Establishment of Human Mesothelioma Cell Lines (MS-1, -2) and Production of a Monoclonal Antibody (Anti-MS) with Diagnostic and Therapeutic Potential

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ABSTRACT

We have established and characterized two mesothelioma cell lines, MS-1 and MS-2, in four attempts at long-term culture of these cells. Both MS-1 and MS-2 cells consistently express cytokeratin and vimentin, and both have long, slender microvilli. The cells that grew indefinitely (MS-1, MS-2) had a higher DNA index and a higher nucleus:cytoplasm ratio than did those cells that failed to grow (MS-3, MS-4). All mesothelioma cells, both in short- and long-term culture, responded to phorbol ester induction by displaying morphological differentiation, such as an increase in the number of microvilli. The distribution of vimentin and cytokeratin in the cells, however, remained the same regardless of the growth pattern or the phorbol-ester treatment of the cells. We have used MS-1 cells to produce a monoclonal antibody (anti-MS) that reacts with mesothelioma cells, but rarely with reactive or normal mesothelial cells or with cells of normal tissues. The antibody does not induce the modulation of antigen, and it causes no direct or complement-mediated cytotoxicity of MS-1 or MS-2 cells. If a toxin or an isotope conjugate of the antibody is used, it may be valuable in the near future to test it for use in immunomaging or immunotherapy.

INTRODUCTION

Mesotheliomas are tumors that arise from the lining of the pleural, pericardial, or peritoneal surface. Many reports have confirmed the relationship between exposure to asbestos and the subsequent development of mesothelioma. Malignant MS2 now causes about 4,000 cancer deaths each year in the United States. Major industrial usage of asbestos in the US began around 1950, and thus it is anticipated that the incidence of this disease may increase (1).

The diagnosis of malignant MS, and in particular its distinction from carcinoma of the lungs and other organs that result in metastases to the pleura or the pericardium, continue to pose difficult enigmas for the pathologist. It is important to distinguish between MS and other tumors because of differences in biological behavior, treatment, and patient survival rates. Recently, it has been shown that the use of antibodies to keratin and vimentin facilitates the diagnosis of MS (2–4). However, these antibodies cannot discriminate between normal and neoplastic mesothelial cells.

In this report, we describe our success in establishing two long-term cultures of MS cells. Established MS cell lines could be used for studies of the efficacy of chemotherapeutic agents and of the biochemical and genetic alterations associated with this disease. In addition, we were successful in obtaining a MAb by immunization of mice with these cells, that neither reacts with normal tissues nor induces antigenic modulation of MS cells. Thus, the use of anti-MS antibody may facilitate the diagnosis of and have a therapeutic potential in MS.

MATERIALS AND METHODS

Source of Cells. Since 1984, five cases of pleural MS have been diagnosed in this laboratory, based on characteristic phenotypes (see below) and histopathological features. None of the MS cells from these five patients was positively stained by anti-Leu M1 or by antibody to carcinoembryonic antigen. All of the patients had pleural effusions. The hemorrhagic pleural fluid from four of the patients was obtained for cell culture immediately after thoracotomies.

Establishment of MS-1 and MS-2 Cell Lines. The cells from the four patients were washed twice and seeded into flask at 4 × 10^5 to 2 × 10^6 cells/ml in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum, 2 mM glutamine, 50 μg/ml penicillin, and 50 μg/ml gentamicin at 37°C in a humidified, 5% CO2 atmosphere (5). The medium was changed every 2–3 days. When we had established two cell lines, the cells (later designated as MS-1 and MS-2) were routinely trypsinized for passage once weekly. Cell viability was determined with the Trypan Blue dye exclusion test.

Immunocytochemistry. We used the MABs anticytokeratin and anti-vimentin to compare the phenotype of the MS cells before and after tissue culture. The antibodies were obtained from Becton-Dickinson Laboratories (Sunnyvale, CA) and Accurate Co. (Santa Barbara, CA), respectively. Biotin-conjugated horse anti-mouse IgG and avidin-biotin-peroxidase complex (ABC) were obtained from Vector Laboratories ( Burlingame, CA). The staining procedure used has been described elsewhere in detail (6–8).

Briefly, the tissue sections were fixed in acetic acid at room temperature for 5 min. The sections were treated with MABs at 2 μg/ml for 1 h. After extensive washing, sections were sequentially incubated with biotin/H/ IgG (1:400) and ABC, and then developed in diaminobenzidine solution (8). The sections were counter-stained with methyl green, dehydrated, and cleared as in routine processing.

