10⁴ cells/well) in the appropriate medium to give a 10:1 ratio of effector to target cells.

These cultures were incubated for 3 days at 37°. The assays were terminated by washing the wells with warm HBSS to remove all nonadherent cells. Adherent cells were removed by lysis with 0.1 ml 0.1 n NaOH and then wiping the wells with cotton-tipped swabs. The radioactivity of these swabs was measured in a NaI crystal well-type γ counter.

The percentage of cytotoxicity, mediated by PEC or M5076 tumor cells, was calculated as follows:

% of cytotoxicity = 100 cpm in target cells cultured without effector cells × — cpm in target cells cultured with effector cells cpm in target cells cultured without effector cells

Chromium Labeling and Sensitization of SRBC. SRBC that were to be used as target cells were washed 3 times with HBSS and counted. These cells (2 x 10⁵) were incubated in CMEM containing 200 μCi ⁵¹Cr for 45 min at 37°. The labeled cells were washed 3 times with CMEM and divided into equal aliquots which were pelleted, and the radiolabeled SRBC were resuspended into 200 μl of heat-inactivated (56° for 30 min) mouse aSRBC, rat aSRBC, or normal murine serum. These cells were incubated for 30 min at 37° and then diluted with CMEM by vigorous agitation to obtain a single-cell suspension. These cells were then pelleted and washed again with agitation.

ADCC. The desired numbers of macrophages or M5076 tumor cells were added to a final volume of 200 μl of CMEM containing 1 x 10⁵ ⁵¹Cr-labeled SRBC. Cultures were established in U-bottom Micro Test plates and incubated for 4 hr at 37°. Following incubation, the plates were centrifuged at 600 rpm for 4 min in a Beckman TJ-6 centrifuge; an aliquot (100 μl) of each supernate was removed carefully, and the radioactivity was measured. Total cpm was determined by counting aliquots of 1 x 10⁶ ⁵¹Cr-labeled SRBC. The percentage of lysis was calculated as follows:

% of lysis = 100 x [(Total — 2 x spontaneous release) — cpm experimental release — 2 x spontaneous release](Total — 2 x spontaneous release)

Assessment of Phagocytosis and Fc Receptors. Macrophages (thioglycollate stimulated or resident peritoneal) or ascitic M5076 tumor cells were added to the wells of a Falcon Micro Test II plate. These cells (1 x 10⁸ in 100 μl of HBSS) were incubated at 37° for 60 min and vigorously washed with HBSS to remove nonadherent cells. Radiolabeled SRBC (5 x 10⁶), sensitized or not sensitized in antiserum, were added to each well in 100 μl of CMEM. The cultures were incubated at 37° for 60 min and washed 3 times with HBSS. Nonphagocytosed SRBC were removed from some of the wells using a brief (10-sec) hypotonic lysis with distilled water. The number of adherent erythrocytes was taken as the difference between the cpm obtained from the wells and the wells with hypotonic lysis.
**NK Activity Assay.** Target cells for this assay were ⁵¹Cr-labeled YAC-1 cells obtained by incubating 2 × 10⁶ cells for 40 min at 37° in RPMI Medium 1640 containing 200 μCi ⁵¹Cr. Labeled cells were washed 3 times with RPMI Medium 1640 with 30-min incubation between washes to remove unbound ⁵¹Cr and to allow for the release of ⁵¹Cr from lysed cells.

The cells were then washed and diluted in medium to a final concentration of 10⁶ cells/ml. Aliquots (50 μl of cells) were added to the wells of Micro Test II dishes containing 200 μl of RPMI Medium 1640. The cells were incubated at 37° for 4 hr and washed once with CMEM, and effector cells were added. Control effector cells were spleen cells, with the adherent cells removed by preincubation for 1 hr at 37° in serum-free medium on 10- x 60-mm tissue culture dishes. The nonadherent, nucleated cells were recovered, counted, and diluted in RPMI Medium 1640, and 200-μl aliquots were added to the target cells. Test effector cells were ascites-passaged or tissue culture-propagated M5076 cells. Either test or control effector cells were added at a ratio of 200:1, 100:1, and 50:1/target cell, and the cell mixtures were incubated for 4 hr. Aliquots of 100 μl of supernatant fluid were removed and counted in a Beckman 300 γ counter. Spontaneous release (background) was computed from wells containing target cells with RPMI Medium 1640 alone and was always less than 10% of the total counts obtained from wells in which the target cells were lysed with 2% sodium dodecyl sulfate. A total of 4 wells was used for each data point, and the percentage of release was computed using the following formula:

\[
\text{% of release} = 100 \times \frac{(2 \times \text{experimental cpm} - 2 \times \text{spontaneous cpm})}{(\text{Total cpm} - 2 \times \text{spontaneous cpm})}
\]

**RESULTS**

**Growth Characteristics In Vitro.** Single-cell suspensions of the M5076 tumor were obtained by mincing a primary tumor and forcing it through a tissue sieve (E-C Apparatus). The single cells attached very rapidly (<15 min) to tissue culture plastic or glass in serum-free medium (Fig. 1). These cells remained attached in the presence of 0.25% trypsin:0.02% EDTA, even after prolonged incubation at 37°. After the primary culture was incubated in RPMI Medium 1640 with 17% equine serum for 48 hr, the tumor cells became nonadherent and grew as a suspension culture (Fig. 1B). These M5076 suspension cultures contained cells that attached lightly to the substratum, generally without extensive spreading, and could be released using gentle agitation.

The M5076 tumor cells grown in RPMI Medium 1640 containing 17% equine serum have a doubling time of 58 hr (Chart 1). Equine serum supported the proliferation of M5076 cells, whereas an equal concentration of fetal bovine serum resulted in reduced growth (Chart 1).

**Growth Characteristics In Vivo.** The s.c. injection of 25,000 M5076 cells into a dorsal-caudal site produced a tumor in 13 days and a tumor-doubling time of 2.7 days. The same number of tumor cells injected into the ventral pinna of the ear produced a tumor in 19 days and had a tumor-doubling time of 4 days. Tumor cells (25,000) injected i.m. into a caudal footpad had an induction time of 19 days and achieved an average tumor diameter of 0.15 ± 0.04 (S.E.) cm 42 days after tumor injection.

**Morphology and Ultrastructure.** M5076 cells were highly anaplastic, whereas the pleomorphic nuclei displayed multiple nucleoli and chromatin margination; the eosinophilic cytoplasm contained a number of granules. Histological sections of primary tumor masses revealed numerous mitotic figures, and silver staining demonstrated intracytoplasmic reticulin-like material, particularly in cells located at the periphery of the tumor mass or in well-vascularized areas (Fig. 2).

Transmission electron microscopy failed to reveal desmosomes or tight junctions but did show a number of cytoplasmic electron-dense granules and phagocytic vacuoles (Fig. 3).

**Fc Receptors and Phagocytosis.** The ascitic and tissue culture-propagated M5076 tumor cells phagocytosed latex beads and carbon particles in vitro. Tumor cell RBC rosettes were formed using SRBC coated with subagglutinating levels (1:2048) of rat αSRBC (Fig. 2). Such rosettes were not found using unsensitized SRBC. During the 60-min incubation period, SRBC were phagocytosed also (Fig. 2).

M5076 tumor cells were more phagocytic than unsensitized resident peritoneal macrophages (Table 1), as assayed using ⁵¹Cr-labeled SRBC coated with mouse or rat αSRBC (agglutinating activity, 1:512 and 1:1024, respectively). M5076 tumor cells phagocytosed SRBC in 60 min, whereas resident PEC did not. However, antibody coating to produce opsonized SRBC enhanced phagocytosis by the M5076 tumor cells (Table 1).

**Enzymatic Activities.** Nonspecific esterase enzyme activities were studied using histochemical stains of cellular cyto-preparations. The ascitic and tissue culture M5076 cells exhibited enzyme activities, although the tissue culture cells exhibited a somewhat more intense staining. However, prepa-
spreads of M5076 tumor cells. Median number of chromosomes, 34. S.D. of 4 assays. 0.65 x 10^7 cells/ml ascites.