Controls for staining specificity were performed by omission of the MAB or by replacement of MAB with BALB/c mouse serum or ascites fluid at a concentration equivalent to that of the MAB.

Ultrastructural and Scanning Electron Microscopic Studies. Cells were placed into 2% cold cacodylate-buffered glutaraldehyde (pH 7.4), postfixed in 1% osmium tetroxide, stained with 2% uranyl acetate, dehydrated in graded alcohol, and embedded in Epon resin. Ultrathin sections were placed on Formvar-coated copper grids, stained with lead citrate, and examined by electron microscopy. For scanning electron microscopic examination, the cells were allowed to adhere to Thermax plastic coverslips (Lux Division, Miles Laboratories, Naperville, IL) in 35- × 10-mm Corning tissue-culture dishes. The coverslips were fixed, processed, and examined in a JEOL JEM 100 CX electron microscope operated at 20 kV with an 8-degree stage tilt.

TPA Induction of Cells. We used a TPA-induction assay to study the differentiation of MS cells. The protocol was the same as described previously (9, 10). Briefly, TPA dissolved in dimethyl sulfoxide (14 μg/ml) was added at a final concentration of 2 ng/ml to cultures of MS cells. Every second day, the medium was replaced with fresh medium containing TPA. The induction was carried out for 5 days. The TPA-induced cells were examined for their phenotype, light microscopic appearance, and ultrastructure.

DNA Content of MS Cells. We examined the DNA content of MS cells at various times before and during culture by using propidium iodide staining and flow cytometry (11). Briefly, the cells were washed.
in RPMI 1640 medium, and then 1 ml aliquots of a cell suspension containing 2 x 10^6 cells were incubated with 75% ethanol (2.5 ml) for 24 h at 2-8°C. After washing in PBS, the cells were incubated with 100 µl RNase A (1000 units/100 µl; Worthington Diagnostic, NJ) for 1 min. They were then stained with propidium iodide (50 µg/ml) for 2 min and vortexed gently. Finally, cells were analyzed by flow cytometry (Ortho Cytofluorograf system 50H).

Cytogetic Analysis. Cytogenetic studies of MS-1 and MS-2 cells were carried out. The cells were cultured in fresh RPMI medium and Colcemid (0.2 µg/ml) for 90 min so that cells were arrested in metaphase. The cell pellet was suspended in a hypotonic solution (1:0.075 M KCl and 1% sodium citrate) for 20 min at room temperature, fixed with modified Carnoy’s fixative solution (3:1 absolute ethanol and glacial acetic acid) for 15 min, and then washed with 2 changes of the fixative solution. The air-dried slides, aged for 1 week, were stained with a conventional Giemsa stain for scoring of chromosome numbers and for the presence of chromosome aberrations, or with trypsin-Giemsa stain for chromosomal banding analysis according to the International System for Human Cytogenetic Nomenclature criteria, or with a fluorescence stain for determination of the Y chromosome (12-14). Fifty metaphases from each cell line were scored, and 10 metaphases were karyotyped.

Heterotransplantation into Nude Mice. Breeding of nude mice (Swiss) was carried out under aseptic conditions. Transplantation experiments were performed on 4-week-old mice. For i.p. transplantation, 1 x 10^6 MS-1 cells in 100 µl were inoculated with a tuberculin syringe.

Production of Monoclonal Antibody. The protocol for production of MAbs 2H9 was used for the production of anti-MS-cell antibodies, except that intrasplenic immunization rather than i.p. injection was performed (15, 16). A total of 1 x 10^6 MS-1 cells in 0.5 ml were injected directly into the spleen of a mouse. A booster injection was given 21 days later, i.e., 3-4 days before hybridization.

To score the reactivities of MAbs, we used an immunoperoxidase technique with cytokin smears of normal and neoplastic MS cells. One antibody (anti-MS), which reacted selectively with neoplastic cells, but not normal MS cells, was subcloned and studied further.

The reactivity of anti-MS with normal tissues and tissues from various types of neoplasms was tested on frozen sections with an ABC immunoperoxidase staining technique. The tumors tested included carcinomas from breast (infiltrating ductal carcinoma, lobular carcinoma, and medullary carcinoma, each three), lung (adenocarcinoma, seven), liver (hepatoma and cholangiocarcinoma, two each), colon (adenocarcinoma, five), kidney (renal cell carcinoma, one), urinary bladder (transitional cell carcinoma, two), skin (melanoma, one), and lymph nodes (follicular lymphoma, three, large cell lymphoma, five).