Triton X lysis fluid control
Thioglycollate-stimulated macrophages

fluid induced by M5076 tumor cells had very high levels of lysozyme that most of the activity was secreted. Cell-free ascites obtained from suspension cultures of M5076 cells, free of tissue culture-grown M5076 tumor cells, but lysozyme activity in thioglycollate-stimulated PEC was 2-fold higher than that in thioglycollate-stimulated PEC.

rations of M5076 tumor cells always exhibited less intense staining than thioglycollate-stimulated PEC.

Lysozyme activity was seen intracellularly in both ascitic and tissue culture-grown M5076 tumor cells, but lysozyme activity in thioglycollate-stimulated PEC was 2-fold higher than that contained in M5076 tumor cells. Tissue culture supernates obtained from suspension cultures of M5076 cells, free of macrophages, contained lysozyme levels high enough to suggest that most of the activity was secreted. Cell-free ascites fluid induced by M5076 tumor cells had very high levels of lysozyme that were not found in control ascites fluid induced by the T-cell lymphoma EL4 (Table 2).

Karyotypic Analysis. Chromosomal analysis of M5076 cells indicated a modal number of 34 chromosomes (normal 2N = 40) with a range from 22 to 56 (Chart 2), indicating that the cells were hypoploid in their chromosome number.

Most of these chromosomes are metacentric (median number of 25), occasionally with 1 to 3 dot-like chromosome fragments (Fig. 4). Using the silver staining method of Goodpasture and Bloom (11), we demonstrated that each metacentric chromosome contained 2 centromeric regions, suggesting...
Characterization of a Macrophage Tumor

Table 5
In vitro cytotoxicity of thioglycollate-stimulated peritoneal macrophages and ascitic or tissue culture-grown M5076 tumor cells against syngeneic B16 target cells

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Radioactivity in surviving target cells (cpm)</th>
<th>Calculated kill (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ LPS</td>
<td>- LPS</td>
</tr>
<tr>
<td>Target (B16) cells alone</td>
<td>2206 ± 243</td>
<td>2286 ± 294</td>
</tr>
<tr>
<td>Ascites M5076 tumor cells</td>
<td>248 ± 64</td>
<td>465 ± 193</td>
</tr>
<tr>
<td>Tissue culture M5076 tumor cells</td>
<td>1037 ± 140</td>
<td>1126 ± 261</td>
</tr>
<tr>
<td>Peritoneal macrophages</td>
<td>1079 ± 248</td>
<td>1920 ± 347</td>
</tr>
</tbody>
</table>

* Mean ± S.D. of quadruplicate cultures.

** Percentage of cytotoxicity compared with target cells cultured alone.

Table 6
NK cell activity by nude mouse spleen cells and ascitic or tissue culture-grown M5076 tumor cells against YAC-1 target cells

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>E:T Ratio a</th>
<th>Cpm b</th>
<th>Cytotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nude spleen cells</td>
<td>10:1</td>
<td>16,924 ± 664 c</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>5:1</td>
<td>11,980 ± 224</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td>2:5:1</td>
<td>9,450 ± 168</td>
<td>12.3</td>
</tr>
<tr>
<td>Ascites M5076 cells</td>
<td>10:1</td>
<td>5,934 ± 308</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>5:1</td>
<td>5,690 ± 674</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2:5:1</td>
<td>5,935 ± 238</td>
<td>1.7</td>
</tr>
<tr>
<td>Tissue culture M5076 cells</td>
<td>10:1</td>
<td>6,108 ± 406</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>5:1</td>
<td>5,930 ± 360</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>2:5:1</td>
<td>6,164 ± 452</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* E:target cell ratio.