Characterization of Antigens. Approximately 2 x 10^6 cultured MS-1 cells were washed twice with RPMI medium, followed by lysis on ice for 5 min in a buffer containing 20 mM Tris-HCl, pH 7.2, 0.15 N NaCl, 1% sodium deoxycholate, 1% NP-40, 0.1% SDS, 10 mM EDTA and 1 mM phenylmethylsulfonyl fluoride, and 10 µg/ml of aprotinin (Boehringer Mannheim). The lysate was clarified by centrifugation in a microcentrifuge for 10 min at 4°C. For immunohblots, proteins in Laemmli buffer (15 µl per lane) were loaded on a 10% SDS polyacrylamide gel and electrophoresed. Prior to transfer, the gel was soaked in transfer buffer (20% methanol, 14.4 g/liter glycine, 3 g/liter Tris) for 20 minutes for removal of SDS. BA-85 nitrocellulose (0.2 µm thick, Schleicher and Schuell) was used as the blotting membrane.

For detection of transferred proteins, one strip of nitrocellulose was stained with 0.5% Fast Green in 20% methanol, 5% acetic acid and then destained. For detection of antigen with MAbs, the filter was first blocked with normal horse serum (1:200 dilution, from Vector Laboratories, ABC kit) in a buffer containing 3% non-fat dry milk in TS (20 mM Tris-HCl, pH 7.6, 0.15 N NaCl) and 1 mM phenylmethylsulfonyl fluoride for 30 min (16). The filter was washed with TS plus 0.1% Triton X-100. An individual strip was reacted with anti-MS (10 µg/ml) for 3 h at room temperature. After washing three times with TS-Triton, the filter was incubated with bio-H/M IgG for 30 min. The filter was washed again with TS-Triton and reacted with the ABC for 30 min (6-8). After a final washing with TS-Triton, the filter strips were developed with 4-chloro-1-naphthol (stock, 3 mg/ml in methanol; diluted 1:5 in PBS prior to use) and hydrogen peroxide (16).

Ultrastructural Distribution of Antigen. The MS cells were washed three times and incubated with anti-MS (10 µg/ml) at 4°C for 1 h. After one washing with cold phosphate buffer, the cells were fixed with paraformaldehyde/periodate/lysine fixative containing 2% paraformaldehyde, 1 x 10^{-3} M periodate, and 2 x 10^{-2} M lysine in 3.75 x 10^{-2} M phosphate buffer, pH 7.4, for 10 min. The cells were then washed, incubated sequentially with bio-H/M IgG and ABC (6-8), and finally developed with a substrate containing diaminobenzidine, nickel chloride, and hydrogen peroxide. Next, the cells were fixed again with 2% glutaraldehyde and processed for electron microscopy (17).

Effects of Antibody on Modulation of Antigen in Vitro. The modulations of MS antigen in vitro by anti-MS was studied by addition of the antibody to cell cultures during incubation at 37°C. Nonreactive ascites fluid or fresh culture medium, added to identical cultures, served as a negative control for modulation (18-20). The quantities of anti-MS used for induction of modulation varied from 0.5 to 200 µg/1 x 10^6 cells. The extent of modulation was examined at various times by means of an immunoperoxidase assay on cytospin smears, or by an immunofluorescence assay with the Ortho Fluoroctystragraph 50H. Briefly, cells were washed three times at 4°C, and MAbs remaining on the surface were detected upon incubation with fluoresceinated rabbit anti-mouse IgG (FITC-R/M IgG), or with bio-H/M IgG followed by ABC (20).

The cells were also stained with additional anti-MS; we used the staining to detect the remaining antigen determinants and/or the remaining antigen determinants plus the bound MAbs added previously.

The percentage of modulated cells was calculated as: No. of reactive cells No. of reactive cells (pre-modulation) = (post-modulation) x 100

Cytotoxicity of MAbs on Cells. Twenty-five µl of cell suspension in RPMI 1640 medium at a concentration of 1.0 x 10^6/ml and 25 µl of diluted hybridoma culture supernatant (1–10 µg/ml) were added to a round-bottom microtiter plate (Linbro), and the cells were incubated at 4°C for 60 min. Then 10 µl of rabbit complement (GIBCO) was added, and the mixture was incubated at 37°C for 60 min. After incubation, 60 µl of 0.24% Trypan Blue dye solution (GIBCO) was added to each well. The contents of each well were mixed by use of a Pasteur pipet and placed into a hemocytometer, and both viable and nonviable (stained) cells were counted. The specific cytotoxicity of the MAbs, as determined in this study, was calculated as follows:

Specific cytotoxicity (%) on unmodulated cells is calculated as:

$$\frac{1 - \frac{\text{Viable cells with antibody and complement}}{\text{Viable cells with complement only}}}{100}$$

RESULTS

Establishment of Cell Lines. In our attempt to achieve long-term cultures of mesothelial cells from four patients, we were able to establish two permanent cell lines from two of these patients (Nos. 1 and 2) (Fig. 1). The mean age of the four patients was 63 years. Two (Nos. 1 and 4) had a history of asbestos exposure. The two cell lines (MS-1 and MS-2) have been cultured continuously for more than 47 and more than 38 months, respectively, in the medium which we use. The cells adhered to plastic and are spindle-shaped (Fig. 2). The doubling times for these cells are approximately 46 and 72 h, respectively, and their viability has been maintained at 95%.

That these cell lines consisted of mesothelial cells was confirmed by immunocytochemical studies and by transmission and scanning electron microscopy. The mesothelial nature of the cells was established by cytogenetic and DNA cycle analysis
Fig. 1. MS cells (MS-1) from a patient (No. 1) with pleural effusion. The cells show aneuploidy, with a DNA index of 1.8 (A). X-axis in A, amount of DNA (fluorescence intensity); Y-axis, number of cells. Ordinate and abscissa are each in linear scales. The tumor cells have slender, elongated villi (B). In the original cytospin smear, the MS cells tended to clump together; these cells have large nuclei and prominent nucleoli (C). After TPA induction, the cytoplasmic:nuclear ratio is increased (D).

Fig. 2. Growth patterns of MS-1 and MS-2 cells. Both cells adhere to plastic. The cells are spindle-shaped. A and B, phase contrast micrographs (original magnification, ×250); C, scanning electron micrograph (×2000).
(Fig. 1A). The cell cytoplasm contained abundant cytokeratin and vimentin, especially around the nuclei. The staining intensity and characteristics were the same before and after culture. Before long-term culture, the MS cells had abundant, long microvilli (Fig. 1B). After culture, however, on light-microscopic or transmission electron-microscopic examination, the number of these microvilli seemed to decrease, and yet they were easily detected on the cultured cells by scanning electron microscopy (Figs. 3 and 4).

The MS cells from the other two patients were maintained in culture for more than 3 months, with gradual loss of viability (Fig. 5). During the course of culture, however, the cells continuously expressed vimentin and cytokeratin. The MS cells which failed to grow indefinitely appeared to be less anaplastic (Fig. 5, B and C) than did those of the permanent cell lines (Fig. 1C). These cells had smaller nuclei and more abundant cytoplasm and were often indistinguishable from reactive mesothelial cells.

DNA Content. The histogram for the cell cycle of MS-1 cells before culture is shown in Fig. 1A, which indicates a DNA index of 1.8. The cultured cells of the MS-1 cell line exhibited a similar, if not identical, DNA index; this is compatible with the aneuploidy (chromosome number between 66 and 90) noted (see below) for these cells. The DNA index of the MS-2 cells was 1.16 (histogram not shown), which is compatible with a modal chromosome number between 48 and 52 in these cells.

The cell cycle of the MS-3 cells (from Patient 3) is displayed in Fig. 5A. These cells were aneuploid, with a DNA index 1.05. The MS-3 cells in the pleural effusion were morphologically similar to reactive mesothelial cells, thus posing a diagnostic problem with respect to their distinction from reactive cells (Fig. 5B). However, the aneuploidy of these cells provides

Fig. 3. MS-1 cells after 28 months of long-term culture. The tumor cells have a large nucleus with two or more prominent nucleoli (A). These cells responded well to TPA induction, and morphologically they resembled reactive atypical mesothelial cells (B). The villi of MS-1 cells seem to decrease in length and in number after long-term culture (C); however, the villi can be reinduced after TPA treatment (D). The cytoplasm of MS-1 cells contains abundant cytokeratin and vimentin. The smears in E, A, and B as well, were prepared after trypsinization. Cells in F were allowed to grow onto the glass coverslip. Note the spindle-shaped cells, which adhere to plastic or glass. Both E and F were stained for vimentin.
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Fig. 4. Scanning electron micrograph of a cultured MS-1 cell shows long, slender, and branched microvilli (arrow heads). Arrow, cell surface (original magnification, 11,000).