** YAC-1 total cpm were 38,677 ± 4,077 (S.D.; n = 4). Spontaneous release was 5,350 ± 228 cpm.

For the ADCC activity, M5076 tumor cells and thioglycollate-stimulated peritoneal macrophages were compared for their ability to participate in ADCC of mouse αSRBC-coated erythrocytes. A representative experiment (Table 3) demonstrates that the lysis of 51Cr-labeled SRBC is dependent on antibody sensitization. The ascites-propagated tumor cells were more active in the lysis of the sensitized SRBC than either the macrophages or the tumor cells grown in vitro. These results could indeed be attributed to ADCC activity and not to phagocytic activity, inasmuch as the inclusion of the phagocytic inhibitor iodoacetamide (0.15 × 10^6 M) in the incubating medium failed to reduce the ADCC activity (Table 4).

Cytotoxicity Assay. In vitro cytotoxicity by M5076 tumor cells and thioglycollate-stimulated peritoneal macrophages was studied using the 72-hr assay as described previously (38). The effector cells were stimulated with endotoxin (LPS), and 5-[125I]iodo-2'-deoxyuridine-labeled B16 melanoma cells were used as targets. Macrophages, even those stimulated by thioglycollate, are not very cytotoxic unless activated by an agent such as LPS (Table 5). The M5076 tumor cells, however, were cytotoxic without activation by LPS, although prior incubation with this agent did slightly increase their cytotoxicity (p < 0.05). The ascitic tumor cells were about 2-fold more active as effector cells than either the tissue culture cells or macrophages.

NK Cell Activity. M5076 tumor cells and spleen cells from nude mice were compared for their ability to lyse the NK cell-sensitizing target YAC-1. Although in every experiment the spleen cells had NK cell activity against YAC-1 target cells, neither the in vitro- nor the in vivo-propagated cells were capable of causing 51Cr release from YAC-1 cells at any effector:target cell ratio (Table 6).

DISCUSSION

The data presented in this paper characterize the M5076 tumor as being of histiocytic (macrophage) lineage. The M5076 tumor, which arose spontaneously in a C57BL/6 mouse, was described originally as an ovarian carcinoma (36) and later tentatively identified as a granulosa cell tumor (22) on the basis of light-microscope morphology alone. Dunn (5) has commented upon the extreme difficulty of differentiating morphologically between granulosa cell tumors and reticulum cell sarcomas when the primary tumor originates in the ovary. Although the in vivo behavior of the M5076 (13) is indicative of a reticulum cell sarcoma (3, 28, 29, 32), this study demonstrates the value of using functional assays to help determine the neoplasm classification.

Reticulum cell sarcomas or histiocytic lymphomas are characterized morphologically by their large cell size and cellular pleomorphism (18, 19, 21). However, functionally and immunologically, many of these tumors have been shown to be composed of malignant lymphocytes (2, 9). A few human (1, 14, 15, 35, 38) and murine (4, 21, 30, 32, 34) tumors have been cultured in vitro and shown to be of macrophage origin by functional, immunological, histochemical, and enzymatic criteria.

M5076 tumor cells propagated either in vivo or in vitro express membrane Fc receptors by both rosetting and radio-label binding studies (Table 1). These cells are also capable of phagocytosing carbon particles, latex beads, and SRBC. The phagocytosis of SRBC does not require antibody coating, although opsonization through antibody coating does result in increased phagocytosis. The demonstration of Fc receptors is considered important in the classification of macrophage tumors (21). Both phagocytic activity and Fc receptors have been reported for human (17) and murine (16, 31) macrophage tumors.

that the metacentrics arose from the fusion of 2 telocentric chromosomes (Fig. 5).

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* Dr. B. Lane (University of Southern California, Los Angeles, Calif.) has shown that M5076 tumor cells have Fc receptors for IgG1, IgG2a, and IgG3b by using monoclonal antibodies and an erythrocyte rosetting assay. Only the Fc receptor for IgG2a is trypsin sensitive. Personal communication.

D. R. Coman (Jackson Laboratories, Bar Harbor, Maine), personal communication.

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The M5076 tumor cells exhibit antibody-dependent cellular cytosis of antibody-coated SRBC (Table 3). Further, when assayed using a 72-hr macrophage cytotoxicity assay, the M5076 tumor cells expressed a macrophage-like effector cell activity against target B16 melanoma tumor cells. However, unlike macrophages, the M5076 cells do not require activation to be cytotoxic, although preincubation with LPS did increase cytotoxicity slightly (Table 4). In both assays of effector cell activity (ADCC and macrophage cytotoxicity), the ascitic tumor cells were about 2-fold more active than the tissue culture-propagated cells. Because host cell contamination of the ascitic tumors was low (<10%), it is unlikely that the observed higher cytotoxicity was caused by these contaminant host cells. Rather, it appears that in vitro cultivation diminishes the cytotoxic capacity of the M5076 cells. The M5076 tumor cells did not express any NK-like activity when assayed against YAC-1 cells in a 4-hr 51Cr release assay (Table 6). M5076 tumor cells express many of the enzyme activities, such as lysozyme and nonspecific esterase or phosphatase, commonly associated with macrophages (25). Lysozyme activity was demonstrated intracellularly, and lysozyme was secreted in vitro into the culture fluid or in vivo into ascitic fluid. Again, this characteristic was unlikely to be caused by contaminating host cells since both intracellular and secreted activity could be detected in cells passaged in tissue culture for 3 to 4 months which were free of contaminant host cells.

Like macrophages, primary M5076 cell cultures adhere very rapidly to glass or plastic without a serum requirement (6). However, on further culture, the cells are released and grow either as a suspension culture or lightly attached to the substrate.

Ultrastructural studies of the M5076 cells failed to reveal any tight junctions, a finding that agrees with the proposed macrophage origin of this tumor line and provides another distinction from granulosa cell tumors which are characterized by the presence of many of these structures (21).

We have included some data on the karyotype of the M5076 tumor line because of the relative rarity of the displayed chromosome pattern. Most murine tumors are aneuploid or hyperdiploid (26), whereas relatively few hypoploid neoplasms have been described (24). Also, the high frequency of metacentric chromosomes, compared with the more characteristic telocentric forms, is unusual (20). Based on the centromeric staining characteristics of these cells, it seems probable that the metacentric chromosomes have arisen as the result of Robertsonian translocations (23). This distinctive chromosomal pattern provides a good marker for the unequivocal identification of M5076 cells.

Based on the foregoing criteria, we suggest that the M5076 is a reticulum cell sarcoma of macrophage origin. In the following paper, we describe the in vivo behavior of this cell line, which provides a good model for site-specific metastasis (13).

**ACKNOWLEDGMENT**

We are grateful to Dr. Cora Bucana for the transmission electron micrographs.

**REFERENCES**


Characterization of a Macrophage Tumor


Fig. 1. Plating behavior of M5076 tumor cells examined by phase microscopy. In A, ascites-derived M5076 cells incubated in serum-free medium for 15 min show initial spreading and attachment to plastic substrate. × 200. In B, incubation of cells for 48 hr in RPMI Medium 1640 supplemented with 17% equine serum results in a general rounding up of the tumor cells and release from attachment. Viability >98% as assessed by trypan blue exclusion. × 40.
Fig. 2. A, silver-stained section of primary M5076 tumor growing in a s.c. site; arrows, intracellular reticulin-like material. × 315; B, tumor cell-SRBC rosettes formed after incubation of M5076 tumor cells with SRBC coated with subagglutinating levels of rat αSRBC antiserum; arrows, phagocytosed SRBC incubation period of 60 min. × 500.

Fig. 3. Electron micrograph of M5076 metastatic deposit in the ovary of C57BL/6 mouse showing presence of what appear to be phagocytic vacuoles in tumor cells. × 7000.

Fig. 4. Two karyotypes derived from metaphase spreads of M5076 cells stained with Giemsa as described in the text. The majority of chromosomes are metacentric. ×1000.
Fig. 5. Metaphase spread from M5076 cell stained with silver nitrate solution as described in the text. Arrows, 2 darkly stained centromeric regions, suggesting that metacentric chromosomes arose by fusion of 2 telocentric chromosomes. × 1260.
Characterization of a Murine Ovarian Reticulum Cell Sarcoma of Histiocytic Origin

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