Fig. 5. MS cells from patient 3 (MS-3) show aneuploidy, with a DNA index of 1.05 (A). X-axis in A, amount of DNA (fluorescence intensity); Y-axis, number of cells. Ordinate and abscissa are each in linear scales. Cytologically, the tumor cells resemble reactive mesothelial cells (B). After TPA induction, the tumor-cell morphology was indistinguishable from that of normal mesothelial cells (C). The MS-3 cells were maintained in culture for more than 3 months, but did not grow indefinitely.

unequivocal evidence for their neoplastic nature. The DNA content of cells from Patient 4 was not determined.

TPA Induction. The MS cells in culture from four of the patients responded to TPA by morphological differentiation toward reactive or normal mesothelium-like cells (Figs. 1D, 3B, and 5C). The nuclei became round and small, and the amount of cytoplasm appeared to increase. The microvilli were significantly increased in length and in number following TPA induction (Fig. 3, C and D). The expression and antigenic distribution of cytokeratin and vimentin in the TPA-induced cells remained unchanged from control, uninduced cells (Fig. 3, E and F).

Cytogenetic Study. The modal number of chromosomes in MS-1 cells was 86, with a range of 66 to 90 per cell. A total of 36% of the cells were seen to have chromosomal aberrations, including chromatid breaks (14%), chromosome breaks (2%), fragments (18%), and partial pulverization (2%). Structural chromosomal abnormalities found in MS-1 cells included the following: t(2;3)(p11;q11), 70%; del(4)(q32), 70%; t(5;22)(p12;p11), 70%; del(6)(q22), 80%; t(6;18)(p11;p11), 100%; del(8)(p12), 70%; del(9)(p21), 100%; i(10q), 100%; del(13)(q21), 100% (Fig. 6).

The modal number of chromosomes in MS-2 cells was 52, with a range of 48 to 52 per cell. Structural chromosomal abnormalities in the MS-2 cells included the following: +7; +11; +14; -19; +21; +22; 4(?); +del(4)(q22); +del(18)(p12); del(X)(q23).

Heterotransplantation Study. Intraperitoneal transplantation of MS-1 cells, but not MS-2 cells, was successful in all three of the nude mice injected with these cells. The tumor cells in...
Production of MAbs. For MAb screening, we selected MAbs based on their reactivity with MS-1 cells, but not with normal or reactive mesothelial cells from noncancer patients. One clone, anti-MS-1, met this requirement. This antibody reacted with the neoplastic cells of all five patients with MS (Fig. 7). Weak reactivity of this antibody was detected in less than 5% of the reactive mesothelial cells in three of 12 pleural-fluid samples tested (Fig. 7, B and C). The antigen-positive cells generally had a large nucleus and prominent nucleoli, and morphologically they displayed reactive atypia. The antibody did not react with normal skin, brain, thyroid, lung, heart, liver, pancreas, stomach, intestine, kidney, adrenal gland, prostate, uterus, cervix, ovary, testis, salivary glands, or skeletal muscle. In lymph nodes, the antibody stained rare, scattered mononuclear cells. The nature of these cells has not been determined. We also observed very weak extracellular staining in germinal centers.

Anti-MS is an IgG1 immunoglobulin. The antibody failed to precipitate or detect antigen when we used immunoprecipitation and Western blotting. The membranous nature of this MS antigen was confirmed by immunoelectron-microscopic examination (Fig. 7, D and E).

Reactivities of Anti-MS with Human Neoplastic Cells. Anti-MS stained the neoplastic cells from all five patients with MS. Because the distinction between MS and adenocarcinoma in the lung may present a diagnostic problem, we screened the reactivity of this antibody primarily on neoplastic cells in lung cancers. None of the lung tumors showed staining with anti-MS. Also, no reactivity was detected in tumors from other organs. The antibody failed to stain MS cells in formalin-fixed and paraffin embedded tissue sections.

Antigen Modulation. In early experiments, antigen modulation was induced by addition of anti-MS to the culture supernatant at a final concentration of 20 μg/ml, or 20 μg per 1 × 10⁶ cells. The MAbs that bound to cell membranes of MS cells were detected on subsequent incubation of the cells with FITC-R/M IgG or with bio-H/M IgG and ABC. The anti-MS could still be detected on the cell surface 3 days after incubation. The MS cells were also stained by additional anti-MS at a concentration of 2 μg/ml, followed by incubation with FITC-R/M IgG or with bio-H/M IgG and ABC. The percentage of cells stained and the intensity of staining were virtually the same as observed in control cells. This result indicates that the antibody-antigen complexes were not lost, or were lost only minimally (<5% modulation), from the surface; it also indicates that no significant amounts of free or unbound antigens were present.

In subsequent experiments, the concentration of anti-MS added to cultures was reduced to 10, 5, 1, or 0.5 μg/ml. Even in these small amounts, the MAb was readily detected on the MS cell surface for more than 48 h. At 72 h, the cells still remained positive for bound antibodies, except in one sample which was incubated with MAb at 0.5 μg/ml, in which the cells were stained only weakly, or not stained at all, by FITC-R/M IgG or bio-H/M IgG and ABC. The result indicates that the antibody-antigen complexes may disappear from the cell surface when small amounts of antibody are applied. When cells were stained with additional anti-MS, they exhibited a staining intensity comparable to that of control cells incubated with non-reactive ascites fluid. This suggests that the amount of these antigens present on the cell surface was similar in MAb-treated and control cells.

Ultrastructural Distribution of Antigen–Antibody Complexes on MS Cells. MS cells were incubated with MAb at a concentration of 10 μg/ml at 4°C for 1 h. At this low temperature, the antigen generally could not be modulated by complexing with antibodies. Thus, the distribution of antigen which we observed on subsequent electron microscopy can be considered a representative pattern for unmodulated cells. Fig. 5 shows a uniform distribution of antigen on the cell membrane. In separate experiments, cells were first fixed with PLP and then stained with
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Fig. 7. Reactivity of anti-MS monoclonal antibody. A, anti-MS reacted with MS cells (MS-4). The slides were prepared after cytocentrifugation and were stored in a freezer at -70°C. The slides can be retrieved at any time for immunostaining. B, a cytospin smear prepared from cells of a uremic patient with pleural effusion. Several reactive mesothelial cells (arrows) and small mononuclear (lymphoid) cells are present. C, most normal or reactive mesothelial cells (arrow) did not react, or reacted extremely weakly (arrow head), with anti-MS. D, anti-MS stained the surface of MS-1 cells after long-term culture. E, the membrane distribution (arrow head) of antigen was verified by immunoelectron-microscopic study.

anti-MS. Although fixation appeared to reduce the antigen reactivity of the cells, as shown by a subsequent weak staining intensity, a similar uniform distribution was observed with this MAb.

Cytotoxicity of MAbs. The viability of MS cells in enriched RPMI medium was generally greater than 95%. The addition of MAb with or without rabbit or fresh human complement did not decrease the viability of the cells. In all of the experiments, the number and viability of cells were essentially identical to those in control cultures.

DISCUSSION

We have described the establishment and characterization of two MS cell lines, MS-1 and MS-2, and the production of a monoclonal antibody, anti-MS. The mesothelial nature of these cell lines has been confirmed by extensive light- and electron-microscopic and phenotypic investigations. Cytogenetic, DNA content, and heterotransplantation studies of the MS-1 and MS-2 cell lines further confirmed their malignant origin. Furthermore, we provided evidence that the MAb anti-MS may be useful in the future for immunotherapy or imaging, and that the antibody can facilitate the diagnosis of MS.

The diagnosis of MS can be very arduous, since MS must be distinguished from adenocarcinoma and from noncancerous disorders in which reactive mesothelial cells are abundant (2-4). For distinction between adenocarcinoma and MS, the use of vimentin antibody has been shown to be helpful, since vimentin is present in mesothelial cells, but generally not in cells from adenocarcinomas (2-4). Other antibodies, such as anti-Leu M1 and B72.3 may also be used (21-22). For distinction between reactive and neoplastic mesothelial cells, the use of vimentin antibody may not be so effective, because both neoplastic and reactive mesothelial cells express vimentin. This is exemplified by the diagnostic problem in our case 3 (Fig. 2), in which the tumor cells had cytological features which it was difficult to distinguish from those of reactive mesothelial cells. The determination of DNA content and the use of anti-MS antibody are helpful for the resolution of this problem.

The aneuploidy of MS cells from three of the patients was detected in cytogenetic studies and determinations of DNA content. For Patient 1, the DNA index was 1.8, which is compatible with the near-tetraploidy of the chromosomes that
was detected by cytogenetic study. The establishment of cell lines from MS cells with a high DNA index (1.8 for MS-1 and 1.16 for MS-2), but not from cells with a low DNA index (1.05 for MS-3) is not surprising, because the majority of human cell lines established from neoplastic cells tend to have a large number of chromosomes. In reports on other MS cell lines, the modal chromosome numbers were 51–52 and 68 for JMNN and H-MESO-1, respectively (23, 24). No chromosome studies was reported for other cell lines, including DND-10B, MT-1, MT-3, HUT28, HUT290, HUT513, and VAMT-1 (25–29).

Although the use of anticytokeratin and antivimentin can facilitate the diagnosis of MS, the expression of cytokeratin and vimentin is highly variable in cultured MS cells. It has been proposed that vimentin may be expressed as a consequence of loss of cell-cell contact after the cells are detached from the epithelial sheet (30). LaRocca and Rheinwald (31), however, showed that vimentin is expressed in normal and neoplastic mesothelial cells in culture, regardless of their growth pattern. The two MS cell lines (MT-1 and -3) reported by Anderson et al. (28) had weak cytokeratin expression, whereas the expression of vimentin was not known. In H-MESO-1 cells, both cytokeratin and vimentin were present, but the expression of vimentin was weak (24). The expression of these intermediate filaments in other MS cell lines was not known. Our MS-1 and MS-2 cells adhered to plastic and only rarely floated in the medium, both adherent and floating MS cells expressed similar amounts of vimentin and cytokeratin, which were not affected by TPA induction. The induction, however, increased the number of microvilli on cultured MS cells.

We have used MS-1 cells as immunogen to produce a specific MAb for MS cells. The antibody anti-MS is of the IgG subclass and recognizes a surface antigen on the MS cells cultured in vitro and in pleural fluid from patients with MS. The nature of the antigen has not been determined because, for unknown reasons, the antibody failed to immunoprecipitate antigen (15). The anti-MS antibody reacted rarely with large reactive mesothelial cells and lymphoid cells in lymphoid tissues. It appears that the antibody may react with a small number of activated or proliferating mesothelial cells. The use of anti-MS should provide a useful supplemental test for the diagnosis of malignant MS.

Anti-MS failed to modulate the antigen. It bound very avidly to the membranes of MS cells for more than 3 days. When MS cells were treated with small amounts of anti-MS (0.5 µg/ml), the antibody-antigen complexes disappeared from the membranes after 72 h of incubation; this may reflect the normal metabolic turnover of surface macromolecules (18–20). The antibodies apparently did not interfere with the expression of antigens during 3 days of incubation. Restaining with anti-MS MAb revealed a staining intensity comparable to that of control cells, indicating that similar amounts of antigen were present. However, the long-term effect of the continuous exposure of cells to this MAb is not yet known.

Since the antibody does not react with most normal cells and fails to induce antigenic modulation in MS cells, it may have clinical applications in immunoimaging or detection of MS. We had noted previously that a nonspecific, unwanted uptake of MAb by the cells or tissues in the reticuloendothelial system always occurs during the first few hours after MAb administration (32). The selective uptake of MAb by tumor cells in vivo is visualized better when the unwanted uptake by the reticuloendothelial system has disappeared, approximately 12–24 h after MAb administration (32).

The fact that anti-MS MAb binds specifically to tumor cells for more than 72 h renders them highly suitable as immunoimaging or therapeutic agents. Anti-MS has neither direct nor complement-mediated cytotoxicity for MS cells. Therefore, treatment of patients with this MAb without its further modification may not be effective. However, the use of antibody-toxin, antibody-drug, or antibody-isotope conjugates appears promising because the antibodies provide an effective means of delivery of drugs or isotopes to tumor cells through specific MAb binding.

In conclusion, we have successfully established two MS cell lines and a specific anti-MS cell MAb. These cell lines maintain characteristics of mesothelial cells, and they are suitable for biochemical and genetic studies of mesothelioma. The anti-MS antibody does not react with most normal tissues and cells, and it does not induce the modulation of antigen. The antibody should be assayed in the near future for its immunoimaging and therapeutic potential. In addition, we suggest that the application of anti-MS, the use of TPA induction, and the determination of DNA content should facilitate the diagnosis of MS.

